ET-13

Thirteenth

International Conference on Endothelin

Celebrating 25 Years of the Discovery of ENDOTHELIN

September 8-11, 2013 Tokyo, Japan

PROGRAM & ABSTRACTS

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Conference Chairs

Noriaki Emoto, MD, PhD, is Professor at the Department of Internal Medicine at Kobe University Graduate School of Medicine and at Kobe Pharmaceutical University. After his residency as a cardiologist in Kobe University Hospital, Japan, he conducted his graduate studies and postdoctoral fellowship under the supervision of Dr. Masashi Yanagisawa in Dallas, Texas. He won the Louis N. Katz Basic Science Research Prizes of the American Heart Association in 1994 for the molecular identification and characterization of endothelin-converting enzymes, ECE-1 and ECE-2. He moved back to Kobe, where he leads his own laboratory as a physician-scientist. His group is staffed by two medical associate professors, two postdoctoral fellows and medical PhD students from various countries. He is in charge of the pulmonary hypertension program in Kobe University Hospital. His major research interest is the translational research of endothelin.

Takashi Miyauchi, MD, PhD, is a Professor of Cardiology (2003 - Present) , Faculty of Medicine, University of Tsukuba, Tsukuba, Japan. He graduated from University of Tsukuba Medical School (1983, MD), and also from Graduate School of University of Tsukuba (1990, PhD). He is a cardiologist who is also familiar with basic sciences of pharmacology, molecular biology, and physiology. The main theme of his research is the investigation of the physiological and pathophysiological roles of vasoactive peptides including endothelin in the cardiovascular system and diseases such as hypertension, pulmonary hypertension, heart failure, e.t.c. He published over 200 manuscripts in Lancet, Nature, Circulation, Circulation Research, Hypertension, American Journal of Physiology, e.t.c. Currently, he works as a Cardiologist (Professor) at Tsukuba University Hospital, and also works at the Life Science Center of Tsukuba Advanced Research Alliance (TARA) at University of Tsukuba.

Conference Faculty

Dennis L. Andress, MD, is the Senior Medical Director for Renal Development with Abbvie. Prior to his employment at Abbvie, he was Clinical Professor of Medicine and Director of the Renal Dialysis Unit at the Division of Nephrology, University of Washington, Seattle, WA. Dr. Andress contributed basic and clinical research discoveries related to complications of chronic kidney disease and end-stage renal failure with over 100 publications in peer-reviewed journals. His research was supported by grants from the National Institutes of Health and the Northwest Kidney Foundation. His current interest is in drug development for diabetic nephropathy particularly as it relates to the endothelin system and to the selective ETA receptorantagonist, atrasentan.

Kazutaka Aonuma is the Professor and Director of Tsukuba University Hospital

EDUCATION

1971-1977 University of Yamaguchi, School of Medicine, Yamaguchi; M.D.

1977-1982 Post Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo; PhD.

POSTDOCTORAL TRAINING

1982-1984 Fellowship in Cardiology, Miami Heart Institute, Miami Beach, Florida, USA.

ACADEMIC APPOINTMENT

1999-Present: Visiting Professor of Cardiology, Department of Internal Medicine, Tokyo Medical and Dental University of Medicine, Tokyo, Japan

2001-Present: Visiting Professor of Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan

2002-Present: Visiting Professor of Internal Medicine, Tokai University School of Medicine, Kanagawa, Japan

2006-Present: Proffessor and Director of Cardiovascular Division, University of Tsukuba, Tsukuba, Japan

Medical Licensure:

National Board License: No.238454 (1977)
Special Board of Internal Medicine: No.3620 (1988)
Special Board of Cardiology: No.02202 (1989)

Board Certification:

Council Member of the Japanese Society of Electrocardiography
Council Member of the Japanese Heart Rhythm Society
Council Member of the Japanese Clinical Cardiac Electrophysiology Society
Council Member of the Japanese Circulation Society
Member of the American Heart Association

Editors of the Journal

Circulation Journal	Associate Editor
Journal of Cardiology	Associate Editor
Journal of Arrhythmia	Editor in Chief

Anna Bagnato, PhD, has been group leader in the Laboratory of Molecular Pathology at the Regina Elena National Cancer Institute of Rome, Italy, since 1998. She is the author of numerous articles published on leading international journals and book chapters and has coordinated cancer research grants by public and private agencies. Her research, defining many critical activities of the endothelin axis in the development and the progression of cancer, in particular in ovarian carcinoma model, represents the translational basis leading to the introduction of novel targeted therapies in the ovarian cancer management. As a member of the Board of the Italian Cancer Society, Dr. Bagnato plays an active role in promoting cancer research and communicating science to the public

Matthias Barton, MD, FAHA, is a graduate of Hannover Medical School, Germany, and since 2007 has been Professor of Cardiology at the University of Zurich in Switzerland. He received his clinical training in internal medicine, cardiology, and anaesthesiology at Hannover Medical School and the University Hospitals of Basel, Bern, and Zurich. From 1999, he held a SCORE Career Development Award from the Swiss National Science

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Foundation to study novel factors involved in coronary artery disease and has a clinical interest in preventive cardiology. For the past 25 years, his research has focussed on atherosclerosis and the molecular mechanisms and endothelial factors contributing to coronary artery disease and cardiovascular risk. Dr. Barton is a Fellow of the American Heart Association and a member of the International Advisory Board of the *International Conferences on Endothelin*. He is Past Chair of the *Twelfth International Conference on Endothelin* (ET-12) held in Cambridge, UK, in 2011, and served as Guest Editor of the ET-12 Conference Proceedings, *Endothelin XII.*

Ariela Benigni read Biological Science (Biol. Sci. D. Degree, University of Milan). She researched problems of anti-cancer and anti-thrombotic drugs in Milan and in Strasbourg before joining the Mario Negri Institute for Pharmacological Research in Bergamo, Italy, where she studied mediators of renal damage including the role of endothelin-1 in progressive renal injury (Ph.D. Degree, University of Maastricht). She is currently the Head of Department of Molecular Medicine and Scientific Secretary of Mario Negri Institute for Pharmacological Research of Bergamo.
She has contributed to more than 230 research publications. Her recent work has looked at therapies to halt renal disease progression or even induce regression of kidney lesions by multidrug approach with the interest to characterize cellular determinants of kidney repair after angiotensin II blockade.
She acted as Associate Editor of *Kidney International, Journal of Nephrology* and *International Journal of Artificial Organs;* actually she is Editor of *Expert Opinion on Therapeutic Patents and PeerJ.*
She was consultant of WHO for a multicentre observational study on the predictive ability of angiogenic factors for Pre-eclampsia. For this latter study she has been appointed as Senior Fellow by the University of Oxford, Nuffield Department of Obstetrics & Gynaecology. She was the Chairman of the 10th International Conference on Endothelin (ET-10), hold in Bergamo 2007.
She has recently been named to take part to the Visiting Committee of AERES – Agence d'Évaluation de la Recherche et de l'Enseignement Supèrieur – for the evaluation of scientists at the Hôpital Necker in Paris and she received the Merit Award of Bergamo City Hall for her contribution to science.

Brian D. Cain, PhD, studied at the University of Colorado and the University of Illinois (Ph.D., 1983). While a postdoctoral fellow at Stanford University, he began a continuing study of the F1F0 ATP synthase. Dr. Cain joined the faculty of the University of Florida in 1988. He has chaired the Physical Biochemistry Study Section for the NIH, and the Bioenergetics Gordon Conference.
In collaboration with Dr. Charles Wingo and later Dr. Michelle Gumz (UF, Dept. of Medicine), the Cain laboratory has also investigated the molecular regulation of ion transport in the renal distal collecting duct. Microarray experiments showed high level induction of the *Edn1* gene in a collecting duct cell line (mIMCD-3) in response to aldosterone leading to our interest in endothelin. Hormone treatment resulted in binding of both the mineralocorticoid and glucocorticoid receptors to a hormone response element located in the 5' regulatory region of the *Edn1* gene. Recently, the miRNA landscape of mIMCD-3 cells was determined by microarray analysis. Ongoing experiments are aimed at determining the mechanisms of miRNA regulation of *Edn1* expression.

Carmine Cardillo is a professor of Internal Medicine, head of a Division of Internal Medicine and chief of the Vascular Physiology Laboratory at the Catholic University Medical School in Rome, Italy. He received his M.D. and his fellowship in Internal Medicine from the Catholic University. He also served for almost 4 years as a Visiting Scientist at the Cardiology Branch, NHLBI, of the National Institutes of Health in Bethesda, MD, USA.
In addition to practicing and teaching, Dr. Cardillo has been involved for long time in research on the mechanisms of vascular dysfunction in patients with risk factors for cardiovascular disease. In particular, he has studied changes in the main endothelial homeostatic mechanisms, including the nitric oxide pathway and the endothelin system, in hypertension, diabetes and hypercholesterolemia. He has also investigated the determinants of impaired vasoactive properties of insulin in conditions like obesity and the metabolic syndrome. He has authored almost one hundred peer-reviewed articles on these topics.

Subrata Chakrabarti, MBBS, PhD, FRCPC, is a Professor and Chair of the Department of Pathology at the University of Western Ontario, London, Ontario, Canada. He also works as a pathologist at the London Health Sciences Centre biology. Dr. Chakrabarti investigates mechanisms of chronic diabetic complications and studies diabetic retinopathy, nephropathy and diabetic heart disease and has published extensively in this field. He has a long-standing interest in the role of endothelins in chronic diabetic complications and other diseases.

Martine Clozel, Actelion Pharmaceuticals, Ltd, Allschwil, Switzerland Martine Clozel, a paediatrician specialized in neonatal intensive care, obtained her MD degree at Nancy University, France, and received further training in physiology and pharmacology from McGill University, Montreal, and the University of California, San Francisco. During her 11 years at F. Hoffmann-La Roche Ltd, she initiated the research project on endothelin and endothelin receptor antagonists (ERAs) which led to the discovery and clinical development of bosentan (Tracleer), tezosentan, clazosentan and macitentan. Her group has published over 150 peer-reviewed papers in the fields of endothelial function, endothelin and ERAs. In 1997 she was awarded the Hoffmann-La Roche Research Prize for her achievements in the field of endothelin research. In 1997 she co-founded Actelion Pharmaceuticals Ltd, where she is Senior Vice President and since 2009, Chief Scientific Officer. Martine Clozel is a member of the Scientific Editorial Board of *Science Translational Medicine*. In 2008, she was honoured as "Chevalier dans l'Ordre de la Legion d'Honneur".

Michael R. Dashwood, PhD, obtained his PhD in Physiology at the University of London in 1986 having previously been at the National Institute for Medical Research in London where his original research was investigating central sites of opioid induced analgesia and cardiovascular control. For the last 20 years his focus has shifted from brainstem regions involved in central

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cardiovascular control to the 'local' vascular effects of endothelium-derived nitric oxide and endothelin. These studies have generated a number of research papers, reviews and book chapters describing the potential involvement of these factors in diseases including atherosclerotic vascular disease such as critical limb ischaemia and venous disease including vein graft failure.

Anthony Davenport, PhD, directs the Human Receptor Research Group in the Clinical Pharmacology Unit, University of Cambridge focusing on understanding the role of G-protein coupled receptors, and their transmitters in the human cardiovascular system and how these are altered with disease. The role of endothelin in human pathophysiology, especially the development of atherosclerosis has been a major research interest since the discovery of the peptide in 1988. These include quantifying and imaging endothelin receptors in normal and diseased human tissue with sub-type selective radioligands and antisera as well as positron emission tomography to non-invasively image endothelin receptors in vivo. Wider research interests are reflected through membership and co-vice chair of the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification which maintains the GPCR and ion channel database at http://www.iuphar-db.org/index.jsp, including latest parings of orphan receptors with their cognate ligands, published this year in *Pharmacological Reviews*. Dr Davenport is a Fellow of the British Pharmacological Society, member of the Editorial Board of the *British Journal of Pharmacology* (including Themed Reviews on *Endothelin 2011*), *Current Opinion in Pharmacology* and editor of *Receptor Binding Techniques*. He is a member of the, International Advisory Board of the International Conferences on Endothelin and was co-chair of the Twelfth International Conference (ET-12) held in Cambridge UK in 2011.

Jo G. R. De Mey, PhD, is Professor of Medical Molecular Pharmacology at the Institute of Molecular Medicine, University of Southern Denmark, and professor of Vascular Pharmacology at the Cardiovascular Research Institute Maastricht (CARIM) of Maastricht University, the Netherlands. He was trained as a zoologist (M.Sc.) and as a pharmacologist (PhD) at the University of Antwerp. Before joining Maastricht University he headed a cardiovascular department in pharmaceutical industry focussing on therapeutic applications of natriuretic peptides. He is the author of more than 160 scientific articles covering early aspects of endothelium-dependent vascular reactivity, endothelial dysfunction in hypertension, endothelium dependent arterial remodeling in experimental models of heart failure and diabetes and, more recently, molecular pharmacology of ET-receptors and endogenous functional antagonists of ET-receptor function such as CGRP. He coordinates the cardiovascular research within the Dutch public–private partnership Top Institute Pharma that addresses among others "metalloproteases and novel targets in endothelial dysfunction".

Neeraj Dhaun (known to most as 'Bean') is a clinical and academic nephrologist in Edinburgh, Scotland. His clinical interests are immune-mediated renal disease especially vasculitis and high-risk renal transplantation. He jointly runs the South-east Scotland Vasculitis & Lupus Clinic. His research is both laboratory and clinical in nature and has focused on the vascular aspects of chronic kidney disease, particularly the role of ET-1.

Pedro d'Orléans-Juste, PhD, is a Professor of Pharmacology (1990 - Present) at Sherbrooke University Medical School, Quebec, Canada. He initiated his research endeavors on endothelins in 1988, as a post-doctoral fellow (supervisor, Sir John Vane, William Harvey Research Institute). Trained in cardiovascular pharmacology during his graduate studies, Dr D'Orléans-Juste was involved in the early report on the nicardipine insensitive- vascular properties of endothelins, in the conversion of big-endothelin-1 to endothelin-1 in vivo and more recently in the first report on the pivotal contribution of chymase in the production of endothelin-1 in vivo. Dr D'Orléans-Juste, among the 100 most cited pharmacologists worldwide, lists 250 publications and currently supervises 5 MSc and PhD students on a CIHR-funded programme on the role of mast cell proteases in the genesis of endothelin-1. Dr. d'Orléans-Juste is Past Chair of the *Sixth International Conference on Endothelin* (ET-6) and the *Eleventh International Conference on Endothelin* (ET-11), both held in Montréal in 1999 and 2009, respectively, and is a member of the International Advisory Board of the *International Conferences on Endothelin.*

Adviye Ergul, MD, PhD, FAHA, is a Professor of Physiology at the Georgia Health Sciences University in Augusta, GA. She received her PhD in Biochemistry and Molecular Biology on a project related to endothelin-1 (ET-1) structure and function. Since then she has been interested in the physiology/pathophysiology of the ET system. She made the seminal observation that plasma ET-1 concentrations are much higher in African-American hypertensives which contributed significantly to our understanding of the ET system in salt-sensitive hypertension. She built a strong research career on the roles of ET and its receptors in diabetes-associated complications. She has over 85 scientific papers and her research has been continuously supported by American Heart Association, American Diabetes Association, National Institutes of Health and Veterans Administration. Dr. Ergul is a Fellow of the *American Heart Association*

Keiichi Fukuda, MD, PhD, is a professor of Department of Cardiology, Keio University School of Medicine in Tokyo, Japan. One of his research interest was the role of endothelin on the cardiovascular development, especially its effect on cardiac sympathetic innervation. He had revealed that cardiomyocyte-produced nerve growth factor (NGF) mediated cardiac sympathetic nerve innervation, and NGF production from cardiomyocytes was determined by autocrine/paracrine secreted endothelin I-mediated signals. Moreover, cardiac sympathetic nerve transdifferentiated to

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cholinergic neurons during heart failure via gp130 mediated signals, especially LIF. He is also interested in regenerative medicine, and developed various techniques for induction, purification, and transplantation of cardiomyocytes obtained from ES cell/iPS cells. He also investigated the disease model of iPS cells obtained from the patients with hereditary heart disease such as long QT syndrome and Brugada syndrome.

Dr. **Adel Giaid** is currently a Professor of Cardiology at the McGill University Health Centre in Montreal, Canada. He was of the original pioneers who worked on the pathological role of endothelin in the cardiopulmonary system. Indeed, Dr. Giaid was the first to report the presence of endothelin in the fetal and adult nervous and respiratory systems. He then reported an association between the pathological characteristics of pulmonary artery disease (pulmonary hypertension) and expression of endothelin-1. Subsequently, he demonstrated increased expression of endothelin-1 in a number of diseased conditions such pulmonary fibrosis, heart failure, atherosclerosis and pulmonary neoplasm.

Hunter C. Gillies, MD, is a Senior Director in clinical research at Gilead Sciences Inc. San Francisco. He spent the past 13 years in the pharmaceutical industry working on clinical drug development, primarily focusing on the cardiovascular effects of phosphodiesterase type 5 inhibitors (PDE5i) and endothelin receptor antagonists (ERA). For the past eight years has worked in the field of pulmonary hypertension including clinical research and trials in PAH and PH secondary to chronic lung disease. Currently his research focus involves clinical trials involving new pharmacological targets and exploring the potential for pharmacological synergy between PDE5i and ERAs. He has a number of peer reviewed publications on cardiovascular physiology and pharmacology, exercise physiology and the development of PD5i for cardiopulmonary disorders.

Katsutoshi Goto, PhD, is a Professor Emeritus of University of Tsukuba, Tsukuba, Japan. He was educated at the University of Tokyo and at West Virginia University Medical Center, USA. He was a Professor of Pharmacology at the Institute of Basic Medical Sciences, University of Tsukuba. He was a member of the team of the discovery of endothelin in 1988. After being a Professor of University of Tsukuba, he also became the Provost of Graduate School of Comprehensive Human Sciences, University of Tsukuba. He has been a councilor of the Japanese Pharmacological Society from 1977 and a member of the American Society for Pharmacology and Experimental Therapeutics and Federation of American Societies for Experimental Biology from1988. His fields of research are pharmacology, molecular biology, and physiology of the cardiovascular systems. The main theme of his research is the analysis of the physiological and pharmacological activity of peptides including endothelin. Form this work, he has written more than 200 papers. He was the chairman of the Eighth International Conferences On Endothelin in 2003 at Tsukuba, Japan. After finishing of his Professor-ship at University of Tsukuba, he became the Director of JST Innovation Satellite Ibaraki, Tsukuba, Japan. Currently, he is a Professor Emeritus of University of Tsukuba, Tsukuba, Japan.

Anil Gulati, MD, PhD, FCP, is a Professor of Pharmaceutical Sciences and Associate Dean for Research at the Midwestern University. He is a United States Fulbright Scholar 2008-2009 and winner of International Ranbaxy Research Award 2007. He obtained his M.D. in 1982 from King George's Medical College, Lucknow, India and became Diplomate American Board of Clinical Pharmacology (1992). He was awarded a Ph.D. in 1996 by Erasmus University Rotterdam, The Netherlands. He is a Fellow of the American College of Clinical Pharmacology. He has more than 265 peer reviewed publications and guided research of more than 70 graduate students and research fellows. Dr. Gulati has 21 patent applications (six issued patents) and has founded three companies. Medications developed by Dr. Gulati are undergoing clinical trials in the United States and India.

Ken-ichi Hirata, MD, PhD, is a Professor of Cardiovascular Medicine at the Kobe University Graduate School of Medicine. His research has focused on atherosclerosis and coronary circulation. He is specifically interested in the vascular biology including inflammation, immune system and endothelial function to develop novel therapies for atherosclerotic diseases. Dr. Hirata is a member of Japanese Circulation Society, Japanese Atherosclerosis Society, ESC and AHA.

Berthold Hocher studied medicine in Berlin and Heidelberg. He started his research career at the department of biochemistry at the Free University of Berlin where he also accomplished his PhD. In the following years he worked clinically in the field of internal medicine with the focus on endocrinology and nephrology. After clinical posts at the Benjamin Franklin University Hospital of the Free University of Berlin and the Charité, he became senior consultant nephrologist and associated professor at the University Hospital Bern/Switzerland. Thereafter, he worked in the pharmaceutical industry in pre-clinical and clinical drug development (Solvay Pharmaceuticals, Hannover, Germany and Roche, Basel, Switzerland). Currently he holds a full professorship at the University in Potsdam for experimental nutritional medicine and is visiting professor at the Jinan University, Guangzhou, China. The main topics of his research group are fetal programming of cardiovascular diseases, exploration of novel targets for the treatment of diabetic complications focusing on diabetic nephropathy as well as biomarker research, see also: http://www.uni-potsdam.de/en/eem/index/prof-hocher-engl.html.

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Satoshi Homma, MD, PhD, is a Professor of Cardiovascular Medicine at the University of Tsukuba. His research interest, since the PhD work in Tsukuba (1987-1991), has focused on the hemo-dynamic changes in microcirculation of visceral organs, dramatically influenced by some potent vaso-active substances such as endothelin, prostacyclin, phospho-diesterase inhibitor, nitroglycerine and CGRP etc. After his clinical career started at National Cardiovascular Center in Osaka (1992-1998), he returned as a medical staff in the critical care unit here in University of Tsukuba Hospital and has begun to develop the treatment of patients with pulmonary hypertension, considering the effects of the potent vaso-active substances on the patient body and its microcirculation. Dr. Homma is also interested in the quality of the hospital care. He is now involved in working as director of QARM (Quality Assurance and Risk Management) department at the University Hospital and as councilor of the Japanese Society of Quality and Safety in Healthcare.

Uichi Ikeda, MD, PhD is a Professor and Chairman of Department of Cardiovascular Medicine at Shinshu University School of Medicine in Matsumoto City, Nagano Prefecture. His research has focused on cardiac regeneration and angiogenesis. He is also interested in epidemiology, vascular function and cell biology, and therapeutics of peripheral artery disease. He has also published several reports concerning pathophysiological roles of endothelin in Takayasu's arteritis, valvular heart disease and coronary artery disease.

Hiroshi Ito MD. PhD. is the Professor of Cardiovascular and Respiratory Medicine, Akita University Graduate School of Medicine. He also appointed as the director of Akita University Hospital and the vice president of Akita University. His research interest is the pathophysiology of heart failure, especially, focused in roles of nerurohumoral factor in the mechanisms of heart failure. He is also involved in several clinical researches in heart failure. He is a committee board member of Japanese Circulation Society, and member of several scientific societies related to cardiovascular diseases.

Hiroshi Ito, MD, PhD, FACC, FESC is a professor of Cardiovascular Medicine, at Okayama University Graduate School of Medicine in Okayama, Japan. He is an executive board member of Japanese Circulation Society and of Japanese Pulmonary Circulation Society. He is going to be a chairman of 3rd annual scientific meeting of Japanese Pulmonary Circulation Society in 2014. He is a chairman of working group on "Indication and application of balloon pulmonary angioplasty to patients with chronic pulmonary thromboembolism" that is supported by Japanese Circulation Society. His research interests include clinical application of echocardiography and development of therapeutic strategy of heart failure. In the field of pulmonary hypertension, he studies the mechanisms of right ventricular dysfunction associated with pulmonary hypertension with echocardiography.

Masaaki Ito, MD, PhD, is a Professor of Cardiology and Nephrology in Mie University Graduate School of Medicine and Physician-in-Chief of Cardiology in Mie University Hospital in Tsu, Japan. He has been working on the regulation of contractile cycle in vascular smooth muscle. He discovered the signal transduction regarding Ca^{2+} sensitization of smooth muscle contraction through Rho-kinase-mediated myosin phosphatase inhibition. He is also interested in clinical pharmacology in hypertension and coronary atherosclerosis. He is recently going to have research-activities focusing on clinical and basic research on pulmonary hypertension and right heart failure. Dr. Ito is a Fellow of Japanese College of Cardiology and the Japanese Society of Hypertension, and a member of the Japanese Circulation Society and the American Heart Association.

Yasuki Kihara, MD, PhD, FJSIM, FACC, FACP, FESC
1987-1989, Instructor in Medicine, Harvard Medical School, Boston
1989-1993, Assistant Professor, Toyama Medical and Pharmaceutical University
1993-2004, Assistant (-2002) and Associate (-2004) Professor, Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine
2005-2007, Chairman, Department of Cardiovascular Medicine, Kobe General Hospital
2008-present, Professor and Chairman, Department of Cardiovascular Medicine, Hiroshima University Graduate School of Biomedical & Health Sciences

Dr. **CheMyong Jay Ko** is an associate professor of the Department of Comparative Bioscience, College of Veterinary Medicine at the University of Illinois at Urbana-Champaign (UIUC). He graduated from the Seoul National University in Seoul, Korea, with a bachelor degree in Biology education in 1986. Dr. Ko taught Biology and General Science for a few years at a junior high middle school before he entered a graduate school at the Seoul National University where studied Developmental Biology and Biology Education. In 1998, he graduated with a PhD degree, and moved to United States to do a postdoctoral training at the University of Kentucky in the field of Reproductive Biology and Molecular Endocrinology. In 2002, he took an assistant professor position in the Department of Clinical Sciences and Department of Biology at the University of Kentucky. In 2011, he moved to the University of Illinois at Urbana Champaign to take his current academic position. His major research interests are in female Reproductive Biology.

Donald E. Kohan, MD, PhD, FASN, is a Professor of Medicine and Physiology at the University of Utah Health Sciences Center in Salt Lake City, UT. His research for over two decades has focused on kidney regulation of blood pressure and salt balance. He is specifically interested in the role of endothelins and nitric oxide in the control of renal sodium and water transport and blood pressure, and uses multiple transgenic and gene-targeted models.

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He is also involved in clinical studies using endothelin receptor antagonists to treat patients with chronic kidney disease. Dr. Kohan is Past Chair of the *Nineth International Conference on Endothelin* (ET-9), held in 2005 in Park City, UT, USA. Dr. Kohan is a Fellow of the American Society of Nephrology and a member of the International Advisory Board of the *International Conferences on Endothelin.*

After graduation from the German School of Athens, Dr. **Theofilos M. Kolettis** obtained his MD degree from Medical School, University of Athens in 1984 and his PhD in 1991. He trained in Cardiology at the Edinburgh Royal Infirmary, U.K and at the Athens General Hospital. He worked as a clinical research associate at the Department of Electrophysiology, Eastern Heart Institute, NJ, and as an attending physician at the Onassis Cardiac Surgery Center, Athens, Greece. He is currently Professor in Cardiology at the University of Ioannina, Greece.
Dr. Kolettis practices in the field of cardiac pacing and clinical electrophysiology. His research interests include the study of the pathophysiological effects of endothelin during myocardial infarction, focusing on ventricular remodeling and tachyarrhythmias.

Issei Komuro
The University of Tokyo Graduate School of Medicine, CREST
Issei Komuro, MD, PhD, FAHA, FISHR, is Professor of Medicine and chairman of Cardiovascular Medicine Department, The University of Tokyo Graduate School of Medicine. After obtaining MD and PhD from The University of Tokyo, he started postdoctoral fellowship at Harvard Medical School in 1989. He has become professor of Chiba University in 2001, of Osaka University in 2009 and of The University of Tokyo in 2012, and PI of CREST in 2011. His research interests are molecular mechanisms of heart failure, cardiovascular development and regeneration. He has published over 500 papers in peer-review journals and received many awards including Outstanding Investigator Prize from ISHR, Sato award from JCS and Balz award. He is an associate editor of Circulation Research and an editorial board member of Journal of Clinical Investigation, Arteriosclerosis thrombosis and vascular biology, Cardiovascular Research and Journal of Molecular Cellular Cardiology.

Takeyoshi Kunieda, MD, PhD, FCCP, is a Professor of Medicine, International University, Health and Welfare, Clinical Medical Research Center, Kaken Hospital, Ichikawa, Chiba, Japan, and former Professor of Medicine, Keio University. Professor Kunieda graduated from Keio University, Tokyo, Japan in 1962 and completed PhD from Keio University in 1968 and has been studying pulmonary circulation for more than 40 years, mostly involved in the study of pulmonary hypertension and pulmonary embolism at National Cardiovascular Center, Osaka, and Keio University, and worked together with many specialized doctors for the treatment of pulmonary hypertension. He currently is a chairman of Japanese Society of Pulmonary Hypertension Reseach.

Hiroki Kurihara, MD, PhD, is a Professor of Molecular and Cellular Biology at the University of Tokyo Graduate School of Medicine. He contributed to the discovery of endothelin in 1988 and first reported endothelin-1 knockout mice in 1994. His present research interests are craniofacial and cardiovascular development and the involvement of neural crest cells. He also acts as a member of Institute for Biology and Mathematics of Dynamical Cell Processes (iBMath), the University of Tokyo, to promote a relationship between medicine and mathematics.

Dr. **Langleben** is a Professor in the McGill University Faculty of Medicine, and former Chief of Cardiology of the Jewish General Hospital. He founded and directs the Center for Pulmonary Vascular Disease, the first pulmonary hypertension clinic in Canada. His research interests include lung vascular metabolism; behaviour and function of lung vascular cells; vascular cell biology; epidemiology of pulmonary hypertension, and the development of new medications for pulmonary hypertension. His work has been supported by the Medical Research Council of Canada, The Canadian Institutes for Health Research, the Fonds de la Recherche en Sante du Quebec, the Heart and Stroke Foundations of Quebec and Canada, and the Quebec Lung Association.

Marilena Loizidou is a Senior Lecturer in the Division of Surgery and Interventional Science, UCL, UK. The Division is situated on 4 different Campuses and Marilena is Head of the Royal Free Campus. She originally trained in biochemistry (Canada) and pharmacology (UK). Her long term research has focused on (a) epithelial-stromal interactions in cancer and (b) translational cancer pharmacology. In colorectal cancer, she investigated tumourigenic actions of endothelin-1. Her group systematically delineated anti-cancer mechanisms of endothelin A receptor antagonists, mainly through cancer-stroma interactions. The antagonists are now in colorectal cancer trials. She currently leads the cancer nanotechnology group which uses nanoparticles and nanoformulations to improve cancer imaging and drug efficacy. Her current research focuses on (a) novel drug nanoformulations, including photochemical internalization and (b) using biofunctionalised (targeted) nanoparticles for theranostics. Marilena is also the Director of the MSc in Surgical Science and Co-Director and Founder of the MSc in Nanotechnology and Regenerative Medicine - cited in Nature as a most innovative course, 2009. Marilena is a council member of the Society of Academic and Research Surgery, UK and was voted UCL Academic Role Model (2013).

Koji Maemura, MD, PhD is a Professor of Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, and Chief of Cardiology of Nagasaki University Hospital, Nagasaki city, Japan. He graduated from Faculty of Medicine, the University of Tokyo in 1986. After trained as a resident and clinical follow in Tokyo University Hospital, he started research under the supervision of Hiroki

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Kurihara, MD, PhD. He was involved in the projects regarding *Edn1* deficiency mice and *Edn1* overexpressing mice. His present major interests are Cardiovascular Medicine, Vascular Biology, Atherosclerosis, and Chronobiology. He is an executive board member of The Japanese Vascular Biology and Medicine Organization, and an editorial board member of Arteriosclerosis Thrombosis Vascular Biology.

Dr. **Janet Maguire** is a Senior Research Associate in the Clinical Pharmacology Unit, University of Cambridge and a Fellow of Queens' College, Cambridge. She is a member of the BHF Receptor Research Group whose focus is the pharmacology of established and novel G-protein coupled receptors in the human cardiovascular system and who have particular expertise in the endothelin peptides and their receptors. Dr. Maguire has co-authored fifty eight peer reviewed papers, eight book chapters and seventeen reviews, and is Editor and contributor of a volume, 'Peptide Research Protocols:Endothelin' for Humana Press. She is also involved in undergraduate and graduate teaching in pharmacology, supervising pre-clinical medical and veterinary students, is a Wellcome Trust/MRC 4-year PhD programme Principal Investigator and a member of the International Union of Pharmacology Receptor Nomenclature Sub-Committee on Endothelin Receptors.

Hiromi Matsubara, MD, PhD, is currently the Director of Department of Clinical Science and Division of Cardiology at National Hospital Organization Okayama Medical Center, Okayama, Japan. After receiving his MD from Okayama University Medical School and finishing his residency in internal medicine at National Okayama Hospital, he began his fellowship at National Cardiovascular Center, Osaka, Japan in 1990. He became an Assistant Professor of Department of Physiology II at Okayama University Graduate School of Medicine and Dentistry in 1993. He then became an Assistant Professor of Department of Cardiovascular Medicine in 1997 and again promoted in 2000 to the Associate Professor of Cardiovascular Medicine. He then became the Director of Division of Cardiology at National Hospital Organization Okayama Medical Center and he also serves as the Director of Department of Clinical Science since 2010.
His investigative interests have focused on clinical and physiologic aspects of pulmonary hypertension. He has made Division of Cardiology at National Hospital Organization Okayama Medical Center as one of the largest pulmonary hypertension center in Japan.

Yasuo Matsumura, PhD, is a Professor of Pharmacology at Osaka University of Pharmaceutical Sciences (Takatsuki, Japan) since 2002. Professor Matsumura is a member of council of the Japanese Pharmacological Society, Japanese Society for Circulation Research, Nitric Oxide Society of Japan, etc. He was a member of Editorial Staff (2007-2010) of Folia Pharmacologica Japonica, an official journal of The Japanese Pharmacological Society. His PhD degree was obtained (1987) at Osaka City University Medical School. His main areas of expertise, research and work include renal, heart, and circulatory pharmacology, health food scuience, and vascular biology.

Soichi Miwa, MD, PhD, is a Professor of the Department of Cellular Pharmacology, Hokkaido University Graduate School of Medicine in Sapporo, Japan. He was a collabolator of Professor Tomoh Masaki in the Department of Pharmacology, Kyoto University Graduate School of Medicine in Kyoto, Japan, from 1992 to 1997, and a Secretary General of the Fifth International Conference on Endothelin (ET-5; President, Tomoh Masaki) held in Kyoto, Japan. His research for over two decades has focused on intracellular Ca^{2+} signaling mediated by endothelin receptor, especially in terms of receptor-operated Ca^{2+} channels and store-operated Ca^{2+} channels. He is also involved in a research on regulatory mechanisms for G-protein coupled receptor (GPCR) recycling/degradation using ET_A and ET_B receptors as a model system. His immediate hope is to clarify the molecular mechanism for the different fate of ET_A and ET_B receptors following ET stimulation. Recently he is also interested in a pathophysiological role of ET system in the development of insulin resistance as a major cause of type 2 diabetes mellitus.

Shin-ichi Momomura, MD is a Professor of Cardiovascular Medicine and also the President of Jichi Medical University Saitama Medical Center. His specialty is heart failure and was participated in publishing Japanese Guidelines for Acute Heart Failure as well as Japanese Guidelines for Chronic Heart Failure. He main interest in heart failure research was cardiac function in diseased heart. However, recently it is shifting to sleep disordered breathing and cardiovascular disease. He was appointed as Chairman of the Committee of Japanese Guidelines for the Diagnosis and Treatment of Sleep Disordered Breathing Associated with Cardiovascular Disease which was published in 2010. He is now conducting a randomized study to evaluate the effect of adaptive servo-ventilation on left ventricular function in patients with heart failure due to left ventricular systolic dysfunction. He is a President of the 2013 Annual Scientific Meeting of the Japanese Heart Failure Society held in Saitama City in November.

Toyoaki Murohara, MD, PhD, FESC is Professor at the Department of Cardiology, Nagoya University Graduate School of Medicine. He obtained M.D. and Ph.D. degrees at Kumamoto University School of Medicine. He started Postdoctoral Fellowship at Thomas Jefferson University in 1993, followed by Department of Cardiology, St. Elizabeth's Medical Center, Tufts University School of Medicine in 1996. His research interests are vascular biology for the prevention of atherosclerosis and regeneration therapy for ischemic diseases. He is an associate editor of Circulation Journal and Hypertension Research, and an Editorial Board Member of Circulation Research, Journal of the American College of Cardiology, Journal of Molecular and Cellular Cardiology and ATVB.

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Norifumi Nakanishi, MD, PhD is a Director of Endowed Department of Pulmonary Hypertension and Pulmonary Vascular Medicine, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan. His clinical and research interests are in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. Dr Nakanishi is a councilor of Japanese Society for Adult Congenital Heart Disease and serves as an executive board member of Japanese Pulmonary Hypertension Society. He is also a head editor of Guideline for Treatment of Pulmonary Hypertension of the Japanese Circulation Society.

Dr. **Kazuwa Nakao** is the professor at Medical Innovation Center, Kyoto University Graduate School of Medicine. Prior to holding the current title, he was Professor and Chairman of the Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine (Kyoto, Japan) for twenty years. He was also Director of Kyoto University EBM Research Center, Kyoto University Translational Research Center, and Vice Dean of Kyoto University Graduate School of Medicine. He has been engaged in Translational Research for natriuretic peptides(ANP,BNP and CNP) and leptin, has achieved clinical application of these hormones and has authored 925 English publications in the areas of Cardiovascular Endocrinology, and Metabolism and Obesity / Metabolic Syndrome.

Professor Nakao is the President of Japan Society for the Study of Obesity (JASSO) and a former Chair of Board of Directors at the Japan Endocrine Society (JES).

He has received numerous awards including Edwin Von Baelt Award (1988), Medical Award (The Japan Medical Association, 2004), The Prize for Science and Technology, Research Category, The Commendation for Science and Technology (Minister of Education, Culture, Sports, Science, and Technology, Japan, 2008), Takeda Medical Award (Takeda Science Foundation, 2009), and Medal with Purple Ribbon (2011).

John Pernow MD, PhD, FESC, is Professor and Head of the Cardiology Unit, Department of Medicine, Karolinska Institutet. He is also senior consultant of cardiology at the Department of Cardiology, Karolinska University Hospital. He is a member of the scientific council of the Swedish Heart and Lung Foundation. His research is focused on mechanisms behind and treatment targeting endothelial dysfunction with special emphasis on the role of endothelin in cardiovascular disease. John Pernow has been active in endothelin research for twenty years and published several papers regarding the pharmacology and pathophysiological role of endothelin in cardiovascular disease and complications to diabetes. An additional research area is protection against myocardial ischemia and reperfusion injury in experimental and clinical studies. Dr. Pernow is a Fellow of the *European Society of Cardiology*

Dr. **David Pollock** earned his Ph.D. degree in Physiology from the University of Cincinnati in 1983. He completed a post-doctoral fellowship at the University of North Carolina at Chapel Hill. He then spent two years as a Senior Scientist at the Institute for Circadian Physiology at Harvard University in Boston before taking a position in the Drug Discovery division of Abbott Laboratories in Chicago. In 1995, he accepted a faculty position in Vascular Biology Center at the Medical College of Georgia (now known as Georgia Regents University) where he has risen to the rank of Regents' Professor. In 2010, he became the founding chief of the Section of Experimental Medicine. He also holds positions in the Departments of Physiology, and Pharmacology & Toxicology.

Dr. Pollock is a fellow of the American Heart Association and the American Society of Nephrology and currently serves as Program Director of a pre-doctoral training grant by the National Heart Lung and Blood Institute. Dr. Pollock was recently elected to become President of the American Physiological Society in 2014. He recently completed a term as Associate Editor for the *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology and Vascular Pharmacology* and is now serving as Editor-in-Chief of *Comprehensive Physiology*. He is also a founding member of the International Advisory Board on Endothelin who organize the bi-annual International Conferences on Endothelin.

Dr. Pollock's research is related to the control of sodium excretion and the role of the kidney in blood pressure regulation. His long-standing interest in natriuretic factors has led to his active involvement in elucidating the actions of endothelin. His research has helped to elucidate the opposing actions of endothelin A versus endothelin B receptors in both renal vasculature and the tubular system. Recent studies from his lab have suggested that defects in the endothelin B receptor system contribute to salt-dependent hypertension. His research has been supported for many years by several grants from the National Heart Lung and Blood Institute and the American Heart Association. He currently serves as the Principle Investigator on a Program Project Grant on Endothelin in the Kidney.

Dr. **Jennifer S. Pollock** is a Weiss Professor and the Director of the University System of Georgia MD/PhD Program at Georgia Regents University. She earned her Ph.D. in Biological Chemistry from The University of North Carolina at Chapel Hill and received post-doctoral training under the tutelage of Dr. Ferid Murad, 1998 Nobel Laureate in Physiology and Medicine. Subsequent to her post-doctoral training, she worked as a Drug Discovery Scientist at Abbott Laboratories before moving to GRU in 1995. Dr. Pollock's research is on the vascular and renal mechanisms of hypertension and diabetes focusing on the role of nitric oxide and endothelin in the relationship of stress on the vasculature, renal function, and immune responses. Her research is currently supported by two Program Project Grants from the National Institutes of Health. Dr. Pollock serves as a member of two AHA study sections and as an ad-hoc reviewer for NIH Program Project Grants. Dr. Pollock has mentored and trained over 60 undergraduate students, medical students, graduate students, post-doctoral fellows, clinical fellows, and junior faculty members in her tenure at GRU.

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Sunu B. Raharjo is a physician-scientist at National Cardiovascular Center Harapan Kita/ Department of Cardiology & Vascular Medicine, University of Indonesia. As a physician, he has been working as cardiologist and cardiac electrophysiologist, taking care of patients and teaching students on a daily basis; while as a scientist, he has been working with other scientists in his Cardiovascular Research Center to continue his research interests.

The journey of Dr Raharjo to become a scientist began with studies on the molecular aspects of endothelin converting enzyme family, and continued with translational researches using animal models. He performed these two research area when he was a PhD student and a JSPS Postdoctoral Fellow at Kobe University, Kobe, Japan. There, he worked with Professor Emoto to apply pharmacological as well as genetic interventions to investigate the pathophysiological roles of endothelin (and bradykinin) system in animal models.

Returning to Jakarta, Indonesia, Dr Raharjo resumed his cardiology training. Besides, his passion on research brought him to continue his basic and translational research in the clinical area. He has been working on the role of endothelin and bradykinin in metabolic syndrome and pulmonary hypertension.

Yoshihiko Saito, MD, FAHA, is a Professor of First Department of internal Medicine at Nara Medical University in Kashihara, Japan. His research has focused on roles of humoral factors in the cardiovascular system, especially heart failure. He proved the usefulness of ANP infusion for the treatment of heart failure in 1987, only 3 years after ANP discovery. He is now the president of the society of cardiovascular endocrinology and metabolilsm (CVEM), that is another scientific society focusing natriuretic peptides, adrenomedullin, ghrelin, endothelin and other cytokines. He held the international symposium on CVEM in Nara in 2010. Recently he is specifically interested in the underling molecular mechanism of the cardiorenal connection.

Tsutomu Saji, MD,PhD, Professor in Pediatrics, has worked in the field of pediatric cardiology for over 30 years. Since 1976, after the graduation from Toho University, Tokyo. he started off his career as a medical intern in the Department of Pediatrics, Toho University Hospital, then became an Assis. professor (1986), an Assoc. professor (1993), and then a professor in chief in Pediatrics (1997-present). He also has experience as an intern in Pediatric Cardiology, Heart Institute of Japan, Tokyo Women's Medical College, (1977-1978), and a research fellow in Children's Hospital of Los Angeles, University of Southern California, CA, U.S.A (1987-1988).

He has expertise in pulmonary hypertension, Kawasaki vasculitis, and myocarditis in children. He serves as FAHA, International Liason of CVDY in AHA, FACC, FSCAI (the Society for Cardiac Angiography and Interventions), FJCC, the Secretary general of the Japanese Society of Ped Card, President of Japanese Pediatric Pulmonary Circulation Society, and holds numerous important roles in many different scientific societies and associations in Japan. He'd also participated in Japanese Guideline Committee members of PAH. He've reported genetic studies of *BMPR2, ALK1, ALK6,* and *Smad8* mutation,and negative HHV-8 in PAH.

Satoshi Sakai, MD, PhD, is an Assistant Professor at the Department of Cardiovascular Medicine, University of Tsukuba in Tsukuba, Japan. After his training as a cardiologist in Tsukuba University Hospital, he started basic studies under the supervision of Professor Katsutoshi Goto and Professor Takashi Miyauchi in Tsukuba, Japan. He won the YIA of the 4th International Conference on Endothelin (ET-4, London) in 1995 for the pathophysiological role of myocardial endothelin system on the development of heart failure. He conducted his postdoctoral fellowship at Baylor College of Medicine in Houston, Texas; he moved back to Tsukuba and he leads his own laboratory as physician-scientist. Dr. Sakai's research deals with mechanisms and treatment of heart failure, pulmonary hypertension, and cardiopulmonary interrelations from the viewpoints not only of the endothelin system but also of the inflammation and metabolism. Dr. Sakai is a council member of Japanese Pharmacological Society, Japanese Heart Failure Society, and Japanese Pulmonary Circulation Society, and serves as a Secretary General of the 13th ET conference (ET-13, 2013).

Shigetake Sasayama,M.D. is an Emeritus Professor of Kyoto University and currently an honorary director of Uji Hospital in Kyoto. Dr. Sasayama was the Chief Director of the Japanese Circulation Society from 2000 to 2002. He has also served as the Chief Director of the Japanese Heart Failure Society and is the past president of the Japanese Society of Internal Medicine. From 2002 to 2005, he committed himself in the WHF as a board member representing Asia-Pacific region. He has a longstanding interest in physiological and biological mechanisms of the development of heart failure. He has also pioneered in an assessment of functional capacity of heart failure patients. Over the past 35 years, he has contributed more than 600 original articles to the cardiovascular literature and has published more than 50 invited articles or book chapters. He also serves on the editor or the editorial board of many major international and domestic journals in Cardiology, including the official journal of the American Heart Association, "Circulation".

Toru Satoh, MD, PhD

1982: Graduated from Keio University School of Medicine

1982-86: Internal Medicine Residency in Keio University School of Medicine.

1986-89: Cardiology Fellow at Keio University School of Medicine.

1989-92: Vice Chief in Cardiology in Ashikaga Red Cross Hospital.

1992-94: Cardiologist at Kawasaki Medical School

1994-99: Cardiologist at the division of Pulmonary Circulation in National Cardiovascular Center in Japan.

1999-2008: Assistant Professor in Cardiology and Associated Professor in Medical Education at Keio University School of Medicine.

2009-: Professor in Cardiology at Kyorin University School of Medicine.

A Leading doctor/ scientist in pulmonary hypertension in Japan. Member of the working group in the 5th world symposium on pulmonary hypertension.

Specialty: pulmonary hypertension, general cardiology, cardiac physical examination, exercise physiology, medical education.

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Dr. **Ernesto Schiffrin** is Physician-in-Chief of the Jewish General Hospital and holds a Canada Research Chair in Hypertension and Vascular Research. He is Professor and Vice-Chair (Research), Department of Medicine, McGill University.

Dr. Schiffrin's research deals with mechanisms and treatment of high blood pressure, from molecules and cells to humans. He is author of more than 500 peer-reviewed publications, many book chapters and is editor of 2 published books, and 2 in preparation, on molecular and clinical aspects of vascular disease and hypertension.

Dr. Schiffrin has been President of the Canadian Hypertension Society (1991-92), Chair of the High Blood Pressure Research Council of the American Heart Association (2002-2004), President of the InterAmerican Society of Hypertension (2005-2007) and President of the Quebec Hypertension Society (2009-2011). Dr. Schiffrin has been Vice-President (2010-2012) and is now President of the International Society of Hypertension (2012-2014). Dr. Schiffrin has been Associate Editor of Hypertension (AHA journal) since 2003.

Dr. Schiffrin received the Senior Investigator Award of the Canadian Society of Internal Medicine in 2003 and the Distinguished Service Award of the Canadian Hypertension Society in 2004. He was elected Fellow of the Royal Society of Canada in 2006, and received the 2007 Irvine Page-Alva Bradley Lifetime Achievement Award of the High Blood Pressure Research Council of the American Heart Association and the 2010 Bjorn Folkow Award of the European Society of Hypertension. He was appointed Member of the Order of Canada (C.M.) in July 2010. He was awarded the 2011 Excellence Award in Hypertension Research of the American Heart Association, in September 2011. In 2013 he was awarded the Queen Elizabeth II Diamond Jubilee Medal.

Hiroaki Shimokawa, MD, PhD is a Professor of Cardiology at Tohoku University.

Dr. Shimokawa is interested in the mechanisms for coronary atherosclerosis and vasospasm. He was the first to develop an animal model of coronary vasospasm and to demonstrate the involvement of Rho-kinase in the pathogenesis of the spasm and atherosclerosis both in animals and humans. Based on these findings, a specific Rho-kinase inhibitor has been developed and now in clinical trials for pulmonary hypertension. Dr. Shimokawa is also interested in endothelium-derived relaxing factors (EDRFs), especially endothelium-derived hyperpolarizing factor (EDHF). He has demonstrated the mechanism for endothelial dysfunction in atherosclerosis and the beneficial effect of fish oils and eicosapentaenoic acid on endothelial function. He has identified that endothelium-derived hydrogen peroxide (H_2O_2) is an EDHF in animals and humans, which notion is widely accepted. Dr. Shimokawa is also interested in applying the recent advances in biomedical engineering to vascular medicine. This includes the development of extracorporeal shock wave therapy for severe ischemic heart disease, and shock wave ablation system for arrhythmias. Finally, Dr. Shimokawa has been conducting several large-scale clinical trials on heart failure, ischemic heart disease, and pulmonary hypertension as principal investigators.

Francesca Spinella, PhD, graduated in Biology from the University of Catania in 1995, obtained her PhD degree in Neurobiology from the same University in 1999. As PhD student she has worked in professor De Vellis' laboratory in Los Angeles, CA. She is actually senior investigator at the Regina Elena National Cancer Institute of Rome where she has been working on endothelins since 2001. As a research fellow funded by Italian Association for Cancer Research (AIRC; 2002-2004), she has been working in Dr. Anna Bagnato's laboratory in Rome studying the role of endothelins on the cell-cell interaction and communication on melanoma cells. Of particular interest is the analysis of signal transduction pathways through which ET-1 triggers cancer progression, with special attention on the role of ET-1 in promoting angiogenesis and tumor cell invasive behavior. In 2010 she was Principal Investigator in a project funded by AIRC in which she defined many critical activities of ET-1 in the induction of lymphangiogenesis. Currently, Dr Spinella's research is focalized on the study of mechanism by which blocking ETBR could lead to tumor angiogenesis and lymphangiogenesis reduction, as potentially candidate in the inhibition of hematic- and lymphatic-driven metastatization.

Dr. **Duncan Stewart** (MD, FRCPC) is the CEO and Scientific Director of the Ottawa Hospital Research Institute (OHRI), as well as an active cardiologist and scientist whose work focuses on developing new regenerative therapies for cardiovascular disease. Dr. Stewart has made a number of important discoveries about the endothelial cells that line our blood vessels, including elucidating the important roles of the endothelial factors endothelin-1 and nitric oxide in vascular disease. He has also initiated Canada's first clinical trials using gene therapy for therapeutic angiogenesis in patients with advanced coronary artery disease and gene-enhanced progenitor cell therapy for pulmonary hypertension. He is currently investigating a gene-enhanced stem-cell therapyto help repair heart muscle after a major heart attack. He is also exploring the use of mesenchymal stromal cells as a therapy for septic shock. Clinical trials for both of these cell therapies are expected to start in 2013.

Pierre-Louis Tharaux, M.D., PhD.
INSERM Research Professor
Professional Experiences
2009-present: Inserm Research Professor and group leader, Inserm Paris Cardiovascular Research Center (PARCC), Hopital Européen Georges Pompidou, Paris, France.
Education and Training

- Medical studies at Faculté Necker-Enfants Malades- Université Paris Descartes.
- Interne des Hôpitaux de Paris (1991) (resident and fellow)
- Fellowship in Nephrology (1991-1998). Nephrology: Board Certified (1998).
- Medical Doctorate from Université Paris Descartes (1998).
- PhD. in Physiology & Pathophysiology from Université Pierre & Marie Curie (2000).
- Curriculum in physiology and pathophysiology of sleep and cardiovascular disorders, Université Paris Sud (2000).

Scientific interests: Pathophysiology of the Renal Circulation
Research in our laboratory is centered on the analysis of mouse

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vascular and renal pathophysiology, with a particular emphasis on genes implicated in signaling pathways. Our work covers three areas:
1- G-proteins coupled receptors (GPCRs) and tyrosine kinase receptors (TKR) signaling in podocytes in response to immune vasculitis with Rapidly Progressive Glomerulonephritis (RPGN), Diabetic Nephropathy and in Focal Segmental Glomerulosclerosis (FSGS).
2- Signaling in podocytes and endothelial cells in Sickle Cell Nephropathy and Vasculopathy.
3- Immunopathogenesis of Sickle Cell Disease.

Rita C Tostes
University of São Paulo
Brazil
B.Sc. in Pharmacy - University of São Paulo (1990), M.Sc. (1993) and Ph.D. (1996) in Biological Sciences (Pharmacology, School of Medicine of Ribeirao Preto, University of São Paulo). Internships at the Albert Einstein College of Medicine - NY, University of Montreal - Clinical Research Institute of Montreal and Georgia Regents University (Medical College of Georgia), GA.
Currently a professor at the School of Medicine of Ribeirao Preto - University of São Paulo, a researcher at the National Council for Scientific and Technological Development (CNPq) and a member of the Foundation for Research Support of the State of São Paulo (FAPESP).
Scientific interests in the field of Cardiovascular Pharmacology and Physiology, focusing on the signalling pathways that control vascular function and its changes in hypertension, diabetes and erectile dysfunction. Specific interests: the role of endothelin,aldosterone, cytokines and glycosylation with N-acetylglucosamine (O-GlcNAc) in vascular (dys)function.

Ivana Vaněčková was born in Prague, Czech Republic. She graduated at the Charles University in 1987. She followed her postgraduate studies at the Institute of Physiology, Czech Academy of Sciences, where she obtained her PhD. Degree for the Thesis „Age-dependent changes in the function of isolated perfused kidney in various forms of experimental hypertension in the rat". She started as a Postdoctoral Fellow at the Renal Physiology Lab at the Institute of Physiology, Prague. In 2001 she became the Assistant Research Professor at the Department of Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, where she was a Head of the Department for Experimental Hypertension. As a Senior Investigator she continued her work in the Institute of Physiology AS CR, in the Department of Experimental Hypertension, where she is a Deputy Head. She is a member of American Society of Physiology, European Society of Hypertension and International Society of Hypertension. Most important topics of her work: mechanisms of contribution of renin-angiotensin system and endothelin systems to blood pressure regulation.

Paul M. Vanhoutte, MD, PhD, FAHA, is a Chair Professor in the Department of Pharmacology and Pharmacy of the Li Ka Shing Faculty of Medicine of the University of Hong Kong. He obtained his M.D. degree at the University of Gent, Belgium. He has been Professor of Pharmacology at the University of Antwerp, the Mayo Clinic, Rochester MN, and Baylor College of Medicine, Houston TX. From 1992 to 2002, he was Director of Discovery Research at Servier near Paris in France. Dr. Vanhoutte is Doctor honoris causa of the Universities of Gent, Antwerp, Zurich, Montréal and Strasbourg, of the RMIT University in Melbourne, Australia, and of the Gr. T. Popa University in Iasi, Romania. Dr. Vanhoutte is a Highly Cited Researcher (ISI) in three categories: Biology & Chemistry, Pharmacology, and Clinical Medicine. Dr. Vanhoutte is the Past Chair of the *Third International Conference on Endothelin* (ET-3) held at Housten in 1993 and Past Honorary Chair of the *Sixth International Conference on Endothelin* ET-11) held in Montréal, Canada, in 1999, the *Ninth International Conference on Endothelin* (ET-9) held in Park City, Utah, in 2005 and the *Twelfth International Conference on Endothelin* (ET-12) held in Cambridge, UK, in 2011.

Nicolas Vignon-Zellweger studied biology at the University Paul Sabatier in Toulouse, France and at the Georg August University in Göttingen, Germany as an ERASMUS Programme fellow. He obtained his master in Pharmacology and Pharmacochemistry from the University Louis Pasteur in Strasbourg, France in 2005. He joined the group of Professors Franz Theuring and Berthold Hocher within the Center for Cardiovascular Research of the Charité Medical School of Berlin, Germany as a fellow of the *Marie Curie Host Fellowship for Early Stage Research Training* program CARDIOVASC funded by the European Commission. He obtained his PhD in natural sciences from the Free University of Berlin, Germany in 2010. He next moved to Japan and joined the research group of Professor Noriaki Emoto in the department of Clinical Pharmacy at the Kobe Pharmaceutical University as a postdoctoral fellow. His research interests include the understanding of the role of the endothelin system in renal and cardiovascular diseases (heart failure, hypertension, diabetic nephropathy…). His publications mostly present basic research and pre-clinical studies using various genetically modified mice. Nicolas Vignon-Zellweger is Secretary General of ET-13.

David J. Webb, MD, DSc, FRCP, FRSE, FMedSci, is Professor of Therapeutics and Clinical Pharmacology in the BHF Centre of Research Excellence at the University of Edinburgh, where he established its Centre for Cardiovascular Science, and consultant physician at the Royal Infirmary of Edinburgh. He is recognised internationally for his work on endothelial function and arterial stiffness, much of which focuses on the endothelin system, and on the investigation and effective treatment of patients with complex hypertension and chronic kidney disease. His work is mainly translational and he provides leadership to two new UK clinical PhD training initiatives in translational medicine and therapeutics (TMAT) based in Scotland, funded by the Wellcome Trust and Medical Research Council. He is a Fellow of the Royal Society of Edinburgh and UK Academy of Medical Sciences, and was awarded the SKB Silver and Lilly Gold Medals from the British

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Pharmacological Society for his research and for contributions to pharmacology, respectively. Dr. Webb was Chair of the Seventh International Conference on Endothelin (ET-7), held at Edinburgh in 2001. He is a member of the International Advisory Board of the International Conferences on Endothelin.

Bambang Widyantoro is an Indonesian medical doctor graduated from University of Indonesia. He joined residency program in Cardiology Department - University of Indonesia and has been working at the National Cardiovascular Center-Harapan Kita Hospital in Jakarta since 2003. During this period, he spent five years completing his research work for PhD thesis investigating the role of endothelin in cardiovascular medicine under the supervision of his mentor, Professor Noriaki Emoto of Kobe University, Japan. His current interests as a clinical cardiologist and researcher include hypertension, heart failure and diabetes-related cardiovascular disease.

Keiko Yamauchi-Takihara, MD, PhD is Presidential Aide and Director of Health Care Center in Osaka University, and the Professor of Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine. She worked in the laboratory of Dr. MJ Sole at The Center for Cardiovascular Research, The Toronto Hospital, as a post-doctoral fellow, where she began learning molecular cardiology. In 1992, she became Assistant Professor in the Department of Molecular Medicine, Osaka University Graduate School of Medicine, conducting research on signal transduction of cytokines and growth factors in cardiac myocytes. Current research interests include pathophysiology of heart failure, pulmonary circulation and cardiopulmonary interrelations.

Masashi Yanagisawa, MD, PhD, is an Investigator of the Howard Hughes Medical Institute (HHMI) and Patrick E. Haggerty Distinguished Professor of Molecular Genetics at the University of Texas Southwestern Medical Center at Dallas. Since 2010, he doubles as Professor and Director of the International Institute for Integrative Sleep Medicine at the University of Tsukuba. Dr. Yanagisawa discovered endothelins, their receptor and processing enzymes, and demonstrated their roles in the embryonic development. Later he also discovered the orexins and their receptors, molecules that regulate sleep and wakefulness. Promising therapeutic applications have emerged from both of his lines of exploratory research. Dr. Yanagisawa is a member of the US National Academy of Sciences, and member of the International Advisory Board of the International Conferences on Endothelin. He was honorary chair of the Tenth International Conference on Endothelin (ET-10) held in Bergamo, Italy, in 2007, the Eleventh International Conference on Endothelin (ET-11) held in Montréal, Canada, in 2009, and the Twelfth International Conference on Endothelin (ET-12) held in Cambridge, UK, in 2011.

Special Guest Lecture

Jun Yamashita, Kyoto, Japan
Born in Kyoto in 1965. Graduated from Kyoto University School of Medicine in 1990, entered Kyoto University Graduate School of Medicine in 1993, specializing in physiology. Gained a Ph.D. in medicine in 1998 and became a special researcher at the Japan Society for the Promotion of Science. After periods as an assistant professor and then an associate professor in Department of Molecular Genetics at Kyoto University Graduate School of Medicine, in 2003 was appointed an associate professor in Department of Stem Cell Differentiation at Kyoto University Institute for Frontier Medical Sciences. In 2008, concurrently appointed an associate professor at the Center for iPS Cell Research and Application (CiRA) at Kyoto University Institute for Cell-Material Sciences. Took up the current position in 2012.

Program at a Glance

[Sunday, September 8]

Tokyo Dome Hotel

		Aurora (B1F)
16:00-19:30	**Registration**	
18:00-20:00	**Welcome Cocktail**	

[Monday, September 9]

University of Tsukuba Tokyo Campus

8:30-8:40	**Opening Remarks**	Room 1 (B1F)
8:40-9:45	**Session 1: New Aspects of Endothelin in Physiology and Disease** *Chairs:* **Pedro D'orleans-Juste**, *Montreal, Canada /* **Soichi Miwa**, *Sapporo, Japan*	Room 1 (B1F)
9:45-10:30	**Session 2: Oncology** *Chairs:* **Anna Bagnato**, *Rome, Italy /* **Marilena Loizidou**, *London, UK*	Room 1 (B1F)
10:30-10:55	Coffee Break	Room 1 (B1F)
10:55-12:05	**Session 3: Renal Diseases** *Chairs:* **David Pollock**, *Augusta, USA /* **Neeraj Dhaun**, *Edinburgh, UK*	Room 1 (B1F)
12:15-13:15	**Lunch Session 1** Pfizer Japan Inc.	Room 1 (B1F)
12:15-13:15	**Lunch Session 2** MSD K.K.	Room 2 (1F)
13:15-14:45	**Poster Session 1** **Oncology, Renal Physiology and Disease, Neurology, Infectious Diseases, New Topics of Endothelin Biology**	Room 122 (1F) Lobby (B1F)
14:45-15:35	**Session 4: Resistant Hypertension** *Chairs:* **Kazutaka Aonuma**, *Tsukuba, Japan /* **Anthony P. Davenport**, *Cambridge, UK*	Room 1 (B1F)
15:35-16:05	Coffee Break	Room 1 (B1F)
16:05-17:05	**Session 5: Neurology, Pain and Stroke** *Chairs:* **Anil Gulati**, *Chicago, USA /* **Yasuo Matsumura**, *Osaka, Japan*	Room 1 (B1F)
17:05-17:45	**Session 6: Actual Applications and Future Perspectives of Dual ETA/ETB Antagonists** *Chairs:* **Janet Maguire**, *Cambridge, UK /* **Ariela Benigni**, *Bergamo, Italy*	Room 1 (B1F)

18:30-21:30	**Social Event for Young Investigators**

[Tuesday, September 10]

University of Tsukuba Tokyo Campus

8:30-10:10	**Session 7: Gene Regulation, Molecular and Cellular Biology** *Chairs:* **Keiichi Fukuda**, *Tokyo, Japan /* **CheMyong Jay Ko**, *Urbana, USA*	Room 1 (B1F)
10:10-10:40	Coffee Break	Room 1 (B1F)
10:40-12:06	**Session 8: Pulmonary Hypertension** *Chairs:* **Masaaki Ito**, *Tsu, Japan /* **Toru Satoh**, *Tokyo, Japan*	Room 1 (B1F)
12:15-13:15	**Lunch Session 3** Actelion Pharmaceuticals Japan Ltd.	Room 1 (B1F)
12:15-13:15	**Lunch Session 4** Nippon Shinyaku Co.,Ltd.	Room 2 (1F)
13:15-14:45	**Poster Session 2** **Gene Regulation, Molecular and Cellular Biology, Pharmacology, Pulmonary Hypertension**	Room 122 (1F) Lobby (B1F)
14:45-16:05	**Session 9: Blocking the ETA Receptors: What's New?** *Chairs:* **Michael Dashwood**, *London, UK /* **Ernesto Schiffrin**, *Montreal, Canada*	Room 1 (B1F)
16:05-16:30	Coffee Break	Room 1 (B1F)
16:30-16:45	**Young Investigator Award Ceremony**	
16:45-17:15	**Honorary Chair Session** *Chair:* **Hiroaki Shimokawa**, *Sendai, Japan* *Invited Lecture:* **Paul M. Vanhoutte**, *Hong Kong, China*	Room 1 (B1F)
17:15-17:45	**Special Guest Session** *Chair:* **Masashi Yanagisawa**, *Dallas, USA / Tsukuba, Japan* *Invited Lecture:* **Jun K. Yamashita**, *Kyoto, Japan*	Room 1 (B1F)
19:00-21:00	**TOKYO DOME HOTEL** **CONFERENCE DINNER** Celebration for the 25th Anniversary of the Discovery of Endothelin	

[Wednesday, September 11]

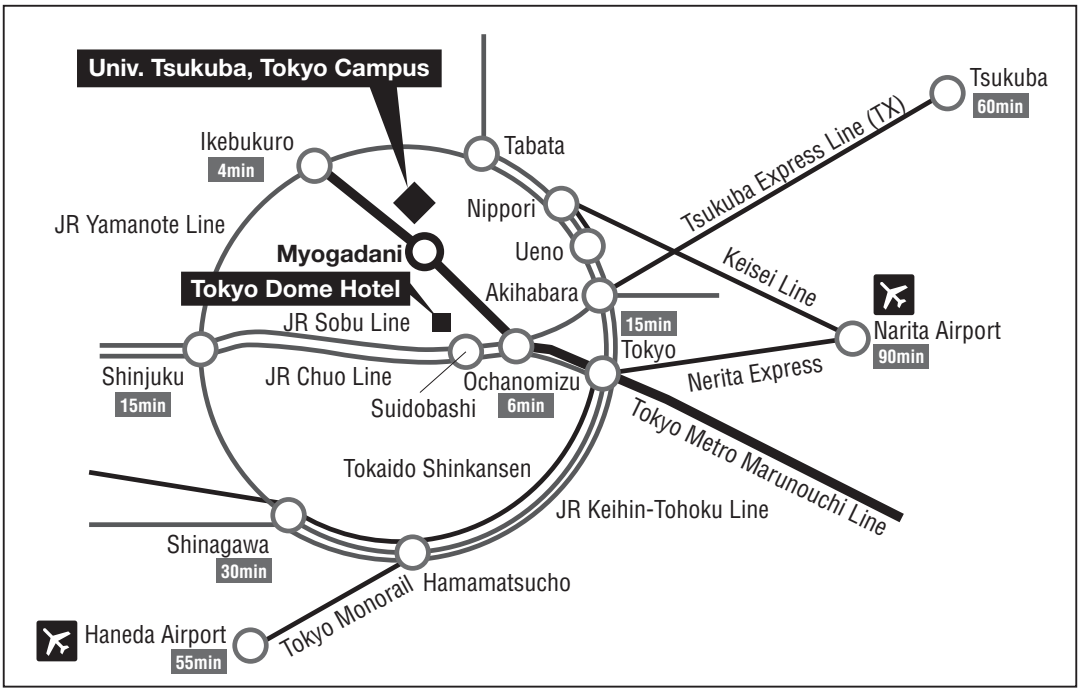
University of Tsukuba Tokyo Campus

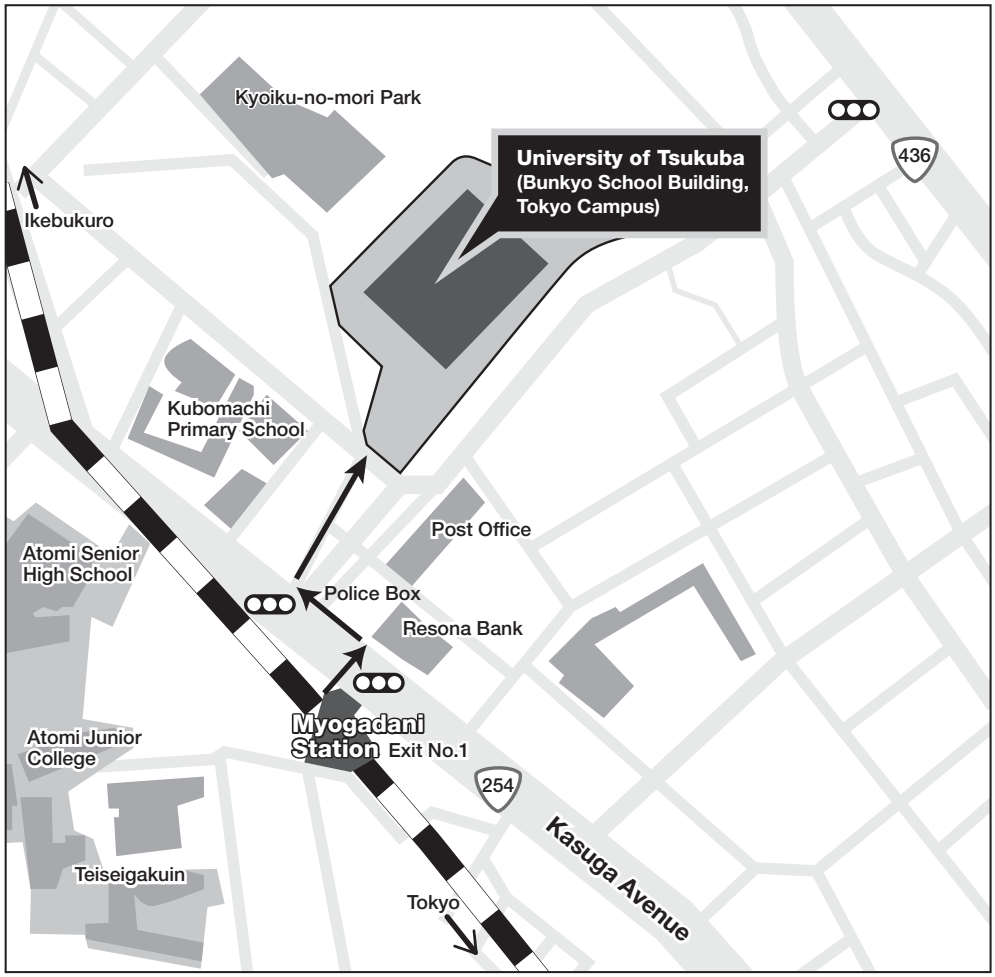
8:30-10:00	**Session 10: Cardiology, Hypertension, Vascular Disease** *Chairs:* **Rita C. Tostes**, *São Paulo, Brazil /* **Jo De Mey**, *Odense, Denmark*	Room 1 (B1F)
10:00-10:30	Coffee Break	Room 1 (B1F)
10:30-12:05	**Session 11: Clinical Studies Update** *Chairs:* **John Pernow**, *Stockholm, Sweden /* **Issei Komuro**, *Tokyo, Japan*	Room 1 (B1F)
12:15-13:15	**Lunch Session 5** GlaxoSmithKline K.K.	Room 1 (B1F)
12:15-13:15	**Lunch Session 6** Takeda Pharmaceutical Company Limited.	Room 2 (1F)
13:15-14:45	**Poster Session 3** **Hypertension, Vascular Diseases, Cardiology, Clinical Studies, Metabolism**	Room 122 (1F) Lobby (B1F)
14:45-15:45	**Session 12: Metabolism, Diabetes and Obesity** *Chairs:* **Adviye Ergul**, *Augusta, USA /* **Subrata Chakrabarti**, *London, ON, Canada*	Room 1 (B1F)
15:45-16:15	Coffee Break	Room 1 (B1F)
16:15-16:30	**Tomoh Masaki Award Ceremony** **Best Presentation and Best Poster Award Ceremony**	Room 1 (B1F)
16:30-17:00	**Session 13: Highlights of ET-13** **Masashi Yanagisawa**, *Dallas, USA / Tsukuba, Japan*	Room 1 (B1F)
17:00-17:15	**Closing Remarks**	Room 1 (B1F)

Access

University of Tsukuba, Tokyo Campus

3-29-1 Otsuka, Bunkyo-ku, Tokyo 112-0012, Japan

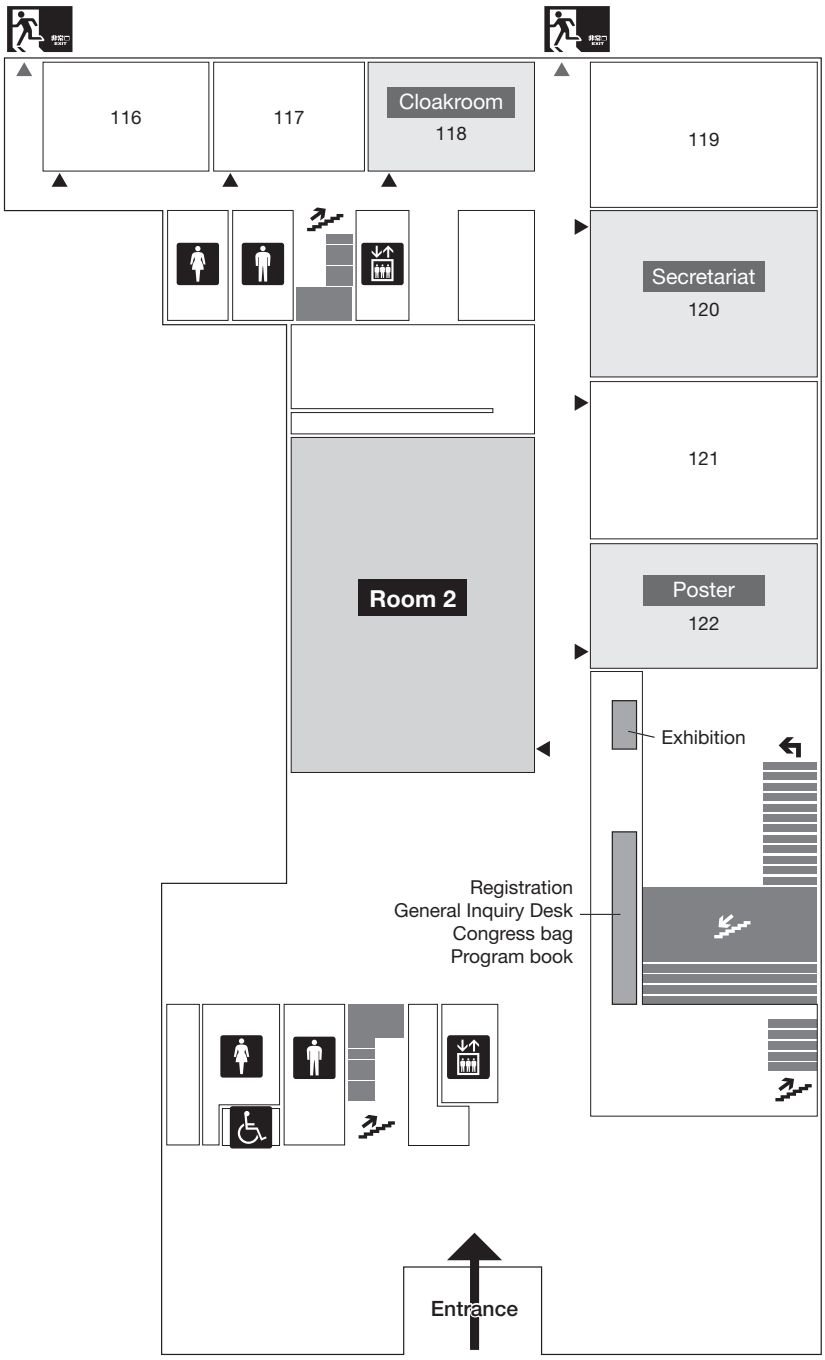




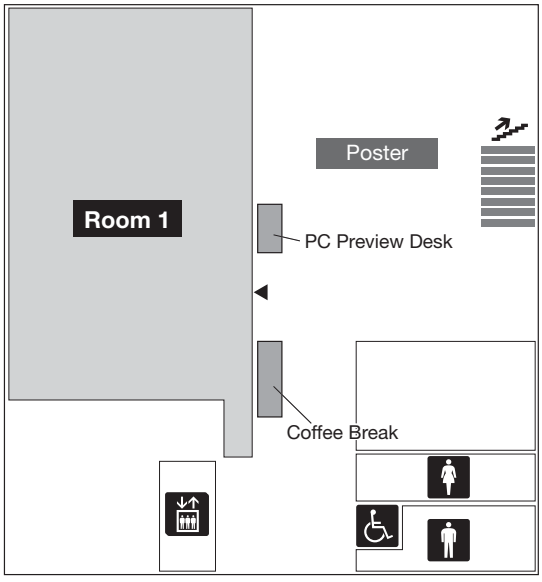
Tokyo Metro Marunouchi Line: Myogadani Station (about two-minute walk)

Floor Plan

1F

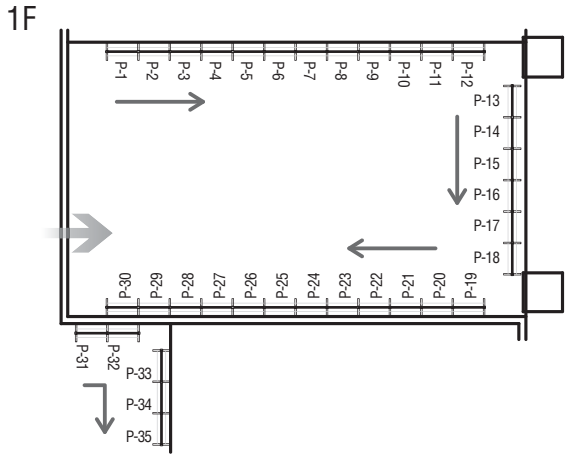


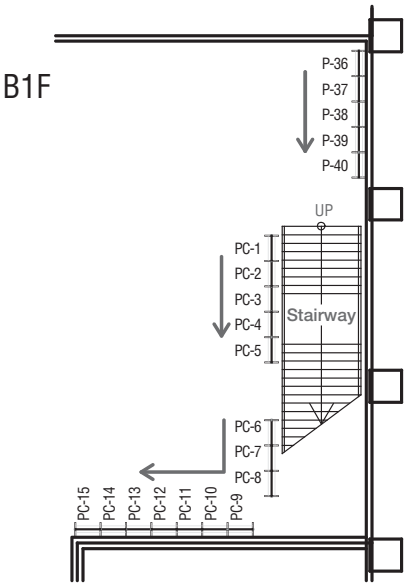
B1F



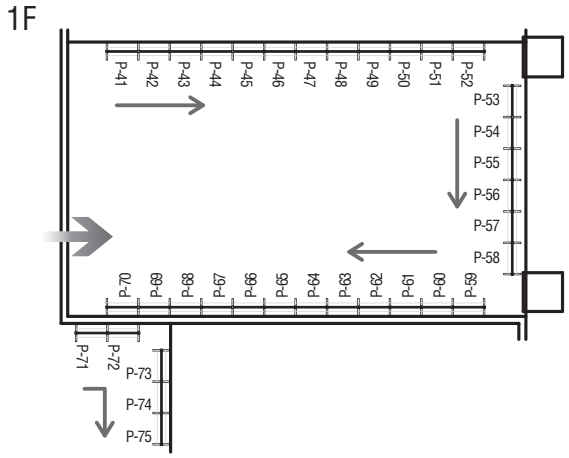
Poster Layout

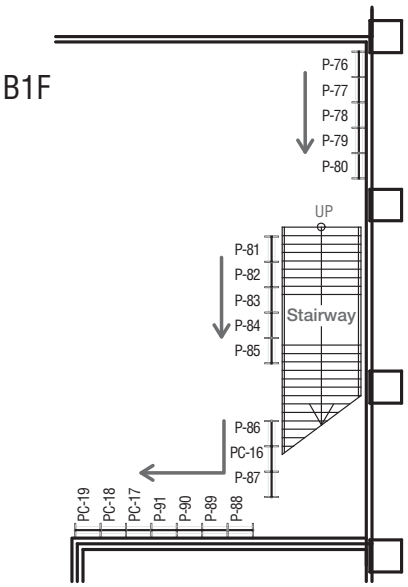
【Monday, September 9】



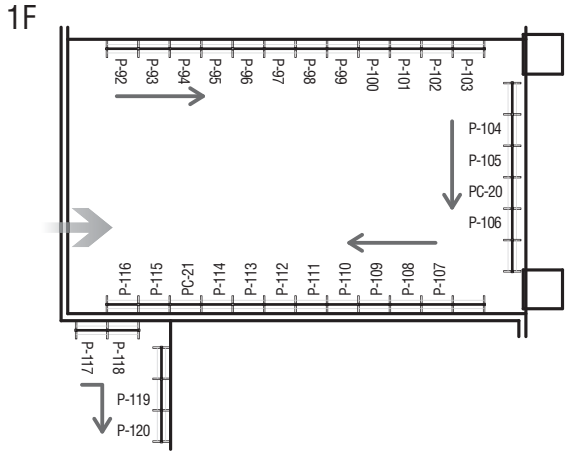


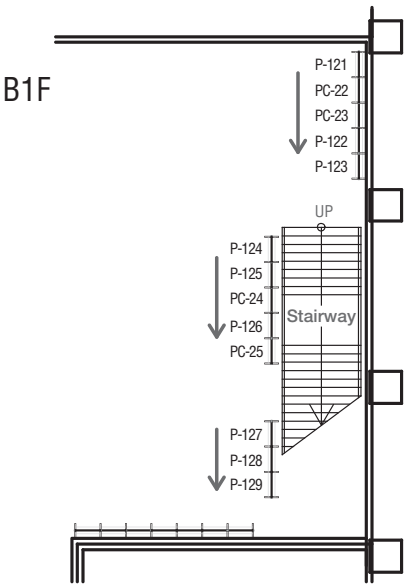
【Tuesday, September 10】





【Wednesday, September 11】





Information for Chairs and Speakers

Information for Scientific Presentations

Guidelines for Chairs

- Chairs should take a seat in the front row of the room specially reserved for the next session's chair, at least 15 minutes prior to the session that he/she is scheduled to chair.
- As there will be no announcement or cue, please proceed to take the stage promptly at the appointed time and initiate the session. We request for your cooperation to ensure that your session proceeds according to the prescribed time limit/schedule.

Guidelines for Speakers

Presentation

Speakers should take a seat in the front row of the room specially reserved for the next speakers, at least 15 minutes prior to his/her presentation time.

Allocated Presentation Time

- Invited Lecture / Keynote Lecture
  15 minutes in total (10 min. presentation + 5 min. discussion) or
  20 minutes in total (15 min. presentation + 5 min. discussion) or
  25 minutes in total (20 min. presentation + 5 min. discussion) or
  30 minutes in total (25 min. presentation + 5 min. discussion)
- Oral Presentation
  12 minutes in total (8 min. presentation + 4 min. discussion)

PC Preview Desk

Speakers are required to upload their presentation at the PC Preview Desk at least 1 hour before the start of the presentation. AV Assistants will be available to help you.

Location: Lobby in front of Room 1, Basement Floor 1

Open Hours for Room 1	September 9	7:30 - 17:00
	September 10	7:30 - 17:00
	September 11	7:30 - 17:00

- Only presentations using a Windows or Mac PC are acceptable. OHP or slides are not acceptable.
- Please bring your presentation data (on your PC, USB flash memory or CD-Rom) to the PC Preview Desk. The data will be temporarily stored for the meeting purposes, and when the meeting is over the organizer will take responsibility for erasing all data.
- At the PC Preview Desk please provide staff with your session name.
- Even if you intend to use your own PC, please come to the PC Preview Desk to check that your presentation functions correctly.
- Please make sure to check the files with anti-virus software before your submission to the Desk.

Presentation Format

Please ensure that your presentation will function on the specifications given below.

OS:	Windows (Windows 7) or Macintosh (MacOS10.4 or later)
Software:	Windows: MS PowerPoint 2003/ 2007/ 2010
	Mac: PowerPoint 2008 / 2011
Fonts:	Standard Fonts for Windows and Macintosh computers
	Ex) Times New Roman, Arial, Arial Black, Arial Narrow, Century, Century Gothic, Courier, Courier New, Georgia
Moving Image:	Windows: Windows Media Player
	Mac: Quick Time Player
Resolution:	up to XGA (1024 X 768)

- If your PowerPoint presentation includes moving images, please bring your own PC to make your presentation, and the back-up data, too.
- When you intend to use your own PC, please bring your own adaptor.
- If your PC is not compatible with a Mini D-sub 15 pin PC cable connector, please bring an adaptor to connect your PC to the Mini D-sub 15pin PC cable connector.
- If you use sound data, please let us know at the PC Preview Desk.

Information for Poster Sessions

Guidelines for Chairs

- Please come to the check-in desk located in front of Room 122, 1st Floor or Lobby, Basement Floor 1, at least 10 minutes prior to your session. As there will be no announcement or cue, Chairs should proceed to start the session promptly at the appointed time.
- We request for your cooperation to ensure that your session proceeds according to the prescribed time limit/schedule.

Guidelines for Poster Presenters

Poster Session

- Posters will be on display in the Room 122, 1st Floor or Lobby, Basement Floor 1.
- Poster presenters are required to be present with their poster during the scheduled sessions. Poster Sessions are from 13:15 to 14:45 through, September 9 to 11.
  The moderation of the posters will start at 13:30.
- Poster presenters are expected to stand ready in front of their poster panels at least 15 minutes prior to their sessions. Since presentation time for each presenter is 7 minutes in total (4 min. presentation + 3 min. discussion), please adhere to the schedule to ensure smooth proceedings, according to chairs moderation.

Schedule

Poster Session 1

Poster Mounting	September 9	8:00 - 8:30 or during coffee break
Poster Session	September 9	13:15 - 14:45
Poster Removal	September 9	16:30 - 17:30

Poster Session 2

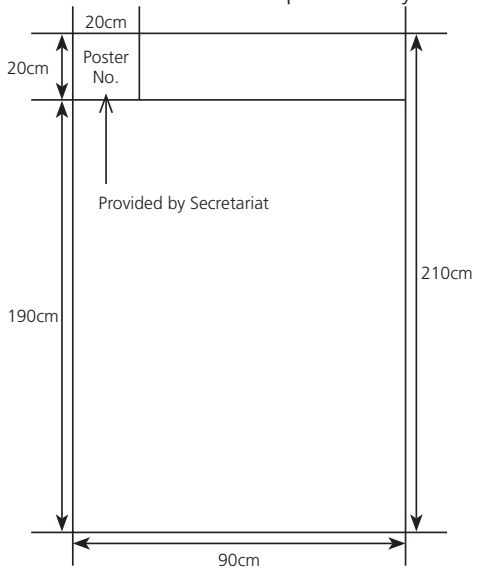
Poster Mounting	September 10	8:00 - 8:30 or during coffee break
Poster Session	September 10	13:15 - 15:45
Poster Removal	September 10	16:00 - 17:00

Poster Session 3

Poster Mounting	September 11	8:00 - 8:30 or during coffee break
Poster Session	September 11	13:15 - 15:45
Poster Removal	September 11	16:00 - 16:45

- All poster boards will be assigned with a number which corresponds to the abstract number. This same number will be cross-referenced in the author index in the Program and Abstract Book.
- Authors should mount their poster on the designated board in the morning of their own session. The schedule of the poster mounting is shown above.
- Please use push-pins to affix your poster presentation to the board firmly. The Secretariat will provide equipment and items required for affixing the posters.
- Any poster left after the scheduled removal time will be disposed of by the Secretariat.

Poster Specifications



Online Publication of the Abstracts

The abstracts of ET-13 will be published online by Elsevier. All e-abstracts will:

- be published online only,
- contain the email address of the presenting author,
- have a unique DOI (digital object identifier),
- be individually indexed in PubMed.

This service is free of charge for the authors and a copyright transfer will not happen.

Video Recording

The oral presentations will be video recorded by a professional team. The recorded material might be published on the website of the Endothelin Conferences (http://www.endothelin-conferences.org/) or used as supplementary materials for the Proceedings of ET-13. If you don't agree with your presentation being recorded, please inform the secretariat (room 120) in time.

ET-13 Group Photograph

Time and date: 13:15, September 10, 2013
Location: In the lobby in front of Room 1 (Basement Floor 1), on the stairs to Room 2 (1st Floor)

About the Conference

Opening Ceremony

Date: September 9 8:30-8:40
Venue: Room 1, Basement Floor 1, University of Tsukuba, Tokyo Campus

Welcome Cocktail

Greet old friends and meet new colleagues from around the world at this reception.
Date: September 8 18:00-20:00
Venue: "Aurora", Basement Floor 1, Tokyo Dome Hotel

Social Event for Young Investigators

Date: September 9 19:00-21:30
Venue: Restaurant "Kyushu Kurodaiko" in Shinjuku
Get there: See "Shutlle Bus Service"
A Contribution of 2,000 Yens will be asked to the participants.

Conference Dinner (Advance application is required)

Date: September 10 19:00-21:00
Venue: "Tenku", Basement Floor 1, Tokyo Dome Hotel
Get there: See "Shutlle Bus Service"

Closing Ceremony

Date: September 11 17:00-17:15
Venue: Room 1, Basement Floor 1, University of Tsukuba, Tokyo Campus

Disclaimer

The ET-13 organizers will not be liable for the safety of any participant, or for personal injury or loss or damage of private property suffered by any registered participant during the congress.

Official Language

The official language of the conference is English.

Secretariat

The Secretariat is located in Room 120 on the 1st Floor, University of Tsukuba, Tokyo Campus

Registration Desk

Location: Lobby, 1st Floor, University of Tsukuba, Tokyo Campus

Open:	September 8	16:00-19:30	Tokyo Dome Hotel*
	September 9	7:30-17:00	
	September 10	7:30-17:00	
	September 11	7:30-16:00	

*Only September 8, we will open the registration in the Tokyo Dome Hotel.

PC Preview Desk

Speakers are required to upload their presentation at the PC Preview Desk at least 1 hour before the start of the presentation. AV assistants will be available to help you.
Location: Lobby in front of Room 1, Basement Floor 1, University of Tsukuba, Tokyo Campus
Open: 7:30-17:00

Internet Connection

Free WiFi is available for participants on the 1st floor and basement floor 1 at the venue except a poster room, Room 122. NO password is required.

Congress Abstracts

All participants will receive the Program & Abstract Book with the Congress Bag.

Lost and Found

Items found will be consigned to the General Inquiry Desk next to the Registration Desk.
For assistance in locating lost property, please contact General Inquiry Desk.

Name Badge

Congress participants are requested to wear their name badges at all times for identification purposes and admission to the scientific and social programs. Should you lose your badge, you may ask for a replacement at the General Inquiry Desk.

Lunch

Lunch boxes will be available to Lunch Session attendees. Please understand that numbers are limited, and lunch boxes will be provided on a first-come, first-serve basis.

Coffee Breaks

Coffee will be available to participants.

Location: Lobby (Basement Floor 1)

Open:	September 9	10:30 - 10:55	15:35 - 16:05
	September 10	10:10 - 10:40	16:05 - 16:30
	September 11	10:00 - 10:30	15:45 - 16:15

Shuttle Bus Service

Complementary shuttle buses will operate according to the following timetable.

Time Table of Shuttle Bus
*Free Shuttle Bus Service is available (15 minutes ride)
Tokyo Dome Hotel ⇔ Tokyo Campus, University of Tsukuba

September 9 (Monday)	
Tokyo Dome Hotel Departure Time	Tokyo Campus, Univ. Tsukuba Departure Time
7:30	18:00
7:40	
7:50	
8:00	
8:10	
8:20	
8:30	

September 10 (Tuesday)	
Tokyo Dome Hotel Departure Time	Tokyo Campus, Univ. Tsukuba Departure Time
7:30	18:00
7:40	18:15
7:50	18:30
8:10	18:35
8:20	
8:30	

September 11 (Wednesday)	
Tokyo Dome Hotel Departure Time	Tokyo Campus, Univ. Tsukuba Departure Time
7:30	18:00
7:40	18:15
7:50	
8:10	
8:20	
8:30	

*Free Shuttle Bus Service will be available for the social event for Young Investigators at 18:20.

General Information

Currency

Currency in Japan is the "yen." You can obtain yen at currency exchange desks at major airports around the world, Haneda Airport, Narita Airport and currency exchange shops in Tokyo. Exchange at the airport is recommended for your convenience. Most foreign currencies and travelers' checks can be exchanged at banks and hotels where you stay. However, we highly recommend purchasing travelers' checks or cash in Yen or U.S. dollars before leaving your home countries. A passport may be required for currency exchange services.

Credit Card

Most hotels, major department stores and restaurants accept VISA, Master, JCB, Amex and Diners. However, small shops like station kiosks, convenience stores, vending machines and train tickets for short distances do not accept credit cards. It is advisable to carry a certain amount of cash.

Traveler's Check

Traveler's checks are acceptable at most hotels and banks.

ATM

Many automatic teller machines (ATMs) in Japan do not accept credit, debit and ATM cards, which are issued outside of Japan. The big exception is the ATMs found at the post offices and 7-Eleven convenience stores. These ATMs allow you to withdraw cash by credit and debit cards issued outside of Japan, including Visa, Plus, Mastercard, Maestro, Cirrus, American Express and JCB cards and provide an English user menu. In addition, international ATMs can be found at international airports, in major department stores and in Citibank and Shinsei Bank branches.

Business Hours

Banks	9:00-15:00, Closed on Saturday and Sunday
Currency Exchange	6:00-22:00 at Airport, 10:00-19:00 at Sakae. Open 365days
Supermarket	Mostly 9:00-20:00, Depending on stores
Convenience Stores	Mostly open 24 hours
Post Offices	9:00-21:00, Closed on Saturday and Sunday
Department Stores	10:00-19:30 Open 7 days
Restaurants	Some open 24 hours, Some open 9:00-23:00
Bars	18:00-Midnight

Electricity

Voltage in Japan is 100V A.C., 2-flat-pin plug Type-A. It is difficult to find 3-pin compatible sockets, 120V, 200V, and 220V. An adapter is advised for the use of electrical equipment.

Water

Tap water is safe to drink anywhere in Japan unless mentioned otherwise. Also you can buy mineral water at convenience stores, supermarkets, and station kiosks, etc.

Smoking

Smoking is prohibited inside most buildings and stations, except in designated smoking areas. In addition, around Tokyo Station, and Myogadani Station, smoking in pedestrian areas is also prohibited.

Tax

Consumption tax rate is 5%, and prices shown on the price tag include tax.

Tipping

Tipping is not customary in Japan, and is not generally done in any situation. However, major hotels or restaurants may add a 10% to 15% service charge to your bill.

Cellular Phone

NTT DoCoMo, Softbank, au KDDI and E-mobile are major cellular phone carriers in Japan. Please contact your own cellular phone carrier to establish whether you can use your cell phone in Japan.

Acknowledgment

As of August 23, 2013

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Sponsored Seminar

Lunch Session 1

Monday, September 9 12:15-13:15 Room 1

Co-sponsored by Pfizer Japan Inc.

Recent Progress in the Management of Pulmonary Arterial Hypertension

Chair: Keiko Takihara *Oasaka University, Suita, Japan*
Speaker: Hiroshi Watanabe *Hamamatsu University School of Medicine, Hamamatsu, Japan*

Lunch Session 2

Monday, September 9 12:15-13:15 Room 2

Co-sponsored by MSD K.K.

Medical Treatment for Type Ⅱ Diabetes Mellitus for Inhibiting Vascular Events

Chair: Kazutaka Aonuma *University of Tsukuba, Tsukuba, Japan*
Speaker: Toyoaki Murohara *Nogoya University, Nagoya, Japan*

Lunch Session 3

Tuesday, September 10 12:15-13:15 Room 1

Co-sponsored by Actelion Pharmaceuticals Japan Ltd.

Twenty-Five Years of Research Leading to a New Generation of Endothelin Receptor Antagonists

Chair: Tsutomu Saji *Toho University, Tokyo, Japan*
Speaker: Martine Clozel *Actelion Pharmaceuticals Ltd., Allschwil, SwitzerLand*

Lunch Session 4

Tuesday, September 10 12:15-13:15 Room 2

Co-sponsored by Nippon Shinyaku Co., Ltd.

The Roles of Endothelin Receptor Antagonists and Phosphodiesterase Type 5 Inhibitors in the Management of Skin Ulcers in Systemic Sclerosis

Chair: Masaru Hatano *University of Tokyo Graduate School of Medicine, Tokyo, Japan*
Speaker: Yoshihide Asano *University of Tokyo Graduate School of Medicine, Tokyo, Japan*

The Challenge to Refractory Pulmonary Hypertension Associated with Scleroderma

Chair: Yoshihide Asano *University of Tokyo Graduate School of Medicine, Tokyo, Japan*
Speaker: Masaru Hatano *University of Tokyo Graduate School of Medicine, Tokyo, Japan*

Lunch Session 5

Wednesday, September 11 12:15-13:15 Room 1

Co-sponsored by GlaxoSmithKline K.K.

Heart Failure in PAH: Focus on the Right Ventricle

Chair: Yasuo Matsumura *Osaka University of Pharmaceutical Sciences, Osaka, Japan*
Speaker: Ronald Oudiz *Harbor-UCLA Medical Center, Torrance, USA*

Lunch Session 6

Wednesday, September 11 12:15-13:15 Room 2

Co-sponsored by Takeda Pharmaceutical Company Limited.

How to Treat the Patients with Hypertension for the Prevention of Heart Failure
-The Back and Forth Strategy between Basic Science and Clinical Medicine-

Chair: Shigetaka Sasayama *Kyoto University and Uji Hospital, Kyoto, Japan*
Speaker: Masafumi Kitakaze *National Cerebral and Cardiovascular Center, Suita, Japan*

Monday, September 9, 2013

O=Oral Presentation, P=Poster, PC=Poster "Cross - border Sessions"

8:30-8:40 **Room 1**

Opening Remarks

Noriaki Emoto *Kobe Pharmaceutical University, Kobe, Japan*
Takashi Miyauchi *University of Tsukuba, Japan*

8:40-9:45 **Room 1**

Session 1: New Aspects of Endothelin in Physiology and Disease

Chairs: **Pedro D'orleans-Juste** *Sherbrooke University, Montreal, Canada*
Soichi Miwa *Hokkaido University, Sapporo, Japan*

Invited Lecture 1 8:40-9:00

Interaction and Sexual Dimorphism of ETB/NOS Signaling in Cardiovascular and Renal Disease

Jennifer S. Pollock, Kelly A. Hyndman
Experimental Medicine, Georgia Regents University, Augusta, USA

Invited Lecture 2 9:00-9:20

Endothelin is Getting Older: How Aging Links Endothelin with Disease

Matthias Barton
University of Zurich, Switerland

O-1 9:20-9:32

Development of Osteoarthritis-Like Changes in Transgenic Endothelin-1 Over-Expressed Mice

Chunyi Wen[1], LimCho Steven Pei[1], Baretella Oliver[2], Sookja Kim Chung[3], Aimin Xu[2], ChunHoi Yan[1], KowngYuen Chiu[1], Weijia William Lu[1]
[1]Department of Orthopaedics & Traumatology, University of Hong Kong, Hong Kong, [2]Department of Medicine, University of Hong Kong, [3]Department of Anatomy, University of Hong Kong

O-2 9:32-9:44

Characterisation of the "Endothelin-Like Domain Peptide" (ELDP) Co-synthesised with Endothelin-1 from the *EDN1* Gene

Jale Yuzugulen[1], Elizabeth G. Wood[1], Inmaculada C. Villar[1], Julie A. Douthwaite[1], Nimesh S. A. Patel[1], James Jegard[1], Alexander Montoya[2], Pedro Cutillas[2], Hubert Gaertner[3], Irene Rossitto-Borlat[3], Keith Rose[3], Oliver Hartley[3], Amrita Ahluwalia[1], Roger Corder[1]
[1]William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, UK, [2]Barts Cancer Institute, Barts and the London School of Medicine, Queen Mary University of London, UK, [3]Faculty of Medicine, University of Geneva, Switzerland

9:45-10:30 **Room 1**

Session 2: Oncology

Chairs: **Anna Bagnato** *Regina Elena National Cancer Institute, Rome, Italy*
Marilena Loizidou *University College London, London, UK*

Keynote Lecture 1 9:45-10:05

The Endothelin Axis: A New Player in Tumor Angiogenesis and Lymphangiogenesis

Francesca Spinella
Regina Elena National Cancer Institute, Rome, Italy

O-3 10:05-10:17

Endothelin-1 Induced Mxi-2/Ago2 Complex Formation Resulting in P53 Downregulation Promoting Breast Cancer Development

Melanie von Brandenstein[1,2], Julia Straube[2], Heike Loeser[1], Luca Ozretic[1], Jochen W.U. Fries[1]
[1]Institute of Pathology, University of Cologne, Cologne, Germany, [2]University of Applied Sciences Bonn-Rhein-Sieg, Department of Natural Sciences, Rheinbach, Germany

O-4 10:17-10:29

Tamoxifen Treatment in Breast Cancer Induces a Cytoplasmic Complex Consisting of Endothelin-1, Estrogen Receptors, and Tamoxifen Leading to Nuclear Transmigration, and Transcription of Target Genes Involved in Metastatic Spread

Julia Straube[1,2], Melanie von Brandenstein[2], Christina Geisbuesch[3], Luca Ozretic[2], Reinhard Depping[4], Jochen W. U. Fries[2]
[1]University of Rhein-Bonn-Sieg, Grantham-Allee 20, 53757 Sankt Augustin, Germany, [2]Institute of Pathology, University of Cologne, Kerpenerstr. 62, 50931Cologne, Germany, [3]Institute of Pediatric and Adolescent Psychiatry, University Hospital, Aachen, Neuenhofer Weg 21, 52074 Aachen, Germany, [4]Institute of Physiology, University of Luebeck, Ratzeburger Allee 160, 23538, Luebeck, Germany

10:30-10:55 **Coffee Break**

10:55-12:05 **Room 1**

Session 3: Renal Diseases

Chairs: **David Pollock** *Georgia Regents University, Augusta, USA*
Neeraj Dhaun *University of Edinburgh, Edinburgh, UK*

Keynote Lecture 2 10:55-11:15

The Role of Endothelin in Glomerular Diseases: Cellular Culprits, Cellular Targets

Pierre-Louis Tharaux
University Paris-Descartes, Paris, France

O-5 11:15-11:27

Heterozygous Overexpression of Preproendothelin-1 in Endothelial Cells Enhances Thromboxane-Prostanoid Receptor-Induced Contractions in the Renal Artery of Obese Mice

Oliver Baretella[1], Sookja K. Chung[2,4], Aimin Xu[1,3,4], Paul M. Vanhoutte[1,4]
[1]Department of Pharmacology & Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [2]Department of Anatomy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [3]Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [4]Research Centre of Heart, Brain, Hormone & Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

O-6 11:27-11:39

The Effect of Proteinuria-Mediated Endothelin-1 Downregulation of PKCα Signalling in Proximal Tubular Cells and Its Successul Treatment is Measurable Using microRNA15a as Biomarker in Vitro and in Vivo

Heike Loeser[1], Melanie von Brandenstein[1], Maike Wittersheim[1], Volker Burst[2], Claudia Richter[1], Bernd Hoppe[3], Jochen W.U. Fries[1]
[1]Institute of Pathology, University Hospital Cologne, Cologne, Germany, [2]Department of Internal Medicine II, Division of Nephrology, University Hospital Cologne, Cologne, Germany, [3]Institute of Pediatrics, Division of Nephrology, University Hospital Cologne, Cologne, Germany

O-7 11:39-11:51

ET-B Receptors in Podocytes Promote Diabetic Glomerulosclerosis with β-Catenin and NFκB Activation

Olivia Lenoir[1,2], Marine Milon[1,2], Anne Virsolvy[3], Yuri Kotelevtsev[4,5], Masashi Yanagisawa[6], David J Webb[4], Sylvain Richard[3], Pierre-Louis Tharaux[1,2,7]
[1]Paris Cardiovascular Research Centre - PARCC, Institut National de la Sante et de la Recherche Medicale (INSERM), Paris, France, [2]Universite Paris Descartes, Sorbonne Paris Cite, Paris, France, [3]INSERM, CHU A. de Villeneuve, Montpellier, France, [4]University of Edinburgh, The Queen's Medical Research Institute, Edinburgh, UK, [5]Pushchino State Institute for Natural Sciences, 142290 Russian Federation, [6]University of Texas Southwestern Medical Center, Dallas, TX, USA, [7]Service de Nephrologie, Hopital Europeen Georges Pompidou, Assistance Publique Hopitaux de Paris, Paris, France

O-8 11:51-12:03

Changes in Urinary ET-1 Excretion in Response to Increased Renal Perfusion Pressure in the Rat

Geoff J. Culshaw, Matthew A. Bailey, Patrick W.F. Hadoke, David J. Webb
The University of Edinburgh/British Heart Foundation Centre for Cardiovascular Science, The Queens Medical Research Institute, Edinburgh, UK

12:15-13:15 Room 1

Lunch Session 1

Chair: **Keiko Takihara** *Osaka University, Suita, Japan*

LS1

Recent Progress in the Management of Pulmonary Arterial Hypertension

Hiroshi Watanabe
Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan

Co-sponsor: Pfizer Japan Inc.

12:15-13:15 Room 2

Lunch Session 2

Chair: **Kazutaka Aonuma** *University of Tsukuba, Tsukuba, Japan*

LS2

Medical Treatment for Type Ⅱ Diabetes Mellitus for Inhibiting Vascular Events

Toyoaki Murohara
Nagoya University, Nagoya, Japan

Co-sponsor: MSD K.K.

13:15-14:45 Poster Area

Poster Session 1: Oncology, Renal Physiology and Disease, Neurology, Infectious Diseases, New Topics of Endothelin Biology

P-1 - P-6 *Moderator:* **Francesca Spinella** 122 (1F)
Regina Elena National Cancer Institute, Rome, Italy

P-1

The Role of Endothelin-1 in the Vascular Pathobiology of Cerebral Malaria

Brandi D. Freeman, Minxian Dai, Mahalia S. Desruisseaux
Albert Einstein College of Medicine, USA

P-2

Dual Endothelin Blockade Exacerbates Upregulated VEGF Angiogenic Signaling in the Heart of Lipopolysaccharide -Induced Endotoxemic Rat Model

Masami Oki[1], Subrina Jesmin[1], Nobutake Shimojo[1], Majedul Islam[1], Tanzila Khatun[1], Satoru Kawano[1], Takashi Miyauchi[2], Taro Mizutani[1]
[1]Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]Department of Cardiovascular Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

P-3

Endothelin Plasma and Tissue Expression in Ductal Carcinoma of the Breast: Correlation with Clinicopathological Characteristics and VEGF

Xeni Provatopoulou[1], Vasileios Kalles[2], Eleni Kalogera[1], Afrodite Nonni[3], Maria Matiatou[2], Ioannis Papapanagiotou[2], George C. Zografos[2], Antonia Gounaris[1]
[1]Research Center, Hellenic Anticancer Institute, Athens, Greece, [2]Breast Unit, 1st Department of Propaedeutic Surgery, Hippokratio Hospital, University of Athens, Athens, Greece, [3]Department of Pathology, University of Athens, Athens, Greece

P-4

The Localisation and Distribution of Endothelin Receptors in Normal and Cancer Colon Tissues: Confirmation by Autoradiography, Immunohistochemistry and Quantum Dot Targeting

Samer-ul Haque[1], Bala Ramesh[1], Hazel Welch[1], David Abraham[3], Olagunju Ogubbiyi[1], Marilena Loizidou[1], Micheal Dashwood[2]
[1]Department of Surgery and Interventional Sciences, University College London, UK, [2]Department of Clinical Biochemistry, University College London, UK, [3]Centre for Rheumatology and Connective Tissue Disorders, University College London, UK

P-5

Novel Molecular Pathways by Which ETA Receptor Mediates Tumourigenic Signals in Colorectal Cancer: Support for ETA Receptor Antagonism as Adjuvant Treatment

Samer-ul Haque[1], Marilena Loizidou[1], Micheal Dashwood[2], Xu Shi-wen[3], David Abraham[3], Hazel Welch[1]
[1]Department of Surgery and Interventional Sciences, University College London, UK, [2]Department of Clinical Biochemistry, University College London, UK, [3]Centre for Rheumatology and Connective Tissue Disorders, University College London, UK

P-6

Serum Big Endothelin-1 as a Clinical Marker in Canine Pulmonary Hypertension and Tumors

Shinya Fukumoto[1], Kiwamu Hanazono[1], Taku Miyasho[4], Tsuyoshi Kadosawa[2], Hidetomo Iwano[3], Tsuyoshi Uchide[1]
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P-7 - P-12 *Moderator:* **Marilena Loizidou** 122 (1F)
University College London, London, UK

P-7

An Unexpected Pulmonary Hypertensive Crisis: Eying the Culprit

Kaori Sato[1], Tsutomu Saji[2], Taku Kaneko[3], Kei Takahashi[4], Kaoru Sugi[1]
[1]Division of Cardiovascular Medicine, Toho University Ohashi Medical Center, Tokyo, Japan, [2]Division of Pediatrics, Toho University Omori Medical Center, Tokyo, Japan, [3]Division of Pathology, Toho University Ohashi Medical Center, Tokyo, Japan, [4]Division of Ophthalmology, Toho University Ohashi Medical Center, Tokyo, Japan

P-8

Pharmacokinetics of SPI-1620 in A Phase I, Open Label, Ascending Dose Study of the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of the Endothelin B Receptor Agonist, SPI-1620, in Recurrent or Progressive Carcinoma

Guru Reddy[1], Anthony Tolcher[2], Anil Gulati[3], Shanta Chawla[1], Lee F. Allen[1]
[1]Spectrum Pharmaceuticals, Irvine, CA, USA, [2]South Texas Accelerated Research Therapeutics, San Antonio, Texas, USA, [3]Midwestern University, Downers Grove, Illinois, USA

P-9

Endothelin-1-Induced β-Arrestin Signalosome is Linked to Chemoresistance, EMT and Stem-Cell Like Properties in Ovarian Cancer Cells

Laura Rosano', Roberta Cianfrocca, Piera Tocci, Elisa Semprucci, Francesca Spinella, Valeriana Di Castro, Anna Bagnato
Experimental Research Center, Regina Elena National Cancer Institute, Italy

P-10

(Pro)renin Receptor in the Breast Cancer and Its Possible Pathophysiological Role in Cancer Cell Proliferation

Kazuhiro Takahashi[1], Koji Ohba[1], Hiroshi Nishiyama[1], Kiriko Kaneko[1], Takuo Hirose[2], Kazuhito Totsune[3], Hironobu Sasano[4], Takashi Suzuki[5]
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P-11

Poly-Gamma-Glutamic Acid Attenuates Angiogenesis and Inflammation in Experimental Colitis

Mi Jeong Sung, Munkhgugs Davaatseren, Jin-Taek Hwnag, Jae Ho Park, Myung Sunny Kim, Shyaiyu Wang
Korea Food Research Institute, Gyeonggi-Do, South Korea

P-12

Identification of Bladder Endothelin-1 Receptors and Binding Characteristics of Bosentan and Ambrisentan

Ayaka Osano
Pharmacokinetics and Pharmacodynamics and Clinical Pharmacol and Genetics, Japan

P-13 - P-18 *Moderator:* **CheMyong Ko** 122 (1F)
University of Illinois at Urbana-Champaign, Urbana, IL, USA

P-13

Endothelin-1 (ET-1) and Its Receptors on Haemorrhoidal Tissue: A Potential Site for Therapeutic Intervention

Michael R. Dashwood[1], Varut Lohsiriwat[2,3], Vincent G. Wilson[3], John H. Scholefield[3]
[1]Clinical Biochemistry, Royal Free Hospital and University College Medical School, London, NW3 2PF, UK, [2]Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [3]The University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK

P-14

Endothelin-1 Modulates Bile Secretory Function in Rats

Nadiia Radchenko, Petro Yanchuk
National Taras Shevchenko University, Kyiv, Ukraine

P-15

Endothelin System in Intestinal Villi: A Possible Role of Endothelin-2 in the Maintenance of Intestinal Architecture

Mariana Bianchi[1], Javier Adur[1], Satoshi Takizawa[2], Kaname Saida[2], Víctor H. Casco[1]
[1]Microscopy Laboratory Applied to Cellular and Molecular Studies, Bioengineering and Bioinformatic School, National University of Entre Ríos, Argentine, [2]National Institute of Advanced Industrial Science and Technology (AIST), Japan

P-16

Vasoprotective Effect of Endothelin Receptor Antagonist in Ovariectomized Female Rats

Kento Kitada[1,2], Mamoru Ohkita[2], Yasuo Matsumura[2]
[1]Department of Pharmacology, Kagawa University, Kagawa, Japan, [2]Laboratory of Pathological and Molecular Pharmacology, Osaka University of Pharmaceutical Sciences, Osaka, Japan

P-17

Increased Cerebrovascular Sensitivity to Endothelin-1 in Obstructive Sleep Apnea Rats is Endothelin-B Receptor Mediated

David J. Durgan, Randy F. Crossland, Eric E. Lloyd, Sharon C. Phillips, Robert Bryan Jr.
Department of Anesthesiology, Baylor College of Medicine, Houston, Texas, USA

P-18

The Akt Pathway Mediates the Neuroprotective Effect of IRL-1620 in A Rat Model of Focal Cerebral Ischemia

Anil Gulati, Anupama K. Puppala, Seema Briyal
Midwestern University Chicago College of Pharmacy, USA

P-19 - P-25 *Moderator:* **Jennifer Pollock** 122 (1F)
Georgia Regents University, Augusta, USA

P-19

Concomitant Downregulation of ET-1-ETB System and VEGF Angiogenic Signaling in the Frontal Cortex of a Murine Model of Endotoxemia: A Double Threat to Cerebral Microcirculation in Sepsis

Aiko Sonobe, Subrina Jesmin, Nobutake Shimojo, Majedul Islam, Tanzila Khatun, Masami Oki, Satoru Kawano, Taro Mizutani
Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

P-20

Changes in Ovarian Constriction by Endothelin-2/ Receptor System in the Feline Ovary

Joseph Cacioppo[1], Patrick Lin[1], Sangwook Oh[1,2], Yongbum Koo[1,3], CheMyong Ko[1]
[1]The Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Biology Education, Chunbuk National University, Jeonju, South Korea, [3]Department of Biotechnology and Biomedical Science, Inje University, Gimhae, South Korea

P-21

ET-1 Overexpression and Endothelial Nitric Oxide Synthase Knock-Out Induce Different Pathological Responses in the Heart of Male and Female Mice

Nicolas Vignon-Zellweger[1], Katharina Relle[1], Jan Rahnenfuhrer[1], Karima Schwab[1], Berthold Hocher[1,2], Franz Theuring[1]
[1]Center for Cardiovascular Research - Institute for Pharmacology, Charite Medical School of Berlin, Germany, [2]Institute of Nutritional Science University of Potsdam, Potsdam, Germany

P-22

Research on the Relationship between Endothelin-1 Gene Polymorphisms and Primary Nephrotic Syndrome in Children

Fang Yang[1], Xinlong Lai[1], Li Deng[1], Xlaoxiao Liu[1], Shuixiu Zeng[1], Cheng Zhang[2]
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P-23

Evaluation of Urinary and Plasma Endothelin-Like Domain Peptide (ELDP) in Chronic Kidney Disease

Jale Yuzugulen[1], Pajaree Lilitkarntakul[2], Elizabeth G. Wood[1], Robert A. Kimmitt[2], Neeraj Dhaun[2], Jane G. Goddard[2], David J. Webb[2], Roger Corder[1]
[1]William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, UK, [2]BHF Centre of Research Excellence, University of Edinburgh, The Queens Medical Research Institute, Edinburgh, UK

P-24

Potential Amelioration of Upregulated Renal HIF1Alpha-Endothelin 1 System Through Landiolol Hydrochloride in A Rat Model of Endotoxemia: A Possible Linkage to the Increased Renal Vascular Resistance Based on Renal Microcirculation Alteration in Sepsis

Yoshiyasu Ogura, Nobutake Shimojo, Subrina Jesmin, Yoshimoto Seki, Hideaki Sakuramoto, Keiichi Hagiya, Satoru Kawano, Taro Mizutani
Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

P-25

p66 Shc Mediates Effect of ET-1 on TRPC Channels Activity and Changes in Intracellular Ca^{2+} in Renal Vascular Smooth Muscle Cells

Andrey Sorokin[1], Oleg Palygin[2], Bradley Miller[1], Alexander Staruschenko[2]
[1]Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA, [2]Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

P-26 - P-33 *Moderator:* **Neeraj Dhaun** 122 (1F)
University of Edinburgh, Edinburgh, UK

P-26

Contractions to Endogenous and Exogenous Endothelin-1 in Segmental Renal Arteries of the Mouse: Up-Regulation in Obesity

Oliver Baretella[1], Aimin Xu[1,2,3], Paul M. Vanhoutte[1,3]
[1]Department of Pharmacology & Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [2]Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [3]Research Centre of Heart, Brain, Hormone & Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

P-27

ET-1-Induced Contraction of Renal Afferent Arterioles of Dahl Salt-Sensitive Rats is Impaired by Targeted Modification of a p66 Shc Regulatory Phosphorylation Site

Andrey Sorokin[1], Bradley Miller[1], Aron M. Geurts[2], John D. Imig[3]
[1]Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA, [2]Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA, [3]Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

P-28

Blocking Endothelin-1 Induced Multiple Drug Resistance Permits Effective Inhibition of Activation of Renal Proximal Tubules Exemplified by the PKC Alpha-microRNA15a Loop

Heike Loeser, Angela Herschung, Melanie von Brandenstein, Jochen W.U. Fries
Institute of Pathology, University Hospital of Cologne, Cologne, Germany

P-29

Evidence for Extrarenal Vascular Endothelin-1 in the Maintenance of Sodium Homeostasis

Joshua S. Speed[1], Kelly A. Hyndman[1], Jennifer S. Pollock[1], Jens M. Titze[2], David M. Pollock[1]
[1]Georgia Regents University, USA, [2]Vanderbilt University, USA

P-30

Endothelin Converting Enzyme Inhibition Attenuates Early Albuminuria and Late Renal Failure in Streptozotocin Induced Diabetic Mice

Kazuhiko Nakayama[1], Nicolas Vignon-Zellweger[1], Susi Heiden[1], Yoko Suzuki[1], Takuya Okano[1], Kazuya Miyagawa[2], Dyah Samti Mayasari[1], Keiko Yagi[1], Masashi Yanagisawa[3], Noriaki Emoto[1,2]
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P-31

High Salt Diet Attenuates ET-1 Mediated Calcium Signaling Responses in Preglomerular Smooth Muscle Cells from WT and ETB Receptor-Deficient Rats

Edward W. Inscho[1], David M. Pollock[2], Jennifer C. Sullivan[2], Shali Zhang[1]
[1]Department of Physiology, Georgia Regents University, Augusta, Georgia, USA, [2]Experimental Medicine, Department of Medicine, Georgia Regents University, Augusta, Georgia, USA

P-32

Collecting Duct NOS1 Knockout Mice Lack ET-1 Mediated Inhibition of Collecting Duct ENaC

Kelly A. Hyndman[1], Vladislav Bugaj[2], Elena Mironova[2], James D Stockand[2], David M Pollock[1], Jennifer S. Pollock[1]
[1]Experimental Medicine, Department of Medicine, Georgia Regents University, USA, [2]Department of Physiology, University of Texas Health Sciences Center, USA

P-33

Increased of Heparanase Expression in Hipoxic Endothelial Cells and Kidney Ischemic-Reperfusion Injury Associates with Endothelin-1 Elevation and eNOS Reduction

Nur Arfian[1], Keiko Yagi[2], Kazuhiko Nakayama[2], Dwi C. Ratna Sari[1], Muhammad M. Romi[1], Untung Tranggono[4], Hary S. Muliawan[3], Noriaki Emoto[2,3]
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P-34 - P-40 *Moderator:* **Pierre-Louis Tharaux**
University Paris-Descartes, Paris, France

P-34 122 (1F)

Endothelial Cells-Derived Endothelin-1 Exaggerates Kidney Fibrosis Through ETAR Activation in Renal Interstitial Cells

Nur Arfian[1], Keiko Yagi[2], Kazuhiko Nakayama[2], Nicolas Vignon-Zellweger[2], Susi Heiden[2], Tran V. Hung[3], Hary S. Muliawan[3], Gahan Satwiko[3], Noriaki Emoto[2,3]
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P-35 122 (1F)

Hypoxia Stimulates Glomerular Reactive Oxygen Species Through an Endothelin-1/ET-A Dependent Mechanism.

J. Brett Heimlich[1], Paul M. O'Connor[1], Dao H. Ho[1], Steffen E. Meiler[2], David M. Pollock[1]
[1]Section of Experimental Medicine, Department of Medicine, Georgia Regents University, Augusta, Georgia, USA, [2]Department of Anesthesiology and Perioperative Medicine, Georgia Regents University, Augusta, Georgia, USA

P-36 Lobby (B1)

Combined Endothelin A Receptor and Renin-Angiotensin System Blockade is Superior to Isolated Renin-Angiotensin System Blockade Against the Progression Renal Damage in 5/6 Nephrectomized Ren-2 Transgenic Hypertensive Rats

Zdenka Vernerova[1,3], Ivana Vaneckova[2], Petra Skaroupkova[1], Zuzana Huskova[1], Ludek Cervenka[1]
[1]Institute for Clinical and Experimental Medicine, Department of Experimental Hypertension, Prague, Czech Republic, [2]Institute of Physiology, Department of Experimental Hypertension, Prague, Czech Republic, [3]Department of Pathology, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic

P-37 Lobby (B1)

Endothelial Cell-Derived ET-1 Contributes to the Severity of Septic Kidney Injury

Daisuke Nakano[1], Noriaki Emoto[2], Kazuhiko Nakayama[2], Masashi Yanagisawa[3], Akira Nishiyama[1]
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P-38 Lobby (B1)

Renal Phenotype of Type 1 Diabetic Endothelial Cell Derived ET-1 Deficient Mice

Susi Heiden[1], Nicolas Vignon-Zellweger[1], Kazuhiko Nakayama[1], Keiko Yagi[1], Masahi Yanagisawa[2], Noriaki Emoto[1,3]
[1]Clinical Pharmacy, Kobe pharmaceutical University, Kobe, Japan, [2]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, USA, [3]Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

P-39 Lobby (B1)

Absence of ETA Receptors on Podocytes is Not Antialbuminuric in Diabetic Mice

Nicolas Vignon-Zellweger[1], Susi Heiden[1], Kazuhiko Nakayama[1], Keiko Yagi[1], Marc Iglarz[2], Masashi Yanagisawa[3], Noriaki Emoto[1,4]
[1]Department of Clinical Pharmacy, Kobe Pharmaceutical University, Kobe, Japan, [2]Actelion Pharmaceuticals Limited, Allschwil, Switzerland, [3]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, USA, [4]Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

P-40 Lobby (B1)

ET-1 Plasma Levels, Choroidal Thickness and Multifocal Electroretinogram in Retinitis Pigmentosa

Alessandro Finzi, Mauro Cellini, Ernesto Strobbe, Emilio Campos
Department of Specialized,. Diagnostic and Experimental Medicine, Ophthalmology Unit, University of Bologna, Bologna, Italy

PC-1 - PC-7 *Moderator:* **Keiko Takihara** Lobby (B1)
Osaka University, Suita, Japan

PC-1

Clinical Value of Plasma Pentraxin 3 Levels for Predicting Cardiac Troponin Elevation After Percutaneous Coronary Intervention

Zheng Wang[1], Akira Sato[2], Taizo Kimura[2], Kazuko Tajiri[2], Tomoya Hoshi[2], Satoshi Sakai[2], Akira Koike[2], Takashi Miyauchi[2], Kazutaka Aonuma[2]
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PC-2

Quantitative Determination of Diastolic Suction Using with Vector Flow Mapping

Yoshie Nogami, Tomoko Ishizu, Akiko Atsumi, Masayoshi Yamamoto, Ryo Kawamura, Yoshihiro Seo, Kazutaka Aonuma
Faculty of Medicine, Division of Clinical Medicine, Department of Cardiology, University of Tsukuba, Japan

PC-3

The Anti-Hypertensive Effect of Radiofrequency Renal Denervation with a Reduction of Renal Tissue Norepinephrine Content in the Spontaneously Hypertensive Rats

Takeshi Machino, Nobuyuki Murakoshi, Akira Sato, Dongzhu Xu, Tomoya Hoshi, Taizou Kimura, Kazutaka Aonuma
Cardiovascular Division, University of Tsukuba, Japan

PC-4

The Case of Pulmonary Veno-Occlusive Disease Succeeded in Administration of Sildenafil by Dose Adjustment

Akinori Sugano, Satoshi Sakai, Hidekazu Maruyama, Taizo Kimura, Tomoko Ishizu, Yoshihiro Seo, Satoshi Homma, Takashi Miyauchi, Kazutaka Aonuma
Cardiovascular Division, Faculty of Medicine, University of Tsukuba, Japan

PC-5

Prognostic Significance of Remaining Severe Left Ventricular Diastolic Dysfunction after Cardiac Resynchronizaion Therapy

Masayoshi Yamamoto[1], Yoshihiro Seo[1], Naoto Kawamatsu[1], Kimi Sato[1], Noriaki Sugano[1], Akiko Atsumi[1], Yoshie Harimura[1], Tomoko Machino-Ohtsuka[1], Fumiko Sakamaki[2], Tomoko Ishizu[1], Takashi Miyauchi[1], Kazutaka Aonuma[1]
[1]Cardiovascular Division, Institute of Clinical Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan, [2]Department of Clinical Laboratory, Tsukuba University Hospital, Tsukuba, Japan

PC-6

Elevated Blood Pressure in Resting Daytime-Phase in A170/p62-Knockout Mice, a Newly Established Obese Model

Satoshi Sakai, Eiji Warabi, Toru Yanagawa, Taizo Kimura, Kazuko Tajiri, Satoshi Homma, Keisuke Kuga, Kazutaka Aonuma, Tetsuro Ishii, Takashi Miyauchi
Faculty of Medicine, University of Tsukuba, Japan

PC-7

Relationship between Plasma Klotho Concentration and Physical Activity Level in Middle-Aged and Elderly Women

Toru Yoshikawa[1], Asako Zempo-Miyaki[1], Tomoko Matsubara[1], Nobuhiko Akazawa[1], Youngju Choi[2], Koichiro Tanahashi[1], Takashi Miyauchi[3], Seiji Maeda[2]
[1]Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan, [2]Faculty of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan, [3]Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

PC-8 - PC-15 *Moderator:* **Satoshi Homma** Lobby (B1)
University of Tsukuba, Tsukuba, Japan

PC-8

Relationship between Plasma Asymmetric Dimethylarginine Concentrations and Aerobic Exercise Capacity in Postmenopausal Women

Koichiro Tanahashi[1], Nobuhiko Akazawa[1], Asako Miyaki[1], Youngju Choi[2], Song-Gyu Ra[1], Tomoko Matsubara[1], Hiroshi Kumagai[1], Satoshi Oikawa[1], Takashi Miyauchi[3], Seiji Maeda[2]
[1]Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan, [2]Faculty of Health and Sport Sciences, University of Tsukuba, Japan, [3]Faculty of Medicine, University of Tsukuba, Japan

PC-9

Relationship between Digit Ratio and Idiopathic Pulmonary Hypertension in Japanese Women

Tsunehisa Yamamoto, Yuichi Tamura, Motoaki Sano, Keiichi Fukuda
Department of Cardiology, School of Medicine, Keio University, Japan

PC-10

The Role of NPBWR1 on Autonomic Nervous System

Yoko Irukayama-Tomobe[1, 2], Nobuyuki Murakoshi[3], Takeshi Sakurai[4]
[1]Department of Pharmacology, Basic Medical Sciences, University of Tsukuba, Tsukuba, Japan, [2]University of Tsukuba Center for Behavioral Molecular Genetics (FIRST Program) Tsukuba, Japan, [3]Cardiovascular Division, Institute of Clinical Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan, [4]Department of Molecular Neuroscience and Integrative Physiology Kanazawa University, Kanazawa, Japan

PC-11

The Hypoxia-Mimetic Agent Cobalt Chloride Induces the Expression of Intrinsic BMP Antagonist Noggin Independently of Endothelin Pathway

Hidekazu Maruyama[1], Celine Dewachter[1], Satoshi Sakai[2], Asmae Belhaj[1], Benoit Rondelet[1], Myriam Remmelink[1], Jean-Luc Vachiery[1], Robert Naeije[1], Laurence Dewachter[1]
[1]Laboratory of Physiopathology, Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium, [2]Division of Cardiovascular Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

PC-12

Combination of Polymorphisms in Angiotensin-Converting Enzyme and Estrogen Receptor-Alpha Genes Increases the Risk for Elevation of Arterial Stiffness

Seiji Maeda[1], Maiko Misono[1], Motoyuki Iemitsu[2], Takeshi Otsuki[3], Jun Sugawara[4], Youngju Choi[1], Asako Miyaki[1], Takashi Miyauchi[1], Shinya Kuno[1], Ryuichi Ajisaka[1]
[1]University of Tsukuba, Japan, [2]Ritsumeikan University, Japan, [3]Ryutsu Keizai University, Japan, [4]National Institute of Advanced Industrial Science and Technology, Japan

PC-13

The Impact of RV/LV Volume Ratio on Biventricular Function

Akihiro Nakamura[1], Hitoshi Horigome[1], Yoshihiro Seo[2], Tomoko Ishizu[2], Kazutaka Aonuma[2], Ryo Sumazaki[1]
[1]Department of Child Health, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan, [2]Cardiovascular Division, Institute of Clinical Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan

PC-14

Immediate Improvement of Pulmonary Hypertension with Out-of-Proportion Physiology After Percutaneous Coronary Intervention for Ischemic Heart Disease

Daiki Akiyama, Tomoko Ishizu, Tomoya Hoshi, Yoshihiro Seo, Satoshi Sakai, Akira Sato, Satoshi Homma, Takashi Miyauchi, Kazutaka Aonuma
Cardiovascular Division, Faculty of Medicine, University of Tsukuba, Japan

PC-15

Diabetes and Obesity Are Significant Risk of Morning Hypertension. From Large Scale Home BP Study: Ibaraki Hypertension Assessment Trial (I-HAT)

Masahiro Toyama[1], Shigeyuki Watanabe[1], Takashi Miyauchi[2], Eiji Ojima[1], Yasuhisa Kuroda[1], Kazutaka Aonuma[2], I-HAT study Investigators
[1]Mito Kyodo Hospital, Tsukuba University Hospital Mito Medical Center, Japan, [2]Graduate School of Comprehensive Human Science, University of Tsukuba, Japan

14:45-15:35 Room 1

Session 4: Resistant Hypertension

Chairs: **Kazutaka Aonuma** *University of Tsukuba, Tsukuba, Japan*
Anthony P. Davenport *University of Cambridge, Cambridge, UK*

Invited Lecture 3 14:45-15:10

Treatment-Resistant Hypertension: The Challenges for Drug Treatment

David Webb
British Heart Foundation Centre of Research Excellence (BHF CoRE), University of Edinburgh, UK

Invited Lecture 4 15:10-15:35

Impact of Neuromodulation on Pressure Dysregulation -From Hypertension to Hypotension-

Kenji Sunagawa
Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

15:35-16:05 Coffee Break

16:05-17:05 Room 1

Session 5: Neurology, Pain and Stroke

Chairs: **Anil Gulati** *Midwestern University, Chicago, USA*
Yasuo Matsumura *Osaka University of Pharmaceutical Sciences, Osaka, Japan*

Invited Lecture 5 16:05-16:25

Targeting Endothelin Axis to Treat Pain

Anil Gulati
Midwestern University, Chicago, USA

O-9 16:25-16:37

Endothelin B Receptor Agonist, IRL-1620, Enhances Neurovascular Remodeling Following Cerebral Ischemia in Rats

Mary G. Leonard[1,2], Anil Gulati[1,2]
[1]Chicago College of Pharmacy, Midwestern University, Downers Grove, IL, USA, [2]University of Illinois at Chicago, Chicago, IL, USA

O-10 16:37-16:49

Endothelin Receptor A as a Modulator of Photoreceptor Signaling

Vicente Bermudez, Melisa D Marquioni Ramella, Angela M Suburo
Facultad de Ciencias Biomedicas, Universidad Austral, Pilar, Argentina

O-11 16:49-17:01

Endothelin-1 Treatment Induces Experimental Cerebral Malaria During *Plasmodium Berghei* NK65 Infection

Yuri C. Martins, Herbert B. Tanowitz, Louis M. Weiss, Mahalia S. Desruisseaux
Department of Pathology, Albert Einstein College of Medicine, New York, USA

17:05-17:45 Room 1

Session 6: Actual Applications and Future Perspectives of Dual ETA/ETB Antagonists

Chairs: **Janet Maguire** *University of Cambridge, Cambridge, UK*
Ariela Benigni *(IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy*

Invited Lecture 6 17:05-17:25

Benefits of Dual Endothelin Receptor Antagonists: Mechanisms of Action, Current Studies, and Future Directions

Martine Clozel
Actelion Pharmaceuticals Ltd., Drug Discovery Department, Switzerland

Invited Lecture 7 17:25-17:45

Effects of Bosentan on Digital Ulcers in Patients with Systemic Sclerosis

Yasushi Kawaguchi
Institute of Rheumatology, Tokyo Women's Medical University, Japan

Tuesday, September 10, 2013

O=Oral Presentation, P=Poster, PC=Poster "Cross - border Sessions"

8:30-10:10 **Room 1**

Session 7: Gene Regulation, Molecular and Cellular Biology

Chairs: **Keiichi Fukuda** *Keio University, Tokyo, Japan*
CheMyong Ko *University of Illinois at Urabana-Champaign, Urabana, IL, USA*

Invited Lecture 8 8:30-8:50

Tissue-Specific and Time-Dependent Regulation of the Endothelin Axis by the Circadian Clock Protein Per1

Michelle L. Gumz, Sean All, George Skopis, Brandy Compton, Kit-Yan Cheng, Jacob Richards
University of Florida, USA

Invited Lecture 9 8:50-9:10

Endothelin Signaling in Craniofacial and Cardiac Development

Hiroki Kurihara
The University of Tokyo, Graduate School of Medicine, Tokyo, Japan

O-12 9:10-9:22

Erythropoietin Induced Blood Pressure Rise, Vascular Inflammation and Oxidative Stress in Mice Overexpressing Human Endothelin-1: Improvement by Exercise

Pierre Paradis[1], Tlili Barhoumi[1,4], Marie Briet[1,2,5], Daniel A. Kasal[1,3], Pascal Laurant[4], Ernesto L. Schiffrin[1,2]
[1]Lady Davis Institute for Medical Research, McGill University, Montreal, PQ, Canada, [2]Department of Medicine, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada, [3]State University of Rio de Janeiro, Brazil, [4]Universite; d'Avignon et des Pays de Vaucluse-Avignon, France, [5]Department of Pharmacology and Institut National de la Sante; et de la Recherche Medicale U970-PARCC, Hopital Europeen Georges Pompidou, Assistance Publique-Hopitaux de Paris, Paris, France

O-13 9:22-9:34

Endothelin-1-Induced Oxidative Stress and Inflammatory Cell Infiltration Contribute to High-Fat Diet Induced-Atherosclerosis and Aneurysm Formation in Apolipoprotein E Knockout Mice

Pierre Paradis[1], Muhammad O.R. Mian[1], Tlili Barhoumi[1], Asia Rehman[1], Melissa W. Li[1], Koren K. Mann[1,2], Ernesto L. Schiffrin[1,3]
[1]Lady Davis Institute for Medical Research, McGill University, Montreal, PQ, Canada, [2]Department of Oncology, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada, [3]Department of Medicine, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada

O-14 9:34-9:46

Flow Regulation of Inner Medullary Collecting Duct Endothelin-1 Production

Meghana M. Pandit, Donald E. Kohan
Division of Nephrology, University of Utah Health Sciences Center, USA

O-15 9:46-9:58

Ubiquitin Modification Plays an Important Role in ET-1-Dependent Endothelin Type B Receptor Trafficking

Koji Terada, Takahiro Horinouchi, Tsunehito Higashi, Prabha Nepal, Mika Horiguchi, Chizuru Hatate, Akimasa Hoshi, Yosuke Mai, Soichi Miwa
Hokkaido University, Japan

O-16 9:58-10:10

Aldosterone Alters Chromatin Structure of the Murine Endothelin-1 Gene

Amanda K. Welch[1,2], Mollie E. Jacobs[3], I. Jeanette Lynch[1,2], Michelle L. Gumz[2], Brian D. Cain[3], Charles S. Wingo[1,2]
[1]Department of Medicine, University of Florida, Gainesville, Florida, USA, [2]North Florida/South Georgia Veterans Health System, Gainesville, Florida, USA, [3]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida, USA

10:10-10:40 **Coffee Break**

10:40-12:06 **Room 1**

Session 8: Pulmonary Hypertension

Chairs: **Masaaki Ito** *Mie University, Tsu, Japan*
Toru Satoh *Kyorin University, Tokyo, Japan*

Keynote Lecture 3 10:40-11:05

The Evolving Paradigm of Pulmonary Arterial Hypertension and the Evolving Role of Endothelin-1

David Langleben
McGill University, Montreal, Canada

Invited Lecture 10 11:05-11:30

Balloon Pulmonary Angioplasty as a Treatment Option for Chronic Thromboembolic Pulmonary Hypertension

Hiromi Matsubara
Department of Clinical Science, National Hospital Organization Okayama Medical Center, Japan

O-17 11:30-11:42

Adipose-Derived Regenerative Cells Therapy Improves Monocrotaline Induced Rat Pulmonary Arterial Hypertension with Suppressing Endothelin-1 Though an Anti-Inflammatory Mechanism

Masamichi Eguchi, Satoshi Ikeda, Daisuke Sato, Saburo Kusumoto, Yuji Koide, Hiroaki Kawano, Koji Maemura
Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

O-18 11:42-11:54

Role of Bradykinin and Endothelin-Converting Enzyme-1 in Pulmonary Hypertension

Sunu B. Raharjo[1], Noriaki Emoto[2], Yoga Yuniadi[1], Kazuhiko Nakayama[2], Ganesja M. Harimurti[1]
[1]Department of Cardiology & Vascular Medicine, Faculty of Medicine, University of Indonesia/National Cardiovascular Center Harapan Kita, Jakarta, Indonesia, [2]Division of Clinical Pharmacy, Kobe Pharmaceutical University, Kobe, Japan

O-19 11:54-12:06

Long-Term Survival in Japanese Patients with Idiopathic/ Heritable Pulmonary Arterial Hypertension

Aiko Ogawa[1], Katsumasa Miyaji[2], Hiromi Matsubara[1]
[1]Department of Clinical Science, National Hospital Organization Okayama Medical Center, Okayama, Japan, [2]Division of Cardiology, National Hospital Organization Okayama Medical Center, Okayama, Japan

12:15-13:15 **Room 1**

Lunch Session 3

Chair: **Tsutomu Saji** *Toho University, Tokyo, Japan*

LS3

Twenty-Five Years of Research Leading to a New Generation of Endothelin Receptor Antagonists

Martine Clozel
Pharmacology & Preclinical Development, Actelion Pharmaceuticals Ltd., Allschwil, SwitzerLand

Co-sponsor: Actelion Pharmaceuticals Japan Ltd.

12:15-13:15 **Room 2**

Lunch Session 4

Chairs: **Masaru Hatano** *University of Tokyo Graduate School of Medicine, Tokyo, Japan*
Yoshihide Asano *University of Tokyo Graduate School of Medicine, Tokyo, Japan*

LS4-1

The Roles of Endothelin Receptor Antagonists and Phosphodiesterase Type 5 Inhibitors in the Management of Skin Ulcers in Systemic Sclerosis

Yoshihide Asano
Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan

LS4-2

The Challenge to Refractory Pulmonary Hypertension Associated with Scleroderma

Masaru Hatano
Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Tokyo, Japan

Co-sponsor: Nippon Shinyaku Co., Ltd.

13:15-14:45 **Poster Area**

Poster Session 2: Gene Regulation, Molecular and Cellular Biology, Pharmacology, and Pulmonary Hypertension

P-41 - **P-48** *Moderator:* **Brian Cain** 122 (1F)
University of Florida, Gainsville, USA

P-41

Endothelin Regulation by miR-218: A Target in Scarring

Andrew Leask, Fen Guo
University of Western Ontario, Canada

P-42

miRNA-1 Regulates Endothelin-1 in Diabetes

Biao Feng[1], Shali Chen[1], Yanan Cao[2], Michael Ruiz[1], Subrata Chakrabarti[1]
[1]Western University, Canada, [2]Mudanjiang Medical University, China

P-43

Endothelin-1 Mediates Downstream Profibrotic Effects by Transforming Growth Factor-Beta 1 in Systemic Sclerosis Skin Fibroblasts

Tomoaki Higuchi, Yasushi Kawaguchi, Kae Takagi, Yuko Ota
Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan

P-44

Effect of Feeding Behavior on Circadian Regulation of Endothelin Expression in Mouse Colon Epithelia

Takaharu Kozakai, Hisato Kobayashi, Katsutaka Oishi, Norio Ishida, Kaname Saida
National Institute of Advanced Industrial Science and Technology (AIST), Japan

P-45

cDNA Cloning and Sequence Analysis of Preproendothelin From Barfin Flounder (Verasper Moseri)

Hongyu Wang[1], Jiexia Quan[1], Tsuyoshi Uchide[2], Tadashi Andoh[3], Kaname Saida[1,4]
[1]National Institute of Advanced Industrial Science and Technology (AIST), Japan, [2]Veterinary Internal Medicine, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, [3]Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Kushiro, Japan, [4]Graduate School, Shibaura Institute of Technology, Japan

P-46

Shark Endothelin: cDNA Cloning, Sequence and Evolutional Analysis

Jiexia Quan[1], Hongyu Wang[1], Tsuyoshi Uchide[2], Hiroyuki Fuse[3], Kaname Saida[1,3]
[1]National Institute of Advanced Industrial Science and Technology (AIST), Japan, [2]Veterinary Internal Medicine, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, [3]Graduate School, Shibaura Institute of Technology, Japan

P-47

Molecular Cloning and Sequence Analysis of Preproendothelin from Medaka, Oryzias Latipes

Jiexia Quan[1], Hongyu Wang[1], Tsuyoshi Uchide[2], Hiroyuki Fuse[3], Kaname Saida[1,3]
[1]National Institute of Advanced Industrial Science and Technology (AIST), Japan, [2]Veterinary Internal Medicine, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, [3]Graduate School, Shibaura Institute of Technology, Japan

P-48

The Signaling Pathways Involved in the Synergistic Effect of ET-1 and cAMP on IL-6 Transcription

Shin-Pei Chai, Jim C. Fong
Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taiwan

P-49 - P-56 *Moderator:* **Jo De Mey** 122 (1F)
University of Southern Denmark, Odense, Denmark

P-49

A Novel Mouse Model to Characterize the Mechanisms of Endothelin-1-Induced Vascular Injury

Pierre Paradis[1], Suellen C. Coelho[1], Yohann Rautureau[1], Ernesto L. Schiffrin[1,2]
[1]Lady Davis Institute for Medical Research, McGill University, Montreal, PQ, Canada, [2]Department of Medicine, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada

P-50

Remodeling of Endothelial Function in Atherosclerotic Mice Overexpressing Endothelin-1 Restricted to Endothelium

Pierre Paradis[1], Noureddine Idris-Khodja[1], Muhammad Oneeb Rehman Mian[1], Melissa W. Li[1], Avshalom Leibowitz[1], Yohann Rautureau[1], Ernesto L. Schiffrin[1,2]
[1]Lady Davis Institute for Medical Research, McGill University, Montreal, PQ, Canada, [2]Department of Medicine, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, PQ, Canada

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Negative Regulation of Endothelin Type A Receptor-Operated TRPC6 Channel by Adenylate Cyclase-cAMP-Protein Kinase A Signaling Pathway

Takahiro Horinouchi, Tsunaki Higa, Tsunehito Higashi, Koji Terada, Yosuke Mai, Takuya Harada, Akimasa Hoshi, Mika Horiguchi, Prabha Nepal, Chizuru Hatate, Soichi Miwa
Department of Cellular Pharmacology, Hokkaido University Graduate School of Medicine, Hokkaido, Japan

P-52

microRNA Regulation of Endothelin-1 in an Inner Medullary Collecting Duct Cell Line

Mollie E Jacobs[1], Lauren A Jeffers[1], Amanda K Welch[2,3], Charles S Wingo[2,3], Brian D Cain[1]
[1]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, 32610, USA, [2]Department of Medicine, Division of Nephrology, Hypertension, and Renal Transplantation, University of Florida, Gainesville FL, USA, [3]North Florida/South Georgia Veterans Health System, Gainesville, FL, USA

P-53

The Relative Contributions of Active Response and Passive Stiffness on the Pharmacological Response of Human Normal and Diseased Coronary Arteries to ET-1

Janet J Maguire[1], Chen Yen Ooi[2], Michael PF Sutcliffe[2], Anthony P Davenport[1]
[1]Clinical Pharmacology Unit, University of Cambridge, Cambridge, UK, [2]Engineering Department, University of Cambridge, Cambridge, CB2 1P2, UK

P-54

Mastocytes Derived or Recombinant Mouse Mast Cell Protease 4 (mMCP-4) Converts Big-ET-1 to ET-1 (1-31)

Louisane Desbiens[1], Robert Day[2], Martin Houde[1], Walid Semaan[1], Shinji Takai[3], Mizuo Miyazaki[3], Gunnar Pejler[4], Pedro D'Orléans-Juste[1]
[1]Department of Pharmacology, Université de Sherbrooke, Sherbrooke, Canada, [2]Department of Biochemistry, Université de Sherbrooke, Sherbrooke, Canada, [3]Department of Pharmacology, Osaka Medical College, Osaka, Japan, [4]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden

P-55

Involvement of Endothelin-1 in Adrenal Catecholamine Regulation

Manabu Murakami[1], Takayuki Nemoto[2], Takayoshi Ohba[3], Hidetoshi Niwa[4], Testuya Kushikata[4], Kyouichi Ono[3], Hiroyuki Watanabe[5], Kazuyoshi Hirota[4]
[1]Department of Pharmacology, Hirosaki University, Graduate School of Medicine, Japan, [2]Department of Pharmacology, Graduate School of Medicine, University of Miyazaki, Japan, [3]Department of Physiology, Akita University School of Medicine, Japan, [4]Department of Anesthesiology, Hirosaki University, Graduate School of Medicine, Japan, [5]Department of Internal Medicine Division of Cardiovascular and Respiratory Medicine, Akita University School of Medicine, Japan

P-56

The Anti-Inflammatory Effects of Endothelin-A Receptor Antagonism During Hyperdinamic Sepsis in Rats

Andras T Meszaros[1], Tamas Buki[1], Kitti Horvath[1], Gabriella Varga[1], Daniel Erces[1], Jozsef Kaszaki[1], Noriko Okada[2], Hidechika Okada[2], Mihaly Boros[1]
[1]Institute of Surgical Research, University of Szeged, Hungary, [2]Department of Immunology, Nagoya City University, Nagoya, Japan

P-57 - P-64 *Moderator:* **Sunu B. Raharjo** 122 (1F)
University of Indonesia, Jakarta, Indonesia

P-57

The Toxicity of Indoxyl Sulfate to Endothelial Progenitor Cells is Rescued by Niacin

Kwan-Dun Wu, Yu-Chin Huang, VinCent Wu
Department of Internal Medicine, National Taiwan University, Taipei, Taiwan

P-58

Endothelin-1 Does Not Alter Macrophage Phenotype

Rebecca C Moorhouse[1], Neeraj Dhaun[1,2], David J Webb[1], David C Kluth[2]
[1]Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK, [2]Centre for Inflammation Research, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

P-59

Endothelin-1 Activates Extracellular Signal-Regulated Kinases 1 and 2 Through Transactivation of Platelet-Derived Growth Factor Receptor in Skeletal Muscle Cells

Tsunehito Higashi, Takahiro Horinouchi, Takuya Harada, Tsunaki Higa, Koji Terada, Akimasa Hoshi, Yosuke Mai, Mika Horiguchi, Prabha Nepal, Chizuru Hatate, Soichi Miwa
Department of Cellular Pharmacology, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

P-60

Endothelin Receptor Transactivates the TGFβ Receptor to Stimulate Proteoglycan Synthesis in Human Vascular Smooth Muscle Cells

Danielle Kamato, Narin Osman, Peter J Little
Discipline of Pharmacy, School of Medical Science and Diabetes Complications Lab, Exercise and Disease Program, Health Innovations Research Institute, RMIT University, Melbourne, Australia

P-61

Structure of the Precursor of Salmon, Oncorhynchus Keta, Endothelins and Phylogenetic Analysis

Hongyu Wang[1], Jiexia Quan[1], Tsuyoshi Uchide[2], Kaname Saida[1,3]
[1]National Institute of Advanced Industrial Science and Technology (AIST), Japan, [2]Veterinary Internal Medicine, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, [3]Graduate School, Shibaura Institute of Technology, Japan

P-62

Decreased MYPT-1 Phosphorylation at Thr696 and Cdc42 Protein Expression are Associated with Decreased Contractile Responses to ET-1 in Corpora Cavernosa and Internal Pudendal Artery from Goto-Kakizaki Diabetic Rats

Rheure Lopes[1], Fernando Carneiro[1], Theodora Szasz[2], Gisele Bomfim[3], Clinton Webb[2], Rita Tostes[1]
[1]University of Sao Paulo, Brazil, Pharmacology, Medical School of Ribeirao Preto, Brazil, [2]Georgia Health Sciences University, USA, [3]Federal University of Mato Grosso, Brazil

P-63

Generation of Edn2-iCre Transgenic Mice

Joseph Cacioppo[1], Patrick Lin[1], Arnon Gal[1], Yongbum Koo[1,2], CheMyong Ko[1]
[1]The Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Department of Biotechnology and Biomedical Science, Inje University, Gimhae, South Korea

P-64

The Calcitonin Gene-Related Peptide (CGRP) Play Beneficial Roles in Myocardial Ischemia Elicited by Endothelin-1

Satoshi Homma[1], Satoshi Sakai[1], Ken-ichi Yanagi[2], Yumi Miyauchi[1], Kazutaka Aonuma[1], Takashi Miyauchi[1]
[1]Department of Medicine, University of Tsukuba, Tsukuba, Japan, [2]Department of Biomedical Sciences, University of Tsukuba, Tsukuba, Japan

P-65 - P-72 *Moderator:* **Janet Maguire** 122 (1F)
University of Cambridge, Cambridge, UK

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The Role of Endothelin Receptors (ETRA/B) in Fibrocyte Differentiation

Sarah L. Trinder[1], Xu Shi-wen[1], Bahja Ahmed Abdi[1], Christopher P. Denton[1], David C. Budd[2], David J. Abraham[1], Alan M. Holmes[1]
[1]Centre for Rheumatology & Connective Tissue Diseases, UCL Medical School, Royal Free Campus, London, UK, [2]Respiratory Drug Discovery, Inflammation, Hoffmann-La Roche Inc., Nutley, NJ, USA

P-66

Improved Relaxations to Acetylcholine in Murine Carotid Arteries with Heterozygous Overexpression of Preproendothelin-1 in the Endothelium

Oliver Baretella[1], Sookja K. Chung[2,4], Aimin Xu[1,3,4], Paul M. Vanhoutte[1,4]
[1]Department of Pharmacology & Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [2]Department of Anatomy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [3]Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China, [4]Research Centre of Heart, Brain, Hormone & Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

P-67

Vascular Pharmacology of Quercetin in Rat

Hiroyasu Satoh
Health Life Science, Shitennoji University, Osaka, Japan

P-68

Effect of Selective Ablation of Endothelin A Receptor in the Granulosa Cells on the Fertility

Jongki Cho[1,2], Joseph Cacioppo[1], Patrick Lin[1], CheMyong Ko[1]
[1]Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL 61802, USA, [2]College of Veterinary Medicine, Chungnam National University, Daejeon, South Korea

P-69

Physiological and Functional Antagonism of Arterial Endothelin$_A$ Receptor Function

Matthijs G. Compeer[1], Merlijn JPMT Meens[1,2], Ger MJ Janssen[1], Jo GR De Mey[1,3]
[1]Department of Pharmacology, Cardiovascular Research Institute Maastricht, The Netherlands, [2]Department of Pathology and Immunology, Universite de Geneve, Switzerland, [3]Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark

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Negative Allosteric Modulation of Endothelin ET$_A$ Receptor Function in Resistance Arteries

Jo G. R. De Mey[1], Misha F Vrolijk[2], Mathijs G Compeer[2], Merlijn J Meens[3], Carsten Hoeltke[4]
[1]Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark, [2]Department of Pharmacology, Cardiovascular Research Institute, Maastricht University, the Netherlands, [3]Department of Pathology and Immunology, Universite de Geneve, Switzerland, [4]Department of Clinical Radiology, University of Muenster, Germany

P-71

Purification Different Forms of Extracellular Superoxide Dismutase and Their Effects on Anti-Hypertension Through Nitric Oxide Induction in Spontaneous Hypertension Rats

Chuan-Mu Chen[1], Hsiao-Ling Chen[1], Ta-Yung Weng[1], Wei Chen[1], Yu-Tang Tung[1]
[1]Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan, [2]Department of Bioresources, Da-Yeh University, Changhwa, Taiwan, [3]Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan, [4]Division of Pulmonary and Critical Care Medicine, Chia-Yi Christian Hospital, Chia-Yi, Taiwan, [5]Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan

P-72

Identification and Characterization of Novel Antihypertensive Peptides Obtained from Fermented Milk

Hsiao-Ling Chen[1], Yu-Tang Tung[1], Geroge Kuo[1], Chuan-Mu Chen[1]
[1]Department of Bioresources, Da-Yeh University, Changhwa, Taiwan, [2]Department of Life Sciences, Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan, [3]Department of Life Sciences, Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan, [4]Department of Life Sciences, Agricultural Biotechnology Center, National Chung Hsing University, Taichung, Taiwan

P-73 - P-79 *Moderator:* **Hunter Gillies** *Gilead, USA*

P-73 122 (1F)

Counteracting Effects of Treprostinil and Endothelin (ET-1) Receptor Antagonists (ETRAs) on Endothelin-1, ETB Receptor and ECE-1 Levels in Pulmonary Smooth Muscle Cells (PASMCs) Derived from Patients with Pulmonary Arterial Hypertension (PAH)

Jigisha Patel, Susan Hall, Lucie Clapp
Clinical Pharmacology Department, Division of Medicine, UK

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Potential Involvement of Functional Tricuspid Regurgitation in the Diagnostic Error to Assess Pulmonary Arterial Pressure by Doppler Echocardiography

Saori Yamamoto, Yasuharu Matsumoto, Kotaro Nochioka, Masanobu Miura, Syunsuke Tatebe, Koichiro Sugimura, Tomoyuki Suzuki, Yoshihiro Fukumoto, Hiroaki Shimokawa
The Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

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Detection of Developing Pulmonary Vasculopathy with Non- Invasive Cardiopulmonary Exercise Testing

Hiroki Kinoshita, Yoshihiro Dohi, Ryoji Sata, Yasuki Kihara
Department of Cardiovascular Medicine, University of Hiroshima, Hiroshima, Japan

P-76 Lobby (B1)

Vascular Endothelial Growth Factor (VEGF) and the Control of Endothelin-1 Synthesis by Human Lung Microvascular Endothelial Cells: A Possible Pathway for Pathogenesis

Gregory Star, Michele Giovinazzo, David Langleben
Center for Pulmonary Vascular Disease and Lady Davis Institute, Jewish General Hospital, Montreal, Canada

P-77 Lobby (B1)

Effect of Bosentan on Exercise Capacity in Patients with Pulmonary Arterial Hypertension or Inoperable Chronic Thromboembolic Pulmonary Hypertension

Akihiro Hirashiki[1], Takahisa Kondo[1], Yoshihisa Nakano[2], Shiro Adachi[2], Shuzo Shimazu[2], Shinya Shimizu[2], Takahiro Okumura[2], Toyoaki Murohara[2]
[1]Department of Advanced Medicine in Cardiopulmonary Disease, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Department of Cardiology, Nagoya University Graduate School of Medicine, Jpan

P-78 Lobby (B1)

Short-Term Drug Interaction of Bosentan and Sildenafil under the Long-Term Use in Patients with Pulmonary Arterial Hypertension

Sachiko Miyakawa[1], Shimako Tanaka[2], Takahiro Goto[3], Shinya Uchida[2], Kazuhiko Takeuchi[1], Naoki Inui[1], Hiroshi Yamada[3], Noriyuki Namiki[2], Hiroshi Watanabe[1]
[1]Department of Clinical Pharmacology & Therapeutics, Hamamatsu University School of Medicine, Japan, [2]Depatment of Pharmacy Practice & Science, School of Pharmaceutical Science, University of Shizuoka, Japan, [3]Division of Drug Evaluation & Informatics, School of Pharmaceutical Sciences, University of Shizuoka, Japan

P-79 Lobby (B1)

Chronic Treatment with Novel Endothelin Receptor Antagonist Macitentan Improved Severe Pulmonary Arterial Hypertension in Rats

Kohtaro Abe[1], Mutsumi Kunita[2], Yoshitaka Hirooka[1], Yukimitsu Kuwabara[2], Katsuya Hirano[3], Kenji Sunagawa[2,3]
[1]Departments of Advanced Cardiovascular Regulation and Therapeutics, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan, [2]Departments of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan, [3]Division of Molecular Cardiology, Research Institute of Angiocardiology, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

P-80 - P-86, PC-16

Moderator: **David Langleben** *McGill University, Montreal, Canada* Lobby (B1)

P-80

Ambrisentan and Tadalafil Synergistically Attenuate Chronic Hypoxia-Induced PAH in Rats

Faquan Liang, Suya Yang, Jessie Jia, Hunter Gillies, Lina Yao, Luiz Belardinelli
Gilead Sciences, Inc, Fremont, CA, USA

P-81

Real World Experience in the DETECT Study for Pulmonary Artery Hypertension Associated with Systemic Sclerosis

Yasuhiro Suyama, Mitsumasa Kishimoto, Hisanori Shimizu, Ryo Rokutanda, Chisun Min, Yuri Ohara, Yoichiro Haji, Ken-ichi Yamaguchi, Yukio Matsui, Masato Okada
Division of Allergy & Rheumatology, St. Luke's International Hospital, Tokyo, Japan

P-82

Reduced Circulating Endothelin-1 Level in Uncorrected ASD Patients with Severe Pulmonary Hypertension

Lucia Krisdinarti, Dyah Wulan Anggrahini, Anggoro Budi Hartopo, Arina Nugraheni, Hariadi Hariawan, Nahar Taufiq, Budi Yuli Setianto
Department of Cardiology and Vascular Medicine, School of Medicine Gadjah Mada University/ Sardjito Hospital, Indonesia

P-83

Current State of Medicine Usage and the Predictor of Mortality in Pulmonary Arterial Hypertension in Japan

Shiro Adachi[1], Akihiro Hirashiki[2], Syuzo Shimazu[1], Yoshihisa Nakano[1], Toyoaki Murohara[1], Takahisa Kondo[2]
[1]Department of Cardiology, Nagoya University Graduate School of Medicine, Aichi, Japan, [2]Department of Advanced Medicine in Cardiopulmonary Disease, Nagoya University Graduate School of Medicine, Aichi, Japan

P-84

Clinical Effect of Ambrisentan in Pulmonary Hypertension

Toshinori Minamishima, Toru Satoh, Masaharu Kataoka, Hideaki Yoshino
Cardiology Division, Department of Medicine, Kyorin University School of Medicine, Japan

P-85

Long-Term Advanced Therapy with Bosentan Improves Symptoms and the Time to Clinical Worsening in the Japanese Patients with Inoperable Chronic Thromboembolic Pulmonary Hypertension

Mami Nishikawa, Shuichi Ueno, Kazuomi Kario
Jichi Medical University School of Medicine, Japan

P-86

Analysis of ET-1 System in Mild and Severe Pulmonary Arterial Hypertension in Mice

Hung Van Tran[1], Noriaki Emoto[1,2], Nicolas Vignon-Zellweger[2], Kazuhiko Nakayama[2], Keiko Yagi[2], Yoko Suzuki[2], Ken-ichi Hirata[1]
[1]Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan, [2] Department of Clinical Pharmacy, Kobe Pharmaceutical University, Kobe, Japan

PC-16

Early Detection of Pulmonary Arterial Hypertension by the Exercise Echocardiography in Patients with Connective Tissue Diseases

Yasuchika Kato[1], Shusaku Fukaya[2], Megumi Kurumizawa[2], Yohko Takakuwa[1], Masatsugu Iwase[2], Yukio Ozaki[1], Shunji Yoshida[1]
[1]Department of Cardiology, Fujita Health University School of Medicine, Japan, [2]Section of Rheumatology and Infectious Diseases, Department of Internal Medicine, Fujita Health University School of Medicine

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P-87 - P-91 , PC-17 - PC-19

Moderator: **Koji Maemura** Lobby (B1)
Nagasaki University, Nagasaki, Japan

P-87

Why Are Endothelin Antagonists Effective in Pulmonary Arterial Hypertension with Right Ventricular Dysfunction?

Rhoda E Kuc[1], Myrna Carlebur[1], Janet J Maguire[1], Peiran Yang[1], Lu Long[2], Mark Toshner[2], Nicholas W. Morrell[2], Anthony P Davenport[1]
[1]Clinical Pharmacology Unit, [2]Department of Medicine, University of Cambridge, UK

P-88

Endothelin-1 Induces Down-Regulation of IP Receptor in Pulmonary Artery Smooth Muscle Cells Obtained from Patients with Pulmonary Arterial Hypertension

Satoshi Akagi, Kazufumi Nakamura, Hiroshi Ito
Department of Cardiovascular Medicine, Okayama University Hospital, Japan

P-89

Efficacy of Oral Triple Upfront Combination Therapy (Long-Acting Prostacyclin Analogue, Endothelin Receptor Antagonist, Phosphodiesterase 5 Inhibitor) in the Patients with Idiopathic /Heritable Pulmonary Arterial Hypertension

Takeshi Ogo, Shigefumi Fukui, Akihiro Tsuji, Norifumi Nakanishi
Pulmonary Vascular Disease Unit, Department of Cardiology, National Cerebral and Cardiovascular Center, Japan

P-90

Combination Therapy of Bosentan and Ambrisentan for Portopulmonary Hypertension

Hironori Muraoka[1], Masaru Hatano[1], Takeo Fujino[1], Shun Minatsuki[1], Teruhiko Imamura[1], Toshiro Inaba[1], Hisataka Maki[1], Atsushi Yao[2], Koichiro Kinugawa[3], Issei Komuro[1]
[1]Department of Cardiovascular Medicine, University of Tokyo, Tokyo, Japan, [2]Division for Health Service Promotion, University of Tokyo, Tokyo, Japan, [3]Department of Therapeutic Strategy for Heart Failure, University of Tokyo, Tokyo, Japan

P-91

Experience in Combination Therapy for Portopulmonary Hypertension in the Young with Intravenous Epoprostenol and Endothelin Receptor Antagonists

Shigetoyo Kogaki, Kunihiko Takahashi, Seiko Mihara, Ryo Ishii, Ryota Higeno, Nobutoshi Nawa, Hiroki Baden, Keiichi Ozono
Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan

PC-17

Peak Systolic Strain at Right Ventricular Free Wall Determined by Two-Dimensional Speckle-Tracking Echocardiography is an Independent Predictor for Pulmonary Hypertension

Satoshi Ikeda[1], Akira Tsuneto[1,3], Sanae Kojima[2,3], Seiji Koga[1], Tomoo Nakata[1], Takeo Yoshida[1], Miyuki Eto[1], Takako Minami[1,3], Katsunori Yanagihara[2], Koji Maemura[1,3]
[1]Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Japan, [2]Central Diagnostic Laboratory, Nagasaki University Hospital, Japan, [3]Ultrasound Diagnostic Center, Nagasaki University Hospital, Japan

PC-18

Prognosis of Sleep-Disorder Breathing for Chronic Heart Failure and the Effectiveness of Nocturnal Home Oxygen Therapy and Continuous Positive Airway Pressure

Hiroyuki Satake, Koji Fukuda, Makoto Nakano, Yuji Wakayama, Masateru Kondo, Yuhi Hasebe, Mohamed Shafee A., Hiroaki Shimokawa
The Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Japan

PC-19

Detection of Hydroxyl Radical in Isolated Goto-Kakizaki (GK) Rat Arteries by Trapping with 4-Hydroxybenzoic Acid

Reiko Ishii-Nozawa, Kohta Kawabata, Yuichi Tomioka, Kyohei Hazama, Mayu Watanabe, Hajime Kagaya
Department of Clinical Pharmaceutics, Meiji Pharmaceutical University, Japan

14:45-16:05 **Room 1**

Session 9: Blocking the ETA Receptors: What's New?

Chairs: **Michael Dashwood** *University College London, London, UK*
Ernesto Schiffrin *McGill University, Montreal, Canada*

Invited Lecture 11 14:45-15:05

Endothelin Receptor Selectivity in Patients with Pulmonary Arterial Hypertension (PAH)

Ronald Oudiz
Harbor-UCLA Medical Center, Torrance, USA

Invited Lecture 12 15:05-15:25

Age-Dependent Antihypertensive and Antiproteinuric Effects of ET_A Receptor Blockade in Ren-2 Transgenic Rats

Ivana Vaněčková[1], L. Červenka[2], Z. Husková[2], Z. Vaňourková[2], Z. Vernerová[3]
[1]Institute of Physiology, Department of Experimental Hypertension, Prague, Czech Republic, [2]Institute for Clinical and Experimental Medicine, Department of Experimental Hypertension, Prague, [3]Department of Pathology, 3rd faculty of Medicine, Charles University, Prague, Czech Republic

Invited Lecture 13 15:25-15:45

Dual Endothelin A and Angiotensin Receptor Blockers in the Treatment of Hypertension and Renal Disease

Donald Kohan
University of Utah, Salt Lake City, USA

Invited Lecture 14 15:45-16:05

Good and Bad News about Single and Dual Biphenyl Endothelin ET_A and Angiotensin AT_1 Receptor Antagonists

Jo G. R. De Mey[1,2], Matthijs G. Compeer[2], Misha F. Vrolijk[2]
[1]Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark, [2]Department of Pharmacology, Cardiovascular Research Institute Maastricht, Maastricht University, NL

16:05-16:30 **Coffee Break**

16:30-16:45 **Room 1**

Young Investigator Award Ceremony

16:45-17:15 **Room 1**

Honorary Chair Session

Chair: **Hiroaki Shimokawa** *Tohoku University, Sendai, Japan*

Invited Lecture 15

End o' the Line Revisited

Paul M. Vanhoutte[1,2]
[1]Department of Pharmacology and Pharmacy, University of Hong Kong, Hong Kong, China, [2]Department of Clinical Pharmacy, King Saud University, Riyadh, Saudi Arabia

17:15-17:45 **Room 1**

Special Guest Session

Chair: **Masashi Yanagisawa** *Southwestern Medical Center, Dallas, USA / University of Tsukuba, Tsukuba, Japan*

Invited Lecture 16

Multiple and Integrative Approaches to Cardiovascular Diseases with Stem Cell Technology

Jun K. Yamashita[1,2]
[1]Department of Cell Growth & Differentiation, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, [2]Department of Stem Cell Differentiation, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Wednesday, September 11, 2013

O=Oral Presentation, P=Poster, PC=Poster "Cross - border Sessions"

8:30-10:00 **Room 1**

Session 10: Cardiology, Hypertension, Vascular Disease

Chairs: **Rita C. Tostes** *University of São Paulo, São Paulo, Brazil*
Jo De Mey *University of Southern Denmark, Odense, Denmark*

Keynote Lecture 4 8:30-8:55

Endothelin in Myocardial Infarction

Theofilos M. Kolettis
University of Ioannina, Greece

O-20 8:55-9:07

Endothelin-1 is a Key Candidate to Exert Pathophysiological Effects on Cardiomyocytes Derived from Hypertrophic Cardiomyopathy-Induced Pluripotent Stem Cell

Atsushi Tanaka[1,2], Shinsuke Yuasa[1], Koichi Node[2], Keiichi Fukuda[1]
[1]Department of Cardiology, Keio University School of Medicine, Tokyo, Japan, [2]Department of Cardiovascular and Renal Medicine, Saga University, Saga, Japan

O-21 9:07-9:19

Endothelin Receptor Antagonists Exacerbate Autoimmune Myocarditis in Mice

Kazuko Tajiri[1], Satoshi Sakai[1], Taizo Kimura[1], Tomoko Machino-Ohtsuka[1], Zheng Wang[1], Akira Sato[1], Takashi Miyauchi[1], Kazutaka Aonuma[1]
[1]Cardiovascular Division, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]Department of Pathology and Matrix Biology, Mie University Graduate School of Medicine, Tsu, Mie, Japan

O-22 9:19-9:31

Imaging of the Binding of ET-1 and of Linear ET-1 in Rat Mesenteric Resistance Arteries

Dimitrios Kapsokalyvas[1,2], Paul M. H. Schiffers[3], Nathan Maij[3], Tilman M. Hackeng[4], Dennis P. Suylen[4], Marrc A. M. J. van Zandvoort[1,5], Jo G. R. De Mey[3,6]
[1]Molecular Cell Biology, Department of Genetics & Cell Biology, Maastricht University, Maastricht, The Netherlands, [2]Institute of Biochemistry and Molecular Cell Biology, RWTH Aachen University, Aachen, Germany, [3]Department of Pharmacology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands, [4]Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands, [5]Institute for Molecular Cardiovascular Research (IMCAR), RWTH Aachen University, Aachen, Germany, [6]Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark

O-23 9:31-9:43

Sympathetic Endothelin a Receptors Contribute to the Development of Heart Failure

Johannes P. Backs[1,6], Lorenz L. Lehmann[1], Julia Rostosky[1], Sebastian J. Buss[1], Walter Mier[2], Michael D. Schneider[3], Rosanna Parlato[4], Hermann-Josef Groene[5], Masashi Yanagisawa[6], Uwe Haberkorn[2], Hugo A. Katus[1]
[1]Cardiology, Heidelberg University, Heidelberg, Germany, [2]Department of Nuclear Medicine, University Hospital, Heidelberg, Germany, [3]British Heart Foundation Centre of Research Excellence, Imperial College London, United Kingdom, [4]Institute of Applied Physiology, University of Ulm, Ulm, Germany, [5]Department of Molecular Pathology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [6]HHMI and Dept. of Molecular Genetics, UTSW Medical Center at Dallas, Dallas, USA

O-24 9:43-9:55

Mouse Mast Cell Protease-4-Dependent Production of ET-1 (1-31) and of Plaque Progression in a Apolipoprotein E Knock-Out Mouse Model of Spontaneous Atherosclerosis

Martin Houde[1], Walid Semaan[1], Louisane Desbiens[1], Zhipeng You[2], Adel G. Schwertani[2], Gunnar Pejler[3], Shinji Takai[4]
[1]Department of Pharmacology, Universite de Sherbrooke, Sherbrooke, QC, Canada, [2]Department of Medicine, McGill University, Montreal, QC, Canada, [3]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden, [4]Department of Pharmacology, University of Osaka, Osaka, Japan

10:00-10:30 **Coffee Break**

10:30-12:05 **Room 1**

Session 11: Clinical Studies Update

Chairs: **John Pernow** *Karolinska Institutet, Stockholm, Sweden*
Issei Komuro *The University of Tokyo, Tokyo, Japan*

Keynote Lecture 5 10:30-10:55

Endothelin Receptor Antagonists in Clinical Research - Lessions Learned

Berthold Hocher
Institute of Nutritional Science, University of Potsdam, Germany

Invited Lecture 17 10:55-11:20

Endothelin Receptor Antagonism Versus Combined ECE/NEP Inhibition in Patients with Type 2 Diabetes and Nephropathy

Ariela Benigni
IRCCS - Istituto Mario Negri, Bergamo, Italy

Invited Lecture 18 11:20-11:35

The Selective Type A Endothelin Antagonist Atrasentan Reduces Residual Albuminuria in Patients with Type 2 Diabetes and Nephropathy

Dennis L. Andress[1], Dick de Zeeuw[2], Hans-Henrik Parving[3]
[1]AbbVie, Chicago, IL, USA, [2]University of Groningen, Groningen, the Netherlands, [3]University Hospital of Copenhagen, Copenhagen, Denmark

Invited Lecture 19 11:35-11:50

A Placebo-Controlled Study of Ambrisentan in Subjects with Idiopathic Pulmonary Fibrosis (ARTEMIS-IPF)

Hunter Gillies, N. Henig[1], P. Pederson[2], L. Shao[1], J. Chien[2], T. O'Riordan[2], ARTEMIS IPF Investigators
[1]Gilead Sciences Inc. Foster City, CA, USA, [2]Gilead Sciences, Inc. Seattle, WA USA

O-25 11:50-12:02

Impact of Short Term Endothelin A Receptor Blockade on Plasma Markers for Remodeling and Neutrophil Activation in Patients with ST Elevation Acute Coronary Syndrome

Raphael Wurm[1], Christopher Adlbrecht[1], Martin Andreas[2], Bassam Redwan[1], Klaus Distelmaier[1], Guenter Klappacher[1], Irene M. Lang[1]
[1]Medical University of Vienna, Department of Internal Medicine II, Division of Cardiology, Austria, [2]Medical University of Vienna, Department of Cardiac Surgery Austria

12:15-13:15 Room 1

Lunch Session 5

Chair: **Yasuo Matsumura** *Osaka University of Pharmaceutical Sciences, Osaka, Japan*

LS5

Heart Failure in PAH: Focus on the Right Ventricle

Ronald Oudiz
Harbor-UCLA Medical Center, Torrance, USA

Co-sponsor: GlaxoSmithKline K.K.

12:15-13:15 Room 2

Lunch Session 6

Chair: **Shigetaka Sasayama** *Kyoto University and Uji Hospital, Kyoto, Japan*

LS6

How to Treat the Patients with Hypertension for the Prevention of Heart Failure -The Back and Forth Strategy between Basic Science and Clinical Medicine-

Masafumi Kitakaze
Department of Clinical Research and Development, and Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita, Japan

Co-sponsor: Takeda Pharmaceutical Company Limited.

13:15-14:45 Poster Area

Poster Session 3: Hypertension, Vascular Diseases, Cardiology, Clinical Studies, Metabolism

P-92 - P-98 *Moderator:* **Bambang Widyantoro** *University of Indonesia, Jakarta, Indonesia* 122 (1F)

P-92

A Potential Role of Endothelins in Rheumatic Mitral Stenotic Valves

Tania M. A. Rodrigues[1], Sydney C. Leao[1], Mateus S. Andrade[1], Williasmin B. Souza[1], Nicolas N. Santos[1], Olivia R. L. L. Teles[1], Carlos A. S. Aragao[1], Manuela Sena[1], Shirlei O. Silva[2], Michael R. Dashwood[3]
[1]Group of Molecular Anatomy, Federal University of Sergipe, Brazil, [2]Department of Morphology, Federal University of Sergipe, Aracaju, SE, Brazil, [3]Department of Clinical Biochemistry, Royal Free Hospital, London, UK

P-93

Blood Pressure Independent Downregulation of Plasma Endothelin-1 Levels in a Lavage-Induced Surfactant Depleted Rabbit ARDS Model: Effects of Various Respiratory Maneuvers on Endothelin-1 Levels

Junko Kamiyama[1], Subrina Jesmin[2], Hideaki Sakuramoto[3], Majedul Islam[2], Tanzila Khatun[2], Nobutake Shimojo[4], Satoru Kawano[4], Taro Mizutani[4]
[1]Master's Program of Medical Sciences, University of Tsukuba, Ibaraki, Japan, [2]The Faculty of Medicine, University of Tsukuba, Ibaraki, Japan, [3]Graduate School of Comprehensive Human Sciences Majors of Medical Sciences, [4]The Department of Emergency and Critical Care Medicine, University of Tsukuba, Ibaraki, Japan

P-94

Upregulated Pulmonary Endothelin-1 in Acute Lung Injury is Not Normalized Through Landiolol Hydrochloride Treatment, an Ultra-Short-Acting β-Blocker, in a Rat Model of Endotoxemia

Nobutake Shimojo, Subrina Jesmin, Yoshimoto Seki, Hideaki Sakuramoto, Majedul Islam, Tanzila Khatun, Taro Mizutani
Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

P-95

Blockade of TRPC6 is a Novel Therapeutic Approach Against Pathological Cardiac Remodeling

Hideyuki Kinoshita[1], Koichiro Kuwahara[1], Motohiro Nishida[2], Hitoshi Kurose[2], Shigeki Kiyonaka[3], Yasuo Mori[3], Chinatsu Yamada[1], Kazuhiro Nakao[1], Yoshihiro Kuwabara[1], Shinji Yasuno[1], Yasuaki Nakagawa[1], Toshio Nishikimi[1], Kenji Ueshima[1], Kazuwa Nakao[1]
[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan, [2]Department of Pharmacology and Toxicology, Kyusyu University Graduate School of Pharmaceutical Sciences, Japan, [3]Department of Synthetic Chemistry and Biological Chemistry, Kyoto University Graduate School of Engineering, Japan

P-96

Effects of Landiolol Hydrochloride, an Ultra-Short-Acting β-Blocker, on Cardiac Endothelin System in a Rat Model of Endotoxemia: A Possible Relevance with Cardiac Functional Compensatory Events at the Early Phase of Sepsis

Yoshimoto Seki, Subrina Jesmin, Nobutake Shimojo, Majedul Islam, Tanzila Khatun, Hideaki Sakuramoto, Keiichi Hagiya, Satoru Kawano, Taro Mizutani
Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

P-97

Inhibitory Effect of Eicosapentaenoic Acid on Cardiomyocyte in Endothelin Induced Hypertrophy Via PPAR-α

Nobutake Shimojo[1,2], Subrina Jesmin[1,2], Satoshi Sakai[1], Seiji Maeda[1], Takashi Miyauchi[1], Satoru Kawano[1,2], Taro Mizutani[2], Kazutaka Aonuma[1]
[1]Cardiovascular Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

P-98

Higher Circulatory Level of Endothelin-1 in Hypertensive Subjects Screened Through a Cross-Sectional Study in Rural Bangladeshi Women

Shamima Akter[1,2,3], Subrina Jesmin[1,2,3], Arifur Rahman[2,4], AKM Ahsan Habib[2,4], Nobutake Shimojo[1], Majedul Islam[1,2,3], Sohel Zaedi[2,3], Naoto Yamaguchi[2,3], Masao Moroi[3], Sosuke Kimura[3], Osamu Okazaki[3], Takashi Miyauchi[1], Satoru Kawano[1], Hidechika Akashi[3], Taro Mizutani[1]
[1]Graduate School of Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]Health & Disease Research Center for Rural Peoples (HDRCRP), Mohammadpur, Dhaka 1207, Bangladesh, [3]National Center for Global Health and Medicine (NCGM), 1-21-1 Toyama, Shinjuku-ku, Tokyo, Japan, [4]Shahid Ziaur Rahman Medical College, Bogra, Bangladesh

P-99 - P-104 *Moderator:* **Rita C. Tostes** 122 (1F)
University of São Paulo, São Paulo, Brazil

P-99

Inverse Correlation between Systemic Endothelin-1 Level and Pulmonary Artery Pressure in Adult Patients with Uncorrected Atrial Septal Defect

Dyah Wulan Anggrahini, Lucia Krisdinarti, Anggoro Budi Hartopo, Arina Nugraheni, Hariadi Hariawan, Nahar Taufiq, Budi Yuli Setianto
Department of Cardiology and Vascular Medicine, School of Medicine Gadjah Mada University, Indonesia

P-100

Synchrotron Radiation Pulmonary Micro-Angiography to Visualize Pulmonary Artery Micro-Vasculature for Measurement of Pulmonary Arterial Flow Velocity in a High Pulmonary Flow Rat Model

Chiho Tokunaga[1], Shonosuke Matsushita[2], Kazuyuki Hyodo[3], Hiroaki Sakamoto[1], Kazunori Miyakawa[4], Misao Kubota[4], Akira Kobayashi[5], Kenkichi Tanioka[6], Yuji Hiramatsu[1], Yuzuru Sakakibara[1]
[1]Department of Cardiovascular Surgery, Faculty of Medicine, Ibaraki clinical education and training center, University of Tsukuba, Ibaraki, Japan, [2]Tsukuba University of Technology, Ibaraki, Japan, [3]High Energy Accelerator Research Organization, Ibaraki, Japan, [4]NHK Science and Technology Research Laboratories, Tokyo, Japan, [5]Hamamatsu Photonics K.K., Hamamatsu, Japan, [6]Tokyo Denki University, Tokyo, Japan

P-101

Effects of Closed vs. Open Repeated Endotracheal Suctioning During Mechanical Ventilation on the Pulmonary and Circulatory Levels of Endothelin-1 in a Lavage Induced Surfactant Depleted Rabbit ARDS Model

Hideaki Sakuramoto, Subrina Jesmin, Nobutake Shimojo, Junko Kamiyama, Majedul Islam, Tanzila Khatun, Satoru Kawano, Taro Mizutani
Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

P-102

Localized Effect of Vascular Aging on NADPH Oxidase-Mediated Contractions to Endothelin

Matthias R. Meyer[1,2], Matthias Barton[3], Eric R. Prossnitz[1]
[1]Department of Cell Biology and Physiology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA, [2]Division of Cardiology, Department of Internal Medicine, Triemli Hospital, Zurich, Switzerland, [3]Molecular Internal Medicine, University of Zurich, Zurich, Switzerland

P-103

Selective Endothelin (ET)-A Receptor Antagonist and Dual ET-A/B Receptor Antagonist are Effective in Preventing the Decrease in VEGF Signaling and Inadequate Coronary Collateral Development in the Diabetic Hearts

Yumi Miyauchi[1], Subrina Jesmin[1,3], Nobutake Shimojo[3], Seiji Maeda[1], Satoshi Sakai[2], Tomoko Yokota[1], Sohel Zaedi[1,3], Taro Mizutani[3], Satoshi Homma[2], Kazutaka Aonuma[2], Takashi Miyauchi[1,2]
[1]Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Tsukuba, Japan, [2]Department of Cardiovascular Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan, [3]Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

P-104

Effect of Sitaxentan on Plasma Biomarkers of Proendothelin-1 Synthesis in Patients with Chronic Kidney Disease

Jale Yuzugulen[1], Robert Kimmitt[2], Neeraj Dhaun[2], Elizabeth G. Wood[1], Jane G. Goddard[2], David J. Webb[2], Roger Corder[1]
[1]William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, UK, [2]BHF Centre of Research Excellence, University of Edinburgh, The Queens Medical Research Institute, Edinburgh, UK

P-105 - P-109, PC-20
Moderator: **John Pernow** 122 (1F)
Karolinska Institute, Stockholm, Sweden

P-105

Aging Selectively Impairs Contractions to Endothelin-1 But Not to Angiotensin II in Murine Carotid Arteries

Matthias R. Meyer[1,2], Matthias Barton[3], Eric R. Prossnitz[1]
[1]Department of Cell Biology and Physiology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA, [2]Division of Cardiology, Department of Internal Medicine, Triemli Hospital, Zurich, Switzerland, [3]Molecular Internal Medicine, University of Zurich, Zurich, Switzerland

PC-20

Inflammatory State Before Catheter Ablation is Associated with Recurrence of Atrial Fibrillation in Patients with Persistent Atrial Fibrillation after Catheter Ablation

Yoko Ito, Akihiko Nogami, Miyako Igarashi, Hiro Yamasaki, Kenji Kuroki, Takeshi Machino, Kojiro Ogawa, Yuko Miki, Nobuyuki Murakoshi, Yukio Sekiguchi, Takashi Miyauchi, Kazutaka Aonuma
Cardiovascular Division, Institute of Clinical Medicine, Graduate School of Comprehensive Human Science,Tsukuba University, Japan

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Pericardial Resistance Artery Contractile Responses to Endothelins

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Regional Differences in the Effect of Hypoxia on Endothelin-1-Induced Contraction in Rat Arteries

Masashi Tawa, Takashi Shimosato, Hirotaka Iwasaki, Takeshi Imamura, Tomio Okamura
Department of Pharmacology, Shiga University of Medical Science, Shiga, Japan

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Urinary ET-1 Excretion after Exposure to Radio-Contrast Media in Diabetic Patient and Patients with Preexisting Impaired Renal Function

Fabian Heunisch[1], Gina-Franziska von Einem[1], Markus Alter[1], Axel Kretschmer[2], Berthold Hocher[3]
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A Predictor to Indicate the Necessity of Measurement of Endothelin and to Give the Calcium Antagonist Medicine in Outpatients

Minako Seki
The Completion of Doctor Course, International University of Health and Welfare Graduate School, Japan

P-110 - P-114, PC-21

Moderator: **Theofilos Kolettis** 122 (1F)
University of Ioannina, Ioannina, Greece

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Assessment of Circulatory Endothelin-1 Level Among Pre- and Post-Menopausal Rural Women in Bangladesh: Result from a Population-Based Study

Most. Tanzila Khatun[1], Subrina Jesmin[1,2,3], Arifur Rahman[2,4], AKM Ahsan Habib[2,4], Majedul Islam[1,2], Shamima Akter[1,2,3], Nobutake Shimojo[1], Sosuke Kimura[3], Osamu Okazaki[3], Masao Moroi[3], Naoto Yamaguchi[2], Hidechika Akashi[3], Farzana Sohael[2,4], Sayeeda Nusrat Sultana[2], Sohel Zaedi[2,3], Saturo Kawano[1], Taro Mizutani[1], Takashi Miyauchi[1]
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Is Excessive Blood Pressure Elevation During Resistance Exercise a Risk Factor for Arterial Stiffening?

Takeshi Otsuki[1], Takahiro Kotato[2]
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A Study of Endothelins and Endothelin Receptors in Rheumatic Mitral Valves

Tania M. A. Rodrigues[1], Sydney C. Leao[1], Maria R.M. Lima[2], Dario G.M. Neto[2], Rosilene C. Soares[2], Ricardo Fakhouri[3], Anderson C. Marcal[1], Michael R. Dashwood[4]
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Complement C5a Antagonism is Associated with Reduced Big-Endothelin Level after Experimental Cardiac Tamponade

Daniel Erces[1], Gabriella Varga[1], Miklos Nogrady[1], Ildiko Laszlo[1], Andras T Meszaros[1], Jozsef Kaszaki[1], Noriko Okada[2], Hidechika Okada[2], Mihaly Boros[1]
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Endothelin A Receptor Blockade and Long Term Outcome in Patients with ST Elevation Acute Coronary Syndrome

Raphael Wurm, Christopher Adlbrecht, Michael Humenberger, Bassam Redwan, Martin Andreas, Klaus Distelmaier, Irene M. Lang
Medical University of Vienna, Austria

PC-21

Clinical Features between Heart Failure and Sleep Disordered Breathing

Yoko Yamada, Hiroshi Wada, Kenichi Sakakura, Naoko Ikeda, Yoshitaka Sugawara, Junya Ako, Shin-ichi Momomura
Division of Cardiovascular Medicine, Saitama Medical Center, Jichi Medical University, Japan

P-115 - P-119 *Moderator:* **Michael Dashwood** 122 (1F)
University College London, London, UK

P-115

Selective Deletion of Endothelin B Receptors from Vascular Smooth Muscle Does Not Inhibit Neointimal Lesion Formation

Patrick WF Hadoke[1], Eileen Miller[1], Karolina Duthie[1], Rhoda E Kuc[2], Anthony P Davenport[2], Elise E Fransen van de Putte[1], Sibylle Christen[1], David J Webb[1]
[1]Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK, [2]University of Cambridge, Cambridge, UK

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Neointimal Lesion Formation Does Not Induce Endothelin (ET) B-Mediated Contraction in Murine Femoral Arteries

Patrick WF Hadoke, Eileen Miller, Karolina Duthie, Raphael Castellan, Matteo Azzolini, Elise E Fransen van de Putte, Sibylle Christen, David J Webb
Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK

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Smooth Muscle Specific Disruption of the Endothelin A Receptor in Mice Reduces Arterial Pressure and Affects Vascular Development

Donald Kohan, Lisa Lesniewski, Anthony Donato, Lise Sorensen, Dean Li, Alfred van Hoek, Deborah Stuart
Department of Medicine, University of Utah Health Sciences Center, USA

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Plasma Endothelin-1 Level is a Predictor of 10-Year Mortality in a General Population the Tanushimaru Study

Kanako Yokoi[1], Hisashi Adachi[1,2], Yuji Hirai[1], Mika Enomoto[1], Ako Fukami[1], Akiko Tanaka-Kasahara[1], Sachiko Nakamura[1], Yume Nohara[1], Tsutomu Imaizumi[1]
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Potential Association between Circulatory Level of Endothelin-1 and Metabolic Syndrome in Bangladeshi Rural Women: A Population-Based Cross-Sectional Study

Majedul Islam[1,2,3], Subrina Jesmin[1,2,3], Arifur Rahman[2,4], AKM Ahsan Habib[2,4], Shamima Akter[1,2,3], Nobutake Shimojo[1], Sohel Zaedi[2,3], Naoto Yamaguchi[2,3], Sayeeda Nusrat Sultana[2,3], Satoru Kawano[1], Hidechika Akashi[3], Takashi Miyauchi[1], Taro Mizutani[1]

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Moderator: **Carmine Cardillo** Lobby (B1)
Catholic University of Rome, Rome, Italy

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Dual Endothelin Antagonism from Early Diabetic Stage is Effective in Preventing Various Diabetic Complications Through Both Improving Organ Microcirculation and Restoration of Altered VEGF Signaling

Subrina Jesmin, Nobutake Shimojo, Sayeeda Nusrat Sultana, Majedul Islam, Sohel Zaedi, Satoru Kawano, Taro Mizutani, Takashi Miyauchi
Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

PC-22

Lifestyle Modification Induces Decreased Central Blood Pressure and Increased Serum Testosterone Concentration in Overweight and Obese Men

Hiroshi Kumagai[1], Asako Miyaki[2], Rina So[2], Takehiko Tsujimoto[2], Takashi Miyauchi[3], Kiyoji Tanaka[2], Seiji Maeda[2]
[1]Guraduate School of Comprehensive Human Sciences, University of Tsukuba, Japan, [2]Faculty of Health and Sport Sciences, University of Tsukuba, Japan, [3]Faculty of Medicine, University of Tsukuba, Japan

PC-23

Characterization of the Binding of [^{125}I]GLP-1(9-36) Amide, the Major Metabolite of the Insulin Secretagogue, Glucagon-like Peptide 1 (GLP-1) and Function of the Unlabelled Peptide in Murine Aorta

Rhoda E Kuc[1], Janet J Maguire[1], Keith Siew[1], Sheena Patel[2], Margaret Jackson[2], Anthony P Davenport[1]
[1]Clinical Pharmacology Unit, [2]Pfizer, Cardiovascular Medicine, Cambridge, MA, U.S.A.

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Dual Endothelin Receptor Antagonism with Bosentan Reverses Established Vascular Remodeling in Diabetic Rats: Relevance to Glycemic Control

Adviye Ergul[1,2,3], Mohammed Abdelsaid[1,2,3], Maha Coucha[3], Handong Ma[1,3]
[1]Charlie Norwood VA Medical Center, Augusta, Georgia, USA, [2]Program in Clinical and Experimental Therapeutics, University of Georgia, College of Pharmacy, Augusta, Georgia, USA, [3]Department of Physiology, Georgia Regents University, Augusta, Georgia, USA

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Bosentan Restores Impaired Cerebrovascular Relaxation in Diabetes

Adviye Ergul[1,2,3], Mohammed Abdelsaid[1,2,3], Handong Ha[1,3], Maha Coucha[3]
[1]Charlie Norwood VA Medical Center, Augusta, Georgia, USA, [2]Program in Clinical and Experimental Therapeutics, University of Georgia, College of Pharmacy, Augusta, Georgia, USA, [3]Department of Physiology, Georgia Regents University, Augusta, Georgia, USA

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ETA Receptor Antagonists in the Treatment of Diabetic Ketoacidosis

Anil Gulati[1], Manish_S Lavhale[2], Birinder_S Marwah[2], Suresh Havalad[3]
[1]Midwestern University, Downers Grove, Illinois, USA, [2]Pharmazz Research Center, Pharmazz India Private Limited, Greater Noida, UP, India, [3]Advocate Lutheran General Children's Hospital, Park Ridge, Illinois, USA

P-124 - P-128, PC-24 - PC-25

Moderator: **Adviye Ergul** Lobby (B1)
Georgia Regents University, Augusta, USA

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Endothelin Antagonism and Diabetic Erectile Dysfunction: Changes in VEGF and NO in Type I Diabetic Penis and Effects of Endothelin Antagonism

Subrina Jesmin[1,3], Sohel Zaedi[1,3], Nobutake Shimojo[1], Satoshi Sakai[2], Seiji Maeda[3], Yumi Miyauchi[3], Tomoko Yokota[3], Taro Mizutani[1], Satoshi Homma[2], Kazutaka Aonuma[2], Takashi Miyauchi[2,3]
[1]Department of Emergency and Critical Care Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan, [2]Department of Cardiovascular Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan, [3]Center for Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Tsukuba, Japan

PC-24

An Endogenous Blocker of Oxidized LDL

Akemi Kakino, Atsushi Nakano, Yoshiko Fujita, Tatsuya Sawamura
Department of Vascular Physiology, National Cerebral and Cardiovascular Center, Japan

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Selective Endothelin ETA and Dual ETA/ETB Receptor Blockade Improves Endothelial Function in Patients with Type 2 Diabetes and Coronary Artery Disease

John Pernow, Arnar Rafnsson, Alexey Shemyakin
Institution of Medicine, Karolinska Institute, Stockholm, Sweden

PC-25

2 Years Follow-Up in Oxidative Stress Levels in Patients with Acute Coronary Syndrome: Insights from the Assessment of Lipophilic vs. Hydrophilic Statin Therapy in Acute Myocardial Infarction (ALPS-AMI) Study

Yuichiro Kashima, Atsushi Izawa, Yusuke Miyashita, Jun Koyama, Uichi Ikeda
Department of Cardiovascular Medicine, Shinshu University School of Medicine, Japan

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Endothelin-Dependent Vasoconstrictor Activity in Metabolically Healthy and Unhealthy Obese Patients

Carmine Cardillo[1], Francesca Schinzari[1], Angelo Adamo[1], Valentina Rovella[2], Manfredi Tesauro[2]
[1]Department of Internal Medicine, Catholic University Medical School, Rome, Italy, [2]Department of Iternal Medicine, Tor Vergata University, Rome, Italy

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Epigallocatechin Gallate Attenuates ET-1-Induced Contraction in Carotid and Thoracic Aorta from Type 2 Diabetic OLETF Rat

Takayuki Matsumoto, Shun Watanabe, Ryusuke Kawamura, Tsuneo Kobayashi
Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Tokyo, Japan

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Association between Endothelin-A Receptor Gene Polymorphisms in Locus rs10305936 and Primary Nephrotic Syndrome in Children

Fang Yang[1], Shuixiu Zeng[1], Cheng Zhang[2], Xiaoxiao Liu[1], Liangzhong Sun[3]
[1]Department of Pediatrics, First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, China, [2]Department of Pediatrics, Zhuhai Hospital of Jinan University, Guangzhou, Guangdong, China, [3]Department of Pediatrics, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China

14:45-15:45 **Room 1**

Session 12: Metabolism, Diabetes and Obesity

Chairs: **Adviye Ergul** *Georgia Regents University, Augusta, USA*
Subrata Chakrabarti *University of Western Ontario, London, ON, Canada*

Keynote Lecture 6 14:45-15:05

Role of Endothelin in Vascular Dysfunction in Human Obesity and Diabetes

Carmine Cardillo
Department of Internal Medicine, Catholic University Medical School, Rome, Italy

O-26 15:05-15:17

Molecular Mechanism for Suppression of Insulin Signaling by Endothelin-1 in Skeletal Muscle Cells

Takahiro Horinouchi, Takuya Harada, Tsunaki Higa, Tsunehito Higashi, Koji Terada, Akimasa Hoshi, Yosuke Mai, Mika Horiguchi, Prabha Nepal, Chizuru Hatate, Soichi Miwa
Department of Cellular Pharmacology, Hokkaido University Graduate School of Medicine, Hokkaido, Japan

O-27 15:17-15:29

Plasma Endothelin-1 Level is Associated with Cardiac Fibrosis and Diastolic Dysfunction in Diabetes

Bambang Widyantoro, Nani Hersunarti, Anna U. Rahajoe, Ganesja M. Harimurti
Department of Cardiology and Vascular Medicine, Universitas Indonesia - National Cardiovascular Center Harapan Kita, Indonesia

O-28 15:29-15:41

Activation of ET-1-Mediated PKC-Epsilon/ERK1/2 Pathway Contributes to the Augmented Contractile Response in Aorta from Young Obese Rats

Fernando P. Filgueira[1], Nubia S. Lobato[2], Victor V. Lima[1], Zuleica B. Fortes[3], Maria Helena C. Carvalho[3], R. Clinton Webb[4], Rita C. Tostes[1]
[1]Department of Pharmacology, School of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil, [2]Department of Biological Sciences, Federal University of Goias, Jatai, Brazil, [3]Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil, [4]Department of Physiology, Georgia Regents University, Augusta, United States of America

15:45-16:15 **Coffee Break**

16:15-16:30 **Room 1**

Tomoh Masaki Award
Best Presentation and Best Poster Awards Ceremony

16:30-17:00 **Room 1**

Session 13: Hightlights of ET-13

Masashi Yanagisawa
Southwestern Medical Center Dallas, USA / University of Tsukuba, Tsukuba, Japan

17:00-17:15 **Room 1**

Closing Remarks

DAY 1

Session 1: New Aspects of Endothelin in Physiology and Disease

Invited Lecture 1

Interaction and Sexual Dimorphism of ETB/NOS Signaling in Cardiovascular and Renal Disease

Jennifer S. Pollock, Kelly A. Hyndman
Experimental Medicine, Georgia Regents University, Augusta, USA

The renal collecting duct is critical in the fine-tuning mechanism of sodium homeostasis. ET, ETB receptors, and nitric oxide synthase (NOS) are all highly expressed in the collecting duct. Our laboratory is interested in the ET/ETB/NOS signaling mechanism regulating sodium homeostasis and blood pressure. Utilizing collecting duct-specific knockout mice, we found that collecting duct ET/ETB activation leads to the stimulation of collecting duct NOS1 pathway to mediate inhibition of sodium reabsorption via epithelial sodium channel activity. While vascular endothelial cell ET knockout mice do not activate collecting duct NOS1. Moreover, the collecting duct NOS1 pathway does not appear to regulate the ET/ETB pathway. The alternative splice variant, NOS1beta, is exclusively expressed in the collecting duct of mice. Thus far, our experiments have not detected a sexual dimorphism in the ET/ETB/NOS pathway in the transgenic mice. However, in rats distinct male and female regulatory pathways of ET/ETB signaling mediated sodium homeostasis are detected. ETA and ETB mediated natriuresis is present in female rats, while only ETB mediates natriuresis in male rats. NOS1 specific activity is significantly higher in female rats compared to male rats. However, NOS1 activity in male rats is ETB dependent while NOS1 activity in female rats is independent of the ET/ETB pathway. Collecting ducts in rats express two NOS1 variants, full-length NOS1alpha and NOS1beta. This difference in NOS1 activity and variant expression may mediate the sexual dimorphism observed in the ET/ETB mediated natriuresis in rats but not seen in mice. In conclusion, the ET/ETB/NOS1 signaling pathway is critical to maintain fluid and electrolyte homeostasis in both males and females.

Invited Lecture 2

Endothelin is Getting Older: How Aging Links Endothelin with Disease

Matthias Barton
University of Zurich, Switerland

Aging is a physiolocical process reflecting the accumulation of changes responsible for the sequential alterations that accompany advancing age and the associated progressive inreases in the chance of disease and death (*PNAS* 1991; 88; 5360). Accordingly, the *American Heart Association* has identified age above 45 years in men and above 55 years in women as an independent risk factor for cardiovascular disease. Within the last century life expectancy in man has doubled to more than 80 years. Increased longevity will result in an overall shift of the world population in the decades to come (*Cardiovasc Res* 2005; 66: 187) which will pose additional challenges to healthcare providers and societies.

Endothelin-1 (ET-1), a cytokine-like pro-inflammatory mitogen with vascular activity and predominant member of the endothelin peptide family, was discovered 25 years ago at the University of Tsukuba in Japan (*Nature* 1988: 332: 411). ET-1 not only has physiological functions such as embryonic development, nociception and natriuresis, but also contributes to disease progression - mainly via ET_A receptor activation. The prevalence of vascular and renal disease in humans show a clear age-dependency (*Nephrol Dial Transplant* 2005; 20: 485), and GFR in humans decreases by 1% per year after age 45.

Research of the past decade has provided new insights into molecular mechanism underlying age-dependent changes in cardiovascular and renal physiology (*Pflugers Arch* 2010; 460: 825). It is now well established that cellular senescence is associated with an overall activation and production of pro-inflammatory cytokines and growth factors (including ET-1), which propagate the development of (patho)-physiological processes such as vascular hypertrophy, cardiomyocyte injuy, renal cell injury, and sarkopenia, among others. New research indicates that aging-associated cellular changes are not inexorable, but that aging - similar to arterial hypertension and obesity - can be considered a *modifiable* risk factor (*JCI* 2013; 123; 906), in which molecular mediators such peptides with cellular and vascular activity may also be involved.

Indeed, preclinical studies targeting either cellular actvitiy or production of ET-1 - using ERAs or ARBs, respectively - have demonstrated that aging-associated changes in vasculature and kidney can indeed be reversed in part (*Hypertension* 2004; 44: 974; *Nephrol Dial Transplant* 2006; 20: 485), suggesting that the endothelin system plays an important role in aging physiology and cellular senescence. The clinical implications of these finding and interventions to promote healthy aging starting in youth will be discussed.

Supported by the SNSF

O-1

Development of Osteoarthritis-Like Changes in Transgenic Endothelin-1 Over-Expressed Mice

Chunyi Wen[1], LimCho Steven Pei[1], Baretella Oliver[2], Sookja Kim Chung[3], Aimin Xu[2], ChunHoi Yan[1], KowngYuen Chiu[1], Weijia William Lu[1]

[1]Department of Orthopaedics & Traumatology, University of Hong Kong, Hong Kong, [2]Department of Medicine, University of Hong Kong, [3]Department of Anatomy, University of Hong Kong

Endothelin-1 (ET-1), known as a potent vasoconstrictor, has been implicated in pathogenesis of osteoarthritis (OA). ET-1 could induce matrix metalloproteinase 13 (MMP13) expressions by chondrocytes in vitro. Use of Endothelin 1 receptor A antagonist, might rescue OA in mice. Yet the direct evidence remains lacking whether overexpressed ET-1 will lead to development of OA. Therefore, we aim to characterize the phenotypes of the articular cartilage and subchondral bone in a transgenic endothelial cell-specific (Tie-1 promoter) overexpressed endothelin-1 (TET-1) mice.Male heterozygous TET-1 mice were generated by microinjection of the ET-1 construct, which contained the mouse ET-1 cDNA with SV40 polyA driven by the Tie-1 promoter. TET-1 mice developed systemic hypertension with altered vascular reactivity since 8 weeks after birth. Tibiae of male, heterozygous TET-1 mice (n=4) and their non-transgenic littermates (n=4) of 35-weeks-old were obtained. PCR were used to confirm their genotypes. Micro-CT scan on tibiae were performed before tissue processed to wax blocks. Tibiae in wax blocks were sectioned and histology was studied on 5μm-thick wax sections. Micro-CT data showed a decrease of bone volume/tissue volume (BV/TV, 10.9±0.4%) in TET-1 mice primary spongiosa when compared to the age- and gender-matched littermates (12.8±0.5%, $p<0.05$). In contrast, there was no significant difference in the density of subchondral trabecular bone (secondary spongiosa) between TET-1 mice (23.5±3.6%) and their littermates (25.7±1.4%, $p>0.05$).It was revealed histologically that articular chondrocytes underwent hypertrophic changes together with thickening of calcified cartilage in TET-1 mice as compared to their littermates.In summary, TET-1 mice present OA-like changes and overexpression of ET-1 contributes to hypertrophic changes of articular chondrocytes.

O-2

Characterisation of the "Endothelin-Like Domain Peptide" (ELDP) Co-synthesised with Endothelin-1 from the *EDN1* Gene

Jale Yuzugulen[1], Elizabeth G. Wood[1], Inmaculada C. Villar[1], Julie A. Douthwaite[1], Nimesh S. A. Patel[1], James Jegard[1], Alexander Montoya[2], Pedro Cutillas[2], Hubert Gaertner[3], Irene Rossitto-Borlat[3], Keith Rose[3], Oliver Hartley[3], Amrita Ahluwalia[1], Roger Corder[1]

[1]William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, UK, [2]Barts Cancer Institute, Barts and the London School of Medicine, Queen Mary University of London, UK, [3]Faculty of Medicine, University of Geneva, Switzerland

Background: Endothelin-1 (ET-1) is strongly implicated in cardiac and renal pathologies both as a potent vasoconstrictor and as an inducer of tissue remodelling and fibrosis. However, ET antagonists demonstrated limited efficacy in clinical trials of heart failure and hypertension. Thus, alternative preproendothelin-1 (ppET-1) derived peptides may contribute/interact with vasoconstrictor responses. Our aim was to characterise ppET-1 biosynthesis and to evaluate potential interactions with ET-1 responses. **Methods and Results:** A combination of specific immunoassays and HPLC were used to characterise ppET-1 processing in conditioned media samples from human endothelial (EA.hy 926) and epithelial (A549) cells. Endothelin-like Domain Peptide (ELDP, ppET-1[93-166]) was identified by immunoassay with LTQ-Orbitrap mass spectrometer confirmation. Specific ELISA for ELDP showed its release from primary cultures of human aortic endothelial cells, which correlated with ET-1.Blood pressure responses were investigated in anaesthetised rats. Although synthetic ELDP alone (3 nmol/kg) had no effect, it significantly increased the duration of the pressor response to ET-1 (0.3 nmol/kg, $p<0.02$). On rat mesenteric resistance arteries (rMRA), ELDP alone had no significant vasoconstrictor effect up to 10 nM, but after pre-treatment with ET-1 (1-3 nM) produced a concentration-dependent vasoconstriction. Pre-incubation of rMRA with 10 nM ELDP increased the response of 1 nM ET-1 by ~5 fold ($p<0.002$). Plasma levels of ELDP, measured by sandwich immunoassay, showed a significant difference between untreated subjects with pre-hypertension/mild hypertension (n=24) compared to patients with chronic heart failure (n=24) (6.45 ± 0.19 and 7.80 ± 0.25 fmol/ml; $p<0.001$, respectively). **Conclusions:** ELDP is co-secreted with ET-1 and modulates its vasoconstrictor responses. ELDP may be a useful biomarker for EDN1-linked pathologies.

Session 2: Oncology

Keynote Lecture 1

The Endothelin Axis: A New Player in Tumor Angiogenesis and Lymphangiogenesis

Francesca Spinella
Regina Elena National Cancer Institute, Rome, Italy

Pathological angiogenesis and lymphangiogenesis are a hallmark of cancer and both serve as the major routes for cancer cell dissemination and metastasis. The tumor-vasculogenic process is the result of the interaction between endothelial and tumor cells and requires the coordinated actions of growth factors with both angiogenic and lymphangiogenic properties. Unrevealing the potential mediators able to modulate this complex process would provide the basis for the development of molecularly targeted therapeutics directed against both tumor and tumor-associated endothelial cells. The multifunctional peptide endothelin-1 (ET-1) and its receptors have been correlated with invasiveness and metastasis and have been shown to be markedly increased in the vasculature of several kind of tumors and associated with tumor grade and poor prognosis. ET-1 acts in both blood and lymphatic endothelial as well as in tumor cells through its G-protein coupled receptors, ETA and ETB, to promote angiogenesis and lymphangiogenesis. The mechanism by which ET-1 promotes these processes are beginning gradually defined. During tumor progression ET-1 exerts crucial roles in the vasculogenic switch promoting, via ET_BR, endothelial cell proliferation, migration, protease production and morphogenesis, and, via both receptors, vascular endothelial growth factor (VEGF) release. Moreover, ET-1 stimulates highly aggressive tumor cells to form vessel-like networks that does not involve endothelial cells. The ET-1-induced vasculogenic effects, progress through the induction of the transcriptional hypoxia-inducible factor (HIF)-1α and HIF-2α, and through a complex interplay with VEGF family members. Furthermore, expression of ET-1 and its receptors is controlled by VEGF and hypoxia in both endothelial and tumor cells, suggesting that a positive interegulation between ET-1/HIF-1α, VEGF and hypoxia is able to amplify the neovasculogenic response. The better mechanistic understanding of these complex interactions is gradually paving the way toward the rationale exploitation of the ET-1/ET receptor signaling pathway as a therapeutically attractive target for neoplastic disease characterized by active neovascularisation.

O-3

Endothelin-1 Induced Mxi-2/Ago2 Complex Formation Resulting in P53 Downregulation Promoting Breast Cancer Development

Melanie von Brandenstein[1,2], Julia Straube[2], Heike Loeser[1], Luca Ozretic[1], Jochen W.U. Fries[1]
[1]*Institute of Pathology, University of Cologne, Cologne, Germany,* [2]*University of Applied Sciences Bonn-Rhein-Sieg, Department of Natural Sciences, Rheinbach, Germany*

Increased Endothelin-1 decreases PKC alpha (PKCα), resulting in high miRNA 15a levels in kidney tumors. Breast cancer cells treated with ET-1, β-estrogen, Tamoxifen, Tamoxifen+β-estrogen and Tamoxifen+ET-1 were analysed regarding miRNA 15a expression. Significantly increased miRNA 15a levels were found after ET-1, becoming further increased in Tamoxifen+ET-1 treated cells. Our group already showed that miRNA 15a induces MAPK p38 splicing resulting in a truncated product called Mxi-2, whose function has yet to be defined in tumors. We described for the first time in ET-1 induced tumor cells that Mxi-2 builds a complex with Ago2, a miRNA binding protein, which is important for the localisation of miRNAs to the 3'UTR of target genes. Furthermore, we show that Mxi-2/Ago2 is important for the interaction with the miRNA 1285 which binds to the 3'end of the tumor suppressor gene p53, being responsible for the downregulation of p53. Tissue arrays from breast cancer patients were performed, analysing Mxi-2, p53 and PKCα. Since the Mxi-2 levels increase in Tamoxifen+ET-1 treated cells, we claim that increasing ET-1 levels in Tamoxifen treated breast cancer patients are responsible for decreasing p53 levels.In summary, ET-1 decreases nuclear PKCα levels, while increasing the amount of miRNA 15a. This causes high levels of Mxi-2, necessary for complex formation with Ago2. The newly identified Mxi-2/Ago2 complex interacting with miRNA 1285 leads to increased 3'UTR p53 interaction, resulting in decreased p53 levels and subsequent tumor progression. This newly identified mechanism is a possible explanation for the development of ET-1 induced tumors.

O-4

Tamoxifen Treatment in Breast Cancer Induces a Cytoplasmic Complex Consisting of Endothelin-1, Estrogen Receptors, and Tamoxifen Leading to Nuclear Transmigration, and Transcription of Target Genes Involved in Metastatic Spread

Julia Straube[1,2], Melanie von Brandenstein[2], Christina Geisbuesch[3], Luca Ozretic[2], Reinhard Depping[4], Jochen W. U. Fries[2]
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Tamoxifen therapy of invasive breast cancer has been associated with increased levels of endothelin-1 (ET-1) so that an endothelin-1 receptor (ETR) blockade has been suggested as new therapeutic approach. This study analysed the relationship between tamoxifen and ET-1 signalling in invasive breast cancer.Using paraffinized tissue from 50 randomly chosen cases of invasive and in-situ ductal carcinoma from our archive, the expression of ETRs was analysed by immune histology. ETRs were regularly detectable in normal breast tissue, but rarely in adjacent tumor areas (3/50 cases). By immunoprecipitation, a complex was found consisting of ET-1, estrogen receptors and Tamoxifen. Consequently, transcription of several target genes of ET-1 and estrogen receptors were detectable (interleukin-6, wnt-11 and a vimentin spliceform). In particular, the combination of tamoxifen, ET-1, and estrogen receptors induced further increasing levels of these target genes. Some of these genes have been found upregulated in metastastically spreading breast cancer cells. We conclude: i) ETRs do not play a role in invasive or in-situ ductal breast cancer; ii) estrogen receptors and Tamoxifen build a complex with ET-1; iii) upregulated transcription of target genes by ET-1-estrogen receptor-Tamoxifen complex may negatively influence breast cancer prognosis. These results indicate a role for ET-1 in Tamoxifen treated breast cancer patients leading to a potentially worsening prognosis.

Session 3: Renal Diseases

Keynote Lecture 2

The Role of Endothelin in Glomerular Diseases: Cellular Culprits, Cellular Targets

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The lecture will review experimental and clinical evidence for an involvement of the endothelin system in promoting glomerular dysfunction and damage. We will propose a network of pathophysiological interactions involving paracrine effects of ET-1 in the distinct glomerular cells. At last, the lecture will tease out specific pathophysiological contexts where clinical development are expected to bring fruits and would meet therapeutic needs.

We will focus on experimental evidence for a regulatory role of ET-1 in podocyte function and hypothesize non-exclusive pathophysiological involvement of the ET-1 system in different glomerular diseases with podocyte injury. First, ET-1 produced by injured glomerular or preglomerular endothelium could be freely filtered through the filtration barrier and act on podocyte ETRs. This is expected to occur in severe hypertension, hemolytic uremic syndrome (HUS) and thrombotic microangiopathy (TMA), sickle cell disease, diabetic nephropathy (DN), connective tissue diseases and vasculitis. We will briefly review evidences for increased ET-1 activity in the vasculature in IgA nephropathy (IgAN) where vascular lesions are frequent and of prognostic value. Second, ET-1 may be produced *de novo* by mesangial cells. We will review the current evidence for ET-1 production by mesangial cells, in particular in DN and IgAN. Third, scarce evidence suggest that podocytes and glomerular parietal epithelial cells produce ET-1 that affects podocyte phenotype in a paracrine and autocrine fashion in vitro. In this regard we will examine the current evidences in focal and segmental glomerulosclerosis (FSGS) and crescentic glomerulonephritis. This review and further studies may assist clinicians in optimally designing clinical trials for patients at increased risk for CKD.

O-5

Heterozygous Overexpression of Preproendothelin-1 in Endothelial Cells Enhances Thromboxane-Prostanoid Receptor-Induced Contractions in the Renal Artery of Obese Mice

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Circulating levels of the endothelium-derived peptide endothelin-1 (ET-1) are elevated in human obesity, and ET-1 mediated vascular tone is increased. The renal artery is important in controlling intrarenal blood flow and is highly sensitive to ET-1. Whether or not ET-1 affects renal artery tone in obesity is unknown. To investigate the role of endogenous ET-1, a mouse model with *tie-1* promoter-driven endothelium-restricted heterozygous overexpression of preproendothelin-1 was used (TET+/-). Obesity was induced in TET+/- and WT littermates by feeding a high fat diet for seven months; lean controls were kept on standard chow. The main renal arteries were studied in wire myographs testing contractions (in the presence of L-NAME) to ET-1, serotonin (5-HT), and U46619, targeting ET_A, 5-HT_2, and TP receptors, respectively. Contractions to ET-1 were comparable between groups (PD_2 8.29±0.05, n=6-8); 5-HT-induced responses were facilitated at lower concentrations in obese mice leading to a shift in PD_2 (lean 7.08±0.02 vs. obese 7.23±0.07, n=5-8, P<0.01). Responses to U46619 were significantly shifted to the left in renal arteries of obese animals (PD_2 8.57±0.06 vs. lean 8.21±0.05, n=5-8, P<0.001), and the area under the curve was significantly different between lean and obese TET+/- mice (AUC 418±23 vs. lean 319±25, n=5, P<0.05). Thus, TET+/- had no effect on responses in lean animals. By contrast, in obesity heterozygous overexpression of ppET-1 enhanced TXA_2-mediated, but not 5-HT or ET-1 induced contractions of the renal artery.

O-6

The Effect of Proteinuria-Mediated Endothelin-1 Downregulation of PKCα Signalling in Proximal Tubular Cells and Its Successul Treatment is Measurable Using microRNA15a as Biomarker in Vitro and in Vivo

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In proteinuric diseases, stimulation of proximal tubule cells (RPTECs) by protein and endothelin-1 result in the activation of different signal pathways, ultimately causing renal insufficiency. Therapeutic interventions are hampered by the lack of specific and easily detectable markers. We described a regulatory pathway in which nuclear migration of protein kinase C α controls the release of pri-miRNA15a. After endothelin-1 stimulation the migration of PKCα is inhibited, and mature miRNA15a is made. Using qRT-PCR we detect miRNA15a in the urine of adult and pediatric patients with membranous or mimimal change nephropathy. By laser-microdissection this miRNA is predominantly located in the proximal tubules. In cell culture, human RPTECs produce the highest miRNA15a levels after ET-1 stimulation. In rats after 5/6 nephrectomy, miRNA15a is increased in the urine. By graded sieving and qRT-PCR, the highest amount of miRNA15a is found in the tubular fraction. Selegiline treatment upregulates PKCα in vitro and in the murine adriamycin model, significantly downregulating ET-1 induced miRNA15a production. Thus measuring urinary miRNA15a levels: i) indicates the regulation of a signal pathway in RPTECs in vivo in proteinuric conditions; ii) allows for the first time to control the effectiveness of a therapy aiming to protect proximal tubules.

O-7

ET-B Receptors in Podocytes Promote Diabetic Glomerulosclerosis with β-Catenin and NFκB Activation

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Recently the endothelin (ET) system has emerged as a novel target for the treatment of diabetic nephropathy (DN) with anti-proteinuric actions. However, no evidence for a direct in vivo pathogenic effect of ET-1 on podocytes has been shown. Despite podocyte dysfunction in DN, specific ET-1 signaling in podocytes has not been investigated. This study investigated ET signaling in podocytes during experimental DN. We first demonstrated that the prominent functional ET-1 receptors eliciting rapid calcium transients in podocytes are ETBRs. Mice with a podocyte-specific double deletion of ETAR and ETBR (Pod-ETRKO) were rendered diabetic by streptozotocin injection. Whereas wild-type diabetic mice developed mild DN with microalbuminuria, mesangial matrix expansion and podocyte loss, Pod-ETRKO mice were protected from diabetes induced glomerulosclerosis and podocyte loss. We next found that total β-catenin and phospho-NFκB expressions are strongly reduced in glomeruli from Pod-ETRKO mice. Moreover, ET-1 could directly activate β-catenin and NFκB signaling pathways in freshly isolated glomeruli. This is the first evidence that ET-1 activation drives development of glomerulosclerosis and podocyte loss through direct activation of ETRs in podocytes, and likely, through NFκB and β-catenin pathways. Surprisingly, both at the expression level and the functional level, the ETBR subtype was found to be prominent. Furthermore, these results indicate that activation of the ET-1 pathways selectively in podocytes is involved in pathophysiological crosstalk that influences mesangial architecture and sclerosis.

O-8

Changes in Urinary ET-1 Excretion in Response to Increased Renal Perfusion Pressure in the Rat

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Urinary ET-1 (UET-1) excretion may result from cellular exposure to shear stress but the relative contributions of renal blood flow and tubular flow are not known. We measured changes in UET-1 in response to changes in renal perfusion pressure (RPP) in the rat in order to identify associations between UET-1, urinary flow rate (UV), urinary sodium excretion rate (UNaV) and RPP. Methods: Seven male Sprague Dawley rats weighing 258±17 g underwent induced pressure natriuresis. Arterial blood pressure was measured directly and urine was collected via tube cystotomy. RPP was initially increased by ligation of both the coeliac and cranial mesenteric arteries and subsequently of the distal aorta. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by FITC-inulin and para-aminohippurate clearance, respectively. UET-1 was measured by a sandwich ELISA assay (Biomedica). Urinary sodium was measured by sodium-selective electrode analysis. Results: GFR and ERPF did not change significantly when RPP increased from 137±9 to 162±11 mmHg but significant increases in UV (4±1 to 97±33 µl/min/g kw) and UNaV (1±1 to 23±9 µmol/min/g kw) had linear relationships with RPP ($r^2 = 0.39$, $p = 0.022$ and $r^2 = 0.51$, $p < 0.001$ respectively). UET-1 increased from 11±7 to 42±26 fg/min/g kw ($p = 0.014$) but was not predicted by RPP. UET-1 did, however, strongly correlate with UV ($r = 0.69$, $p = 0.001$) and UNaV ($r = 0.65$, $p = 0.002$). Conclusions: In the rat, UET-1 is more associated with renal tubular flow than renal blood flow at higher RPPs. Funded by Moray Endowment Fund and British Heart Foundation.

Lunch Session 1

LS1

Recent Progress in the Management of Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a disease characterized by extensive remodeling of the small pulmonary arteries resulting in increased elevated pulmonary arterial pressure and right ventricular heart failure. PAH is fatal if not treated, with only 34% survival rate after 5 years. Pathobiologically, PAH is the result of a combination of many factors. Simplistically, abnormal proliferation of endothelial cells is an important mechanism, which leads to increased arginase activity and down regulation of prostacyclin (PGI_2) synthase in lung tissues, and subsequent reduction in the production of the vasodilators nitric oxide and prostacyclin. Endothelial hyperproliferation also increases the production of endothelin-1, one of the most potent vasoconstrictors. Endothelin-1 overexpression in turns reduces the production of NO and PGI_2. These changes are associated with many other interrelated pathological changes, including vasoconstriction, hyperproliferation of vascular smooth muscle cells and fibroblasts, vascular wall hypertrophy, inflammation, platelet aggregation, and thrombosis, all contributing to the remodeling of the pulmonary vasculature in PAH.
Based on these key pathophysiological features, currently approved therapies for PAH include phosphodiesterase 5 inhibitors (PDE5I), endothelin receptor antagonists (ERA) and PGI_2 derivatives. PDE5Is prevent cyclic GMP breakdown and therefore enhances the vasodilatory effect of NO, ameliorating the impact of reduced NO production. ERAs prevent ET-1 interaction with its receptors, thereby alleviating effects of excessive ET-1 in PAH patients. PGI_2 derivatives increase PGI_2 levels, thereby alleviating the vasoconstriction caused by reduced concentrations of PGI_2 in PAH patients. Generally, PDE5Is and ERAs improve symptoms and survival; however, as the disease progresses, prostacyclin derivatives are required, and there is evidence that disease progression and survival are improved by timely prostacyclin usage. PAH guidelines have proposed combination therapy (ERA and/or PDE5I and/or prostanoid) if the clinical response to monotherapy is not adequate. On the other hand, upfront combination therapy targeting different pathologic processes seems to be a more theoretical option in PAH compared with mono- or sequential-combination therapy. Nevertheless, successful treatment with an upfront combination of multiple drugs may offer the perspective of reverse remodeling, that is, the regression of established pulmonary vascular lesions. The aim of this presentation is to summarize recent advances in the management of PAH with particular focus on the possibility of reverse remodeling.

Lunch Session 2

LS2

Medical Treatment for Type Ⅱ Diabetes Mellitus for Inhibiting Vascular Events

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Treatment of hyperglycemia is an important way to prevent diabetic vascular complications. However, intensive therapy to target glycated hemoglobin levels below 6.0% paradoxically increased all cause mortality and did not significantly reduce major composite cardiovascular events compared to standard therapy. However, it is also true that the same trial confirmed that the incidence of nonfatal myocardial infarction was significantly lower in the intensive glucose lowering group. Moreover, the UKPDS 80 demonstrated that a better control of blood glucose was associated with a continued risk reduction for myocardial infarction and all-cause death during 10 years of post-trial follow-up, a phenomenon called "legacy effect". Considering these clinical findings, it is desirable to use anti-diabetic drugs that can effectively lower the blood glucose levels but hardly induces hypoglycemia in patients with T2DM. One class of such ideal drugs is dipeptidyl peptidase-4 (DPP-4) inhibitors. Recent studies suggested that DPP-4 inhibitors have not only glucose lowering effects but also pleiotropic effects including cardiovascular protection. In this seminar, I would like to discuss these points.

Poster Session 1: Oncology, Renal Physiology and Disease, Neurology, Infectious Diseases, New Topics of Endothelin Biology

P-1

The Role of Endothelin-1 in the Vascular Pathobiology of Cerebral Malaria

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Cerebral malaria (CM) is a serious complication of Plasmodium falciparum infection associated with cerebral vasculopathy, high mortality, and risk of neurological sequelae. In human CM, infected RBCs adhere to the brain endothelium, occlude the cerebral blood vessels causing cerebral vascular damage, impaired perfusion, vasospasms, vasoconstriction, and inflammation. Vasoactive factors, including endothelin (ET-1), have become increasingly important in the pathogenesis of CM. We previously demonstrated that antagonism of the ET-1 type A receptor (ET_A) improved survival and attenuated brain hemorrhage in murine CM. In this study we tested the hypothesis that ET-1 contributes to CNS inflammation and BBB disruption in experimental CM (ECM) via its actions on ET_A. To test this hypothesis we used our model of Plasmodium berghei ANKA (PbA) infection of C57BL/6 mice. PbA-infection resulted in activation of monocytic CNS cells, microglia, important in inflammation. ECM was also associated with an increase in brain microvascular endothelial cell activation, critical for leukocyte adhesion. Treatment of PbA-infected mice with ET_A receptor antagonists attenuated the increase in microglial and endothelial cell activation, suggesting that ET-1 contributes to CNS inflammation during ECM. Furthermore, leakage of Evans blue bound-albumin into the brain was reduced in ECM mice receiving ET_A receptor antagonism, providing further support that disruption of the BBB and inflammation during ECM result, in part, from increases in ET-1 and its actions on the ET_A receptor. Together these findings illustrate a role for ET-1 in the immunopathology and vasculopathy associated with ECM, and highlight the peptide as a potential target for adjunctive therapy for the protection of neurological function in patients with CM.

P-2

Dual Endothelin Blockade Exacerbates Upregulated VEGF Angiogenic Signaling in the Heart of Lipopolysaccharide -Induced Endotoxemic Rat Model

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Sepsis, a heterogeneous class of syndromes, is associated with the development of progressive damage in multiple organs. The pathogenesis of sepsis-induced myocardial dysfunction is still not fully understood. The present study examined the alteration of key angiogenic signaling pathway mediated by vascular endothelial growth factor (VEGF) in sepsis heart and the effects of dual endothelin (ET) antagonism on it. Normal male wistar rats at age 8 wks were administered with lipopolysaccharide (LPS : 15 mg/kg) and then sacrificed at different time points (1h, 3h, 6h and 10h), some rats without LPS administration was considered as control group. Some of the LPS-administered rats were treated with dual endothelin blocker (SB209670, 1mg/kg body weight) for six hours and then sacrificed. Administration of LPS resulted in increases in the serum levels of TNF-alpha (maximum at 1 hour after LPS, 1200-fold compared to control rats), and ET-1 (maximum at 3 hour after LPS, 25-fold compared to control rats). At 6 h after LPS administration, we found decreased percent of fractional shortening in heart. The expression of VEGF, and its downstream angiogenic signaling molecules namely eNOS and NO were significantly increased in heart tissues after LPS administration compared to control group which was also accompanied by increased cardiac ET-1 level. Dual endothelin blockade for 6 hours further upregulated the VEGF angiogenic signaling in endotoxemic heart.

P-3

Endothelin Plasma and Tissue Expression in Ductal Carcinoma of the Breast: Correlation with Clinicopathological Characteristics and VEGF

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Purpose: Endothelin-1 (ET-1) is overexpressed in breast carcinomas, while circulating levels of its precursor (Big ET-1) have also been found elevated. In the present study, we evaluated plasma ET-1 and Big ET-1 levels, and tissue expression of ET-1 in patients with ductal carcinoma of the breast. Methods: Peripheral venous blood samples were collected prior to diagnostic biopsy from women with suspicious non-palpable mammographic lesions. Plasma ET-1 and Big ET-1 levels were determined in 30 patients with IDC, 30 with DCIS and 30 with benign lesions (controls), performing ELISA. ET-1 and VEGF tissue expression was immunohistochemically determined. Potential correlations with histological grade, hormone receptor status, Her2/neu amplification, tumor size, lymph node involvement and disease stage were investigated in IDC. Results: Big ET-1 plasma levels were significantly higher in IDC and DCIS patients compared to controls ($p<0.001$ and $p<0.01$, respectively). No significant differences in ET-1 levels were observed between the three groups. Moderate to strong IHC staining for ET-1 was observed in 3/29 and 7/23 IDC and DCIS patients, respectively. VEGF was significantly expressed in 8/27 and 8/23 IDC and DCIS patients, respectively. In IDC, plasma and tissue expression of ET-1 and plasma expression of Big ET-1 did not correlate with any of the analyzed clinicopathological characteristics or VEGF tissue expression. Conclusions: Plasma levels of Big ET-1 were a more sensitive indicator of ET-1 deregulation than those of ET-1 in our study. Our results support the potential clinical application of Big ET-1 as a breast cancer biomarker.

P-4

The Localisation and Distribution of Endothelin Receptors in Normal and Cancer Colon Tissues: Confirmation by Autoradiography, Immunohistochemistry and Quantum Dot Targeting

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Background: Endothelin-1 (ET-1) acts via two G-protein-coupled receptors, ETA and ETB. Overexpressed ET-1 and ETA in colorectal cancer (CRC) promote tumour growth and progression. **Aim:** To investigate (1) ETA and ETB distribution in normal and cancer tissues from patients with CRC and (2) determine ETA and ETB localisation to cell types and tissue structures. **Methods:** ETA and ETB distribution was determined using in vitro autoradiography with competitive inhibition, using receptor antagonists (BQ123, ZD4054, BQ788) on normal and cancer tissues resected from patients with CRC (N=8). Immunohistochemistry (IHC) confirmed ETA and ETB expression and identified associated cells/structures. ETA distribution was also investigated by quantum dots (QDs) conjugated to BQ123 (ETA-antagonist). **Results:** In normal bowel epithelium, ETA was observed closer to the luminal surface and ETB towards the muscularis mucosa/lamina propria. There was greater ETA than ETB binding in CRC. Both cancer and normal tissues demonstrated strongest binding to stromal cells, particularly fibroblasts (IHC). QD-BQ123 demonstrated an ETA punctate pattern in stromal areas surrounding epithelial cells; and an ETA increase in CRC compared to normal. **Conclusions:** ET-1 binds strongly to CRC stromal structures, with ETA greater than ETB, and is consistent with ET-1 signalling contributing to tumourigenesis. Within normal tissue, differential ETA and ETB distribution (luminal versus muscularis mucosa/lamina propria) has not been reported previously. This may relate to trophic, growth arrest and differentiation signalling. This study demonstrates the effective, novel use of receptor-antagonist-conjugated QDs; reveals possible ET-1 roles in normal tissue; and provides further evidence for the potential therapeutic use of ETA antagonists as CRC adjuvant treatment.

P-5

Novel Molecular Pathways by Which ETA Receptor Mediates Tumourigenic Signals in Colorectal Cancer: Support for ETA Receptor Antagonism as Adjuvant Treatment

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Background: The endothelin A receptor (ETA) mediates tumourigenic signals in colorectal cancer (CRC). The ETA ligand, endothelin-1 (ET-1), stimulates not only cancer cells but also surrounding fibroblasts and may promote the creation of a supporting tumour stroma. **Aim:** To identify ET-1 regulated genes associated with oncogenic pathways in colonic fibroblasts. **Methods:** Micro-array analysis following 4hr ET-1 stimulation of colonic fibroblast strains (isolated from patients undergoing resection for CRC, n=4) identified differentially expressed genes (n=19) at significant levels. Three were investigated further: COLXI, AML-1, EGFR (collagen type-XI; acute myeloid leukemia-1; epidermal growth-factor receptor). Quantitative RT-PCR (qRT-PCR) and immunoblotting evaluated AML-1 and COLX expression levels, following treatment with ET-1 and/or receptor antagonists (ETA: BQ123, ZD4054; ETB: BQ788). ETA and ETB regulation of EGFR was investigated by gene silencing (siRNA); these assays and ET-1 regulation of EGFR over 24hrs were evaluated by qRT-PCR. **Results:** ET-1 stimulated expression of AML-1 and COLXI at both gene (>1.5-fold; $p<0.01$) and protein ($p<0.05$) levels; stimulation was inhibited by ETA, but not ETB, antagonism (AML-1:75.1-77.1% by BQ123, ZD4054; COLXI:65.1% by ZD4054; $p<0.05$). EGFR expression demonstrated a biphasic increase at 4hr and 24hr (3.8-fold; :4.5-fold). Silencing ETA, but not ETB, returned EGFR levels to control. **Conclusions:** ETA antagonism has potential for targeting oncogenic pathways: AML-1 is linked to c-Jun N-terminal kinase which inhibits apoptosis/promotes proliferation; and abnormal TGF-β (transforming growth-factor-beta) signalling. COLXI is linked to CRC tumourogenesis. The ET-1-stimulated biphasic EGFR response and ETA antagonism have not been reported before in CRC. These findings identify mechanisms by which ETA promotes tumourigenesis and support addition of ZD4054 to existing EGFR antagonism therapy.

P-6

Serum Big Endothelin-1 as a Clinical Marker in Canine Pulmonary Hypertension and Tumors

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In human medicine there have been many studies reporting the importance of evaluating serum ET-1or big ET-1 in various diseases as a clinical marker, especially related to pulmonary hypertension and several tumors. In veterinary medicine, however, few studies have been performed on the clinical importance of serum ET-1or big ET-1. In this study we explored the feasibility of using the serum big ET-1 as a clinical marker in dogs with various cardiopulmonary and neoplastic diseases. Pulmonary hypertension was diagnosed in dogs by echocardiography based on the velocity of tricuspid valve regurgitation. Serum big ET-1 and NT-pro BNP concentrations in these dogs were measured by ELISA and compared with those of healthy dogs. Serum big ET-1 concentration in dogs with various neoplastic diseases was also assessed and compared.Our results showed a significant increase of serum big ET-1 in dogs with pulmonary hypertension, lung tumor and hemangiosarcoma when compared to normal dogs. No significant difference was observed in NT-pro BNP concentration between healthy and pulmonary hypertension dogs.Although further studies are necessary, these findings point to the potential of serum big ET-1 as a clinical marker in canine pulmonary hypertension and for some tumor types.

P-7

An Unexpected Pulmonary Hypertensive Crisis: Eying the Culprit

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A 56-year-old man developed sudden dyspnoea after resection of choroidal melanoma in his left eyeball. Worsening hypoxia required intensive treatment, including percutaneous cardiopulmonary support. On contrast-enhanced computed tomography there was no evidence of either thrombi in the pulmonary arteries or obvious lung diseases. A Swan-Ganz catheter showed increased mean pulmonary arterial pressure and no elevation of pulmonary capillary wedge pressure. These findings were consistent with a diagnosis of pulmonary arterial hypertension. Because reports have described a significant relationship between melanoma and endothelin (ET)-1, we hypothesized that a substantial amount of ET-1 had been released from malignant melanoma cells during resection, thus triggering the pulmonary hypertensive crisis in our patient. The patient fully recovered after intensive treatment and administration of the endothelin receptor antagonist bosentan. The success of bosentan treatment, along with an extremely high level of ET-1 on pathologic analysis, confirmed our hypothesis regarding an increase in plasma ET-1 level - 9.60 pg/mL (normal range <2.3 pg/mL).

P-8

Pharmacokinetics of SPI-1620 in A Phase I, Open Label, Ascending Dose Study of the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of the Endothelin B Receptor Agonist, SPI-1620, in Recurrent or Progressive Carcinoma

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Objective: The primary objective of the Phase I study was to assess the safety and tolerability of SPI-1620 administered to patients with recurrent or progressive carcinoma who had failed all standard therapy. Secondary objectives were to assess PK and PD profiles of SPI-1620, and to identify the optimum dose of SPI-1620 to be used in future Phase II studies. The pharmacokinetic properties of SPI-1620 will be presented. Methods: Eligible patients received SPI-1620 by intravenous infusion over one minute in an accelerated dose escalation scheme. SPI-1620 doses ranged from 0.5 µg/m2 to 15.1 µg/m2. Serial blood samples were collected from each patient prior to infusion (0 min) and at pre-specified intervals from the start of the infusion. Human plasma samples were analyzed by a validated HPLC-MS/MS method. Descriptive PK parameters were determined by standard model independent methods based on the concentration-time data of each subject. Results & Conclusion: The highest concentration of SPI-1620 was achieved by the end of infusion. SPI-1620 C max increased proportionally as a function of SPI-1620 dose while the AUC (0-T) increased in a more than dose proportional manner. The SPI-1620 T 1/2 was short and ranged from 4.38 minutes to 8.29 minutes. SPI-1620 had a low systemic clearance and small VD (approximately equal to the intravascular volume).

P-9

Endothelin-1-Induced β-Arrestin Signalosome is Linked to Chemoresistance, EMT and Stem-Cell Like Properties in Ovarian Cancer Cells

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The epithelial-mesenchymal transition (EMT) is known to play a crucial role in the aggressiveness of epithelial ovarian cancer (EOC), contributing to chemoresistance and cancer stem cell populations. In this tumor, the endothelin (ET)-1/endothelin A receptor (ET_AR) axis, by regulating EMT and invasion, endows EOC cells with an increased chemoresistance. Here we examined whether β-arrestin-1 (β-arr1) can act as nuclear hub orchestrating nuclear signaling in ET_AR-driven EMT and chemoresistance. A significant higher expression of β-arr1 and ET-1/ET_AR and the stronger presence of β-arr1 in the nuclear compartment upon ET_AR activation is present in chemoresistant cells, compared to sensitive cells. In the nuclei, β-arr1 robustly interacts with β-catenin to form a nuclear complex localized on the ET-1 promoter region, leading to transcription of ET-1, demonstrating that β-arr1 drives the positive inter-regulation of ET-1 itself. This autocrine circuit is involved in β-arr1-driven appearance of EMT features and acquisition of stem-cell like properties. Moreover, at functional level, chemoresistant cells, with high nuclear β-arr1, display higher invasive potential and increased resistance to chemotherapeutic drugs. These effects were inhibited by ET-1 receptor blockade with macitentan, or by β-arr1 nuclear mutant. Moreover, in vivo, silencing of β-arr1 or macitentan treatment inhibited metastasis in sensitive and resistant EOC xenografts, providing evidence that blockade of ET_AR/β-arr1-driven EMT can overcome chemoresistance and inhibit tumor progression. Collectively, our findings provide insights into how ET_AR controls EMT transcriptional responses and tumor initiating trait, deciphering a novel function for β-arr1 for nuclear compartmentalization of ET_AR signalling influencing the mechanism of acquired resistance, EMT and stem-cell like features.

P-10

(Pro)renin Receptor in the Breast Cancer and Its Possible Pathophysiological Role in Cancer Cell Proliferation

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The endothelin system is an important paracrine or atutocrine system for cancer cell proliferation. Endothelin-1 and endothelin receptors are expressed in various types of cancers including breast cancer. (Pro)renin receptor ((P)RR) is a specific receptor for renin and prorenin. Receptor-bound prorenin becomes enzymatically active in converting angiotensinogen to angiotensin I, and binding then activates phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), independently of angiotensin II generation. Furthermore, (P)RR is associated with vacuolar-type H^+-ATPase (V-ATPase), which may be related to cell proliferation. The aim of the present study is to clarify the pathophysiological role of (P)RR in the breast cancer. We investigated (P)RR expression in 69 clinical cases of breast carcinoma by immunohistochemistry and its correlation with clinicopathological parameters. Effects of (P)RR on cell proliferation and ERK1/2 phosphorylation were examined in cultured human breast carcinoma cells. Immunohistochemistry showed that (P)RR immunoreactivity was detected in carcinoma cells of breast carcinoma tissues, and was correlated with Ki-67 expression. The (P)RR specific small interference RNA or bafilomycin A1 (an inhibitor of V-ATPase activity) inhibited cell growth of breast carcinoma cell lines (MCF-7 and SK-BR-3). Prorenin stimulated phosphorylation of ERK1/2 in MCF-7 cells. Treatment of MCF-7 cells with endothelin-1 had no significant effects on (P)RR expression levels. The present study has raised the possibility that, in addition to the endothelin system, (P)RR is involved in the pathophysiology of the breast cancer by stimulating the proliferation of breast carcinoma cells via the association of V-ATPase and/or phosphorylation of ERK1/2.

P-11

Poly-Gamma-Glutamic Acid Attenuates Angiogenesis and Inflammation in Experimental Colitis

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Poly-gamma-glutamic acid (PGA), naturally secreted from various strains of Bacillus, has anti-inflammatory activity. In inflammatory bowel disease (IBD), inflammation is promoted and sustained by angiogenesis; however, the role played by PGA in this condition is unclear. Therefore, we evaluated PGA effects on angiogenesis and inflammation in a dextran sulfate sodium (DSS)-induced mouse colitis model. Experimental colitis was induced in male C57BL/6 mice by administering 3% DSS. Disease activity index (DAI), histopathological scores, microvascular density, myeloperoxidase activity, and VEGF-A and VEGFR2 expression were compared among control mice, DSS-treated mice, and mice receiving 3% DSS along with PGA at 50 mg/kg body weight per day, or 3% DSS with PGA at 200 mg/kg body weight per day. We found that PGA significantly attenuated weight loss, DAI, and colon shortening. PGA also significantly reduced histopathological evidence of injury. Moreover, PGA significantly attenuated DSS-induced blood vessel densities. Furthermore, PGA attenuated DSS-induced expression of VEGF-A and its receptor, VEGFR2. In addition, PGA treatment led to reduced recruitment of leukocytes to the inflamed colon. Therefore, our results indicate that PGA has potential application in conditions marked by inflammatory-driven angiogenesis and mucosal inflammation.

P-12

Identification of Bladder Endothelin-1 Receptors and Binding Characteristics of Bosentan and Ambrisentan

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Endothelin (ET)-1 induces prolonged contractile responses in isolated bladder muscle strips. ET-like immunoreactivity was identified in detrusor muscles, epithelium and vascular endothelium. Selective ETA receptor antagonists have ameliorating effects on urinary dysfunctions. The current study aimed to identify bladder ET-1 receptors using radioligand binding assay and characterize receptor binding of clinically used ET-1 receptor antagonists. ET-1 receptors were measured in rat bladder using [125I]ET-1, and binding parameters of dissociation constant (Kd) and maximal number of binding sites (Bmax) for [125I]ET-1 were estimated. The inhibition of specific [125I]ET-1 binding was measured in the presence of ET-1 and its receptor antagonists. Specific [125I]ET-1 binding in rat bladder was saturable and of high affinity, which characterized selective labeling of bladder ET-1 receptors. ET-1, bosentan, ambrisentan, and CI-1020 inhibited specific [125I]ET-1 binding in a concentration-dependent manner at nanomolar ranges of IC50. Nonlinear least squares regression analysis revealed the presence of high- and low-affinity ET-1 receptor sites for ambrisentan and CI-1020. Bosentan significantly increased Kd for bladder [125I]ET-1 binding without affecting Bmax, while ambrisentan increased Kd with a concomitant reduction in Bmax. Thus, bosentan seems to bind bladder ET-1 receptor in competitive and reversible manner while ambrisentan may bind to bladder ET-1 receptors, partially in noncompetitive manner in addition to a competitive manner. Oral administration of bosentan caused a dose-dependent decrease in Bmax for bladder [125I]ET-1 binding, suggesting significant binding of bladder ET-1 receptors in vivo. These results indicate that pharmacologically relevant ET-1 receptors exist in rat bladder and they may become a promising target for the development of novel therapeutic agents for bladder dysfunction.

P-13

Endothelin-1 (ET-1) and Its Receptors on Haemorrhoidal Tissue: A Potential Site for Therapeutic Intervention

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Objectives: Haemorrhoids is a common anorectal condition that affects millions worldwide. We studied the potential role of ET-1 and its (ETA and ETB) receptors in haemorrhoid tissue. **Methods:** ET-1, ETA and ETB receptor localisation was studied in haemorrhoids using autoradiography and immunohistochemistry. Protein expression was compared between haemorrhoids and normal rectal submucosa using Western blot analysis. ETA and ETB receptor antagonist effects on ET-1- and Sarafotoxin 6a-induced contraction of human mesenteric artery and vein was assessed by myography. **Results:** There was dense [^{125}I]-ET-1 binding to haemorrhoidal sections with ETB>ETA binding (12.7 ± 3 vs 4.4 dpm x 10^3/mm^2, n=3, NS). Immunohistochemistry revealed a higher ETB than ETA receptor immunostaining in haemorrhoidal than in control rectal tissue. This was confirmed by Western blot analysis where haemorrhoidal ETB receptor protein levels were about 4-times higher than ETA receptors (78.3 ± 28.0 vs 18.8 ± 3.6 densitometric units, p=0.026). ETA and ETB receptors were localised to smooth muscle of mesenteric arteries and veins with ETB receptors also on endothelium. Myograph studies showed the sensitivity and maximum contractile response to ET-1 and sarafotoxin 6a was greater in mesenteric veins than arteries (p<0.05). **Conclusions:** ETA and ETB receptors are present in haemorrhoids with ETB receptors predominating. Mesenteric veins are more sensitive than arteries to ET-1-induced contraction, an effect that is blocked to a greater extent by ETB compared with ETA receptor antagonists. Since ETB receptors are located on smooth muscle of both the mesenteric artery and vein ETB agonists may have therapeutic potential via a constrictor action on haemorrhoidal blood vessels.

P-14

Endothelin-1 Modulates Bile Secretory Function in Rats

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Study was conducted in acute trials on rats anaesthetized with urethane 100mg/100g of body weight. Endothelin-1 0.1 µg/100g was injected into the portal vein and 0.9% NaCl (100µl/100g) was administrated to the control animals. Choleresis was estimated in microlitres each 10 minutes during three-hour experiment. Six half-hour bile samples were collected. Concentration of the bile acids, and lipids in bile was determined by thin-layer chromatography. Endothelin-1 in vasoactive concentration (0.1µg/100g) decreased choleresis with maximum reduction by 15.5% (p<0.05) in 40 minutes following its administration and exhibited diverse effect on concentration of different bile acids. Taurocholic acid was gradually reduced in control and experimental animals, but its level was higher by 9.3% (p<0.05) in the sixth sample of experimental animals. Concentration of glycocholic acid was reduced in the control animals, but increased following endothelin-1 administration by 12.3% (p<0.05), 19.7% (p<0.01), 16.3% (p<0.01) in the last three bile samples correspondingly. Endothelin-1 caused an increase of free bile acids. Concentration of cholic acid increased in the third and sixth samples by 20.6% and 19.8% (p<0.05) correspondingly. Phospholipids increased in the fifth bile sample in endothelin-1 action by 16,7% (p<0.05). Free cholesterol decreaced slightly but ether-coupled cholesterol increased in the last two samples by 43,8% (p<0.05) and 45,7% (p<0.05) correspondingly. Triglycerides increased gradualy and 50% (p<0.05) elevation was the fourth bile sample. So endothelin-1 intensifies conjugation of the free bile acids, biosynthesis of ether-coupled cholesterol, trihydroxycholane acids and phospholipids in liver that assists improvement of bile colloidal system characteristics.

P-15

Endothelin System in Intestinal Villi: A Possible Role of Endothelin-2 in the Maintenance of Intestinal Architecture

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The endothelin system consists of three ligands (ET-1, ET-2 and ET-3) and at least two receptors (ETA and ETB). In intestinal villi, fibroblasts-like cells express endothelin's receptors and response to ET-1 and ET-3 peptides, changing their cellular shape. Several functions have been attributed to these peptides in the "architecture" maintenance of intestinal villi acting over sub-epithelial fibroblasts. Despite this, ET-2 has not been analyzed in depth. In this work we show the intestine gene expression and immunolocalization of ET-1, ET-2 and the ETA and ETB receptors from duodenum to rectus and in the villus-crypt axis in mice, allowing a complete analysis of their functions. While ET-1 is expressed uniformly, ET-2 had a particular distribution, being higher at the bottom of the villi of duodenum, ileum and jejunum and reverting this pattern in the crypts of colon and rectus, where the higher expression was at the top. We postulated that ET-2 would act in a cooperative manner with ET-1, giving to the villus the straight enough to withstand mechanical stress.

P-16

Vasoprotective Effect of Endothelin Receptor Antagonist in Ovariectomized Female Rats

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The effects of hormone replacement therapy with estrogen on cardiovascular disease in post menopausal women are still controversial. We previously reported that endothelin (ET)-1/ET receptor system is involved in sex differences in the development of neointimal formation after vascular injury. In the present study, we hypothesized that dual ETA/ETB receptor antagonist (ERA) could exhibit vasoprotective effects after menopause. To confirm it, we examined the efffects of ERA and/or angiotensin receptor blocker (ARB) on neointimal formation after vascular injury in intact and ovariectomized (OVX) female rats. The right carotid artery was exposed to balloon injury, and harvested 2 weeks after the injury. In intact female groups, treatment with ARB for two weeks after the injury significantly decreased neointimal formation, whereas treatment with ERA did not affect neointimal formation. On the other hand, in OVX groups, ET antagonist markedly decreased neointimal formation after the injury although neointimal formation was not significantly improved by ARB. Combined treatment with 17β-estradiol and ARB markedly suppressed neointimal formation after the injury in OVX groups, whereas there were no additive effects during combined treatment with 17β-estradiol and ERA. These ERA or 17β-estradiol-induced vasoprotective effects in OVX groups were related to the suppression of NADPH oxydase-dependent reactive oxygen species production. Taken together, ERA has an estrogen-like vasoprotective action on neointimal formation via inhibition of oxidative stress. ERA may be an alternative therapy for the prevention of vascular disease in postmenopausal women.

P-17

Increased Cerebrovascular Sensitivity to Endothelin-1 in Obstructive Sleep Apnea Rats is Endothelin-B Receptor Mediated

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Obstructive sleep apnea (OSA) has been identified as a significant risk factor for stroke. However, little is known regarding the effects of OSA on the cerebrovascular wall. We hypothesized that OSA augments endothelin-1 (ET-1) induced constrictions of cerebral arteries. Using rats chronically instrumented with an inflatable endotracheal obstruction device we simulated a moderate severity of sleep apnea, 30 apneas/hour for 8 hours/day (sleep phase) for 1 month. Cerebral arteries were harvested for analysis of gene-expression, immunohistochemistry, and vascular reactivity. Following 1 month of OSA, blood pressure and plasma/cerebral vessel ET-1 levels were similar in sham and OSA rats (n=4-7, NS). Using the pressurized cerebral artery preparation, we observed a 17.5-fold increase in sensitivity to ET-1 (n=5-6, p<0.05). The increased sensitivity of OSA cerebral arteries to ET-1 was abolished by the ET-B receptor antagonist BQ-788 (n=6, NS). However, increased ET-1 sensitivity of OSA cerebral arteries persisted in the presence of the ET-A receptor antagonist BQ-123 (n=3-6, p<0.05). Additionally, constrictions to the ET-B specific agonist IRL-1620 were significantly greater in OSA, versus sham, cerebral arteries (n=6, p<0.05). Gene expression analysis revealed no difference in the mRNA levels of *et-1*, *et-a* or *b* receptors, or endothelin converting enzyme (*ece1*) (n=6, NS). However, immunohistochemical analysis demonstrated elevated levels of ET-B receptor in the smooth muscle of OSA cerebral arteries. These data demonstrate that OSA increases the sensitivity of cerebral arteries to ET-1, which appears to be driven by increased ET-B receptor signaling. These observations suggest an important role of ET-1 signaling in the adverse cerebrovascular outcomes associated with OSA.

P-18

The Akt Pathway Mediates the Neuroprotective Effect of IRL-1620 in A Rat Model of Focal Cerebral Ischemia

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We found that ET_B receptor agonist, IRL-1620, provides significant neuroprotection following permanent cerebral ischemia. The serine/threonine kinase Akt plays a role in regulating cell survival and death cascades, and may mediate neuroprotective effect of IRL-1620. Present study investigated the effect of IRL-1620 on the phosphorylation state of Akt in a rat model of cerebral ischemia. Male Sprague-Dawley rats underwent permanent middle cerebral artery occlusion. Following surgery, rats received three intravenous injections of vehicle or IRL-1620 (5 µg/kg) at 2, 4, and 6 hours post-occlusion. Evaluation of behavioral parameters confirmed the induction of stroke. Animals were sacrificed 7 and 24 hr following occlusion and brains processed to evaluate protein expression of total Akt and Akt phosphorylated at Ser473. There were no significant changes in behavioral parameters between vehicle and IRL-1620 groups 7 hr post-occlusion. However, at 24 hr there was a marked improvement in the behavioral parameters of IRL-1620 compared to vehicle treated rats. There were no changes in total Akt expression levels in the brains of sham, vehicle, and IRL-1620 treated rats, however, there was an increase in the phosphorylation of Ser473 of Akt (p473-Akt) in the IRL-1620 treated rats compared to sham (P&lt0.01) or vehicle treated (P&lt0.05) rats 7 hr post-occlusion. No difference in total Akt or p473-Akt levels was observed 24 hr post-occlusion in the brains of sham, vehicle, or IRL-1620 treated rats. It is concluded that IRL-1620 causes a transient elevation in phosphorylation of Ser473 after 7 hr of cerebral ischemia, suggesting that Akt pathway may be involved in mediating neuroprotective effect of IRL-1620.

P-19

Concomitant Downregulation of ET-1-ETB System and VEGF Angiogenic Signaling in the Frontal Cortex of a Murine Model of Endotoxemia: A Double Threat to Cerebral Microcirculation in Sepsis

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Sepsis is a disease of the microcirculation, with endothelial dysfunction playing a key role in its pathogenesis and subsequent associated mortality. Pathophysiology of brain dysfunction due to sepsis remains poorly understood. Cerebral microcirculatory alterations may play a potential role; however, experimental data are scarce. The present study sought to investigate whether key angiogenic pathways are altered in frontal cortex in a clinically relevant animal model of endotoxemia/sepsis. Male mice at 8 weeks of age were administered either saline or 20 mg/kg lipopolysaccharide (LPS) at different time points (1, 3, 6, and 10 hrs). Mice that did not receive LPS were considered to be controls. We confirmed the induction of endotoxemia by measuring circulatory TNF-alpha level as well as cerebral mRNA levels of TNF-alpha, IL-1beta, and IL-6. Vascular endothelial growth factor (VEGF), a major vascular multi-factorial cytokine involved in all the three types of vascular growth namely, angiogenesis, arteriogenesis and atherogenesis, mediates its angiogenic action through its receptor VEGF-R2. In the frontal cortex of mice endotoxemic model, the expression of VEGF and VEGF-R2 with downstream molecule eNOS was downregulated time-dependently implying the great disturbances in the maintenance of cerebral microcirculation in sepsis. Concomitantly, endothelin-1 (ET-1) which also is pro-angiogenic in function through its receptor ETB was downregulated time-dependently with similar pattern as VEGF angiogenic system. Recent study reported a significant decrease in cerebral capillary density in a sheep model of sepsis which is strongly associated with the progression of cerebral pathologies. We believe our current findings would shed some light to the mechanisms underlying the microcirculation based brain dysfunction in sepsis.

P-20

Changes in Ovarian Constriction by Endothelin-2/Receptor System in the Feline Ovary

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Endothelin-2 (ET-2) is transiently expressed in the granulosa cells of periovulatory ovaries immediately prior to ovulation. Ex vivo experiments showed that ET-2 treatment induces ovarian constriction. Endothelin receptor pathway antagonization inhibits ovulation in rodent animals. We postulated ET-2 induced constriction of ovarian smooth muscles is a final trigger for follicle rupture at the time of ovulation. Similar to humans, feline ovaries possess a layer of contractile, smooth muscle-like cells around developing follicles, known as the theca externa. In addition, feline ovaries have been documented to contract ex vivo. However, the function of this constriction and whether ET-2 induces ovulation remain to be determined. Here, we investigated the characteristics of contraction in feline ovaries using a myogram in the absence or presence of physiological doses of ET-2. Whole live feline ovaries were collected after spay procedures through the Junior Surgery Program of the College of Veterinary Medicine at the University of Illinois. Of 13 ovaries tested, all demonstrated a period of strong and sustained constriction when treated with 50 nM ET-2, with an average increase in base tensile force of 2.48 ± 0.40 mN. Additionally, treatment with the dual ET receptor antagonist Tezosentan reduced constriction in a dose-dependent manner. Measurement of mRNA expression by polymerase chain reaction showed that feline ovaries express mRNA for ET-2, both isotypes of endothelin receptors (ET-A and ET-B), and endothelin converting enzymes 1 and 2 (ECE-1 and ECE-2). Taken together, this study demonstrates that ET-2 produces a strong constriction in feline ovaries, and may be important for rupture of follicles at the time of ovulation.

P-21

ET-1 Overexpression and Endothelial Nitric Oxide Synthase Knock-Out Induce Different Pathological Responses in the Heart of Male and Female Mice

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The nitric oxide and endothelin systems are closely related to another. We previously reported that diastolic dysfunction observed in mice lacking the endothelial nitric oxide synthase (eNOS-/-) can be prevented by a genetic overexpression of ET-1. Sexual dimorphisms have been reported in both ET-1 and NO systems. Particularly, eNOS-/- mice present sex related differences. We report here that the cardiac level of ET-1 expression in eNOS-/- mice was elevated in males but not in females. Systolic blood pressure and cardiomyocyte diameter was higher in male eNOS-/- mice and male ET-1 overexpressing (ET+/+) mice compared to the females of same genotype. Cardiac interstitial fibrosis was similar between the groups, but perivascular fibrosis developed in female ET+/+ mice but not in males. Additionally, the cardiac expression of metalloprotease-9 was higher in eNOS-/- males compared to females. Finally, cardiac proteome analysis revealed that the protein abundance of the oxidative stress related enzyme superoxide dismutase presented with sexual dimorphism in eNOS-/- and ET+/+ mice. These results indicate that both an elevated ET-1 and reduced NO production, which are typical characteristics of cardiovascular diseases, induce different pathological responses in male and female mice.

P-22

Research on the Relationship between Endothelin-1 Gene Polymorphisms and Primary Nephrotic Syndrome in Children

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AIMS: To investigate the relationship between endothelin-1 gene polymorphisms in locus rs1630736, rs5370, 3A/4A and the morbidity and progress of Nephrotic syndrome (NS) in children. METHODS: 36 children with primary NS were as case group, 94 healthy children were as control. All subjects were genotyped for endothelin-1 polymorphisms in locus 3A/4A, rs5370 and rs1630736 by using the polymerase chain reaction and direct gene sequence test technique. RESULTS: (1) In locus rs5370, the frequencies of GG, GT, TT genotype were statistically significant difference within case group and control group (χ^2=6.25, P=0.044). (2) In locus 3A/4A, the frequencies of 3A/3A, 3A/4A, 4A/4A genotype were statistically significant difference within case group and control group(χ^2=5.47, P=0.048). (3) In locus rs1630736, the frequencies of CC, CT, TT genotype were no statistically significant difference within case group and control group. (4) In GG+CT+3A/4A combined genotype, the difference between case group and control group on gene frequency distribution was statistical significance (χ^2=4.65, P=0.03). (5) The plasma level of ET-1 between case group and control group (t=2.85, P=0.007), the active phase and remittent phase of case group had statistical significance (t=3.27, P=0.003). (6) The difference between gene frequencies distribution in locus rs5370, 3A/4A and plasma of cholesterol had statistical significance (P&LT0.05). CONCLUSIONS: (1) The endothelin-1 gene polymorphisms in locus rs5370 and 3A/4A are related to the morbidity of Nephrotic syndrome. (2) In locus rs5370, rs1630736 and 3A/4A, the GG+CT+3A/4A combined genotype show greater risk of Nephrotic syndrome. (3) In locus rs5370 and 3A/4A are related to the plasma cholesterol level in NS children.

P-23

Evaluation of Urinary and Plasma Endothelin-Like Domain Peptide (ELDP) in Chronic Kidney Disease

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Background: Current evidence indicates that endothelin-1 (ET-1) plays a physiological role in kidney collecting ducts by inhibiting Na^+ reabsorption. Conversely, experimental models of renal disease suggest ET-1 contributes to pathological processes by increasing vasoconstriction and stimulating fibrosis. ELDP is a recently identified *EDN1* gene product (preproET-1 [93-166]) that is co-synthesised and co-released with ET-1. To investigate whether ELDP plays a role in the pathological changes occurring in chronic kidney disease (CKD), or may act as a biomarker for disease severity we assayed ELDP levels in urine and plasma samples from control subjects and patients with CKD. **Methods and Results:** A specific double-recognition site sandwich ELISA for ELDP was optimised for urine and plasma measurements. Patients recruited to this study had renal disease without co-existing morbidities. Venous plasma and urine samples were collected after a 12 h fast and stored frozen at -80°C until analysis. Plasma levels of ELDP (mean ± SD) were 6.34 ± 1.4 for control subjects and 6.16 ± 1.43, 7.01 ± 2.15, 7.74 ± 1.64, 8.96 ± 3.82 and 12.35 ± 4.45 pmol/L for CKD stages 1 to 5 respectively ($P < 0.05$ for the trend). Urine samples showed marked variation with no statistically significant pattern. Mean urine levels were 1.08 ± 1.19 pmol/L, with >200 fold difference between the minimum and maximum values of 0.03 and 6.65 pmol/L. **Conclusions:** The trend for increased plasma ELDP in patients with CKD adds further evidence to the concept that increased expression of *EDN1* contributes to the vascular changes in these patients. The factors affecting urinary levels of ELDP merit further investigation.

P-24

Potential Amelioration of Upregulated Renal HIF1Alpha-Endothelin 1 System Through Landiolol Hydrochloride in A Rat Model of Endotoxemia: A Possible Linkage to the Increased Renal Vascular Resistance Based on Renal Microcirculation Alteration in Sepsis

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Alterations in the microcirculation in the renal cortex or renal medulla has been shown as a factor contributing to the development of renal dysfunction in sepsis despite normal or increased global renal blood flow (RBF). Sepsis-induced renal microvascular alterations (vasoconstriction, capillary leak syndrome with tissue edema, leukocytes and platelet adhesion with endothelial dysfunction and/or microthrombosis) could contribute to an increase in renal vascular resistance in sepsis. Endothelin (ET)-1, a potent vasoconstrictor that has been implicated in the pathogenesis of sepsis and in our previous study we have shown that ET-1 is highly upregulated in renal tissues as well as in plasma after LPS administration and there is a potential imbalance in the renal tissue expression of vaso regulatory molecules. In the current study we investigated whether landiolol hydrochloride, an ultra-short-acting β-blocker, can play an important role in ameliorating the LPS-induced upregulated renal HIF-1alpha-ET-1 system with inflammatory cytokine (TNF-alpha) in a rat model of endotoxemia. Male Wistar rats at 8 weeks of age were administered lipopolysaccharide (LPS) for three hours and some LPS-administered rats were continuously treated with landiolol for three hours. At 3h after LPS administration, both circulatory and renal TNF-alpha level increased. In addition, LPS induced a significant upregulated expression of ET-1and HIF-1alpha in the renal tissues compared to control. Finally, treatment of LPS-administered rats with landiolol for three hours potentially normalized the upregulated renal TNF-alpha level as well as HIF-1alpha-ET-1 system. These data taken together, led us to conclude that landiolol may be renal protective in endotoxemia modulating the renal microvascular alteration as well as renal vascular resistance.

P-25

p66 Shc Mediates Effect of ET-1 on TRPC Channels Activity and Changes in Intracellular Ca^{2+} in Renal Vascular Smooth Muscle Cells

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Hypertension-induced nephropathy is accompanied by impaired renal vascular responsiveness and structural changes, but molecular mechanisms involved remain elusive. Elevation of intracellular Ca^{2+} $[Ca^{2+}]_i$ is strongly linked to renal microvascular responses and is crucial for ET-1-induced contraction of smooth muscle cells (SMC). The adaptor protein p66 Shc is overexpressed in renal vascular SMC of hypertensive Dahl S rats. In patch clamp electrophysiology experiments carried out with primary SMCs isolated from renal vessels of Zinc Finger Nuclease-mediated p66 Shc rat knockouts we established that p66 Shc deficiency results in a dramatic increase in TRPC channels activity in response to ET-1. Knockout of p66 Shc resulted in increase of channel activity. Next we showed that ET-1 produced dynamic changes in cytosolic Ca^{2+} concentration in SMCs derived from p66 Shc knockout rats, when compared with SMCs derived from their WT littermates. Fura 2-AM was used to measure changes in the $[Ca^{2+}]_i$ before and after administration of 100 nM ET-1. We also tested activation of $[Ca^{2+}]_i$-dependent signaling pathways in renal SMCs isolated either from p66 Shc knockouts or from WT littermates. We have previously shown that ET-1-mediated activation of calcium regulated cytoplsmic tyrosine kinase Pyk2 caused an activation of p38 MAP kinase which is known to contribute to actin remodeling in SMC. Accordingly, we detected increased activation of Pyk2 and p38 MAP kinase in ET-1-treated SMCs isolated from p66 Shc knockout rat. Our data suggest that p66 Shc restrains activity of TRPC channels, which mediate influx of Ca^{2+} in SMC in response to ET-1, contributing to renal vascular dysfunction.

P-26

Contractions to Endogenous and Exogenous Endothelin-1 in Segmental Renal Arteries of the Mouse: Up-Regulation in Obesity

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Endothelin-1 (ET-1) is implicated in cardiovascular risk factors such as obesity, and the endothelin system is prominent in the kidney. In murine arteries, the contractile profile of the peptide is heterogeneous among different preparations, and the renal vascular bed is largely unexplored. Segmental renal arteries branching from the main renal arteries of age-matched lean and 30 weeks diet-induced obese WT mice were investigated by isometric tension recording in Halpern-Mulvany myographs. Contractions after administration of big endothelin-1 (bET-1) or ET-1 (both 10 pM to 100nM) were determined in the absence and presence of L-NAME, followed by full concentration-response curves to serotonin (5-HT) or the TP receptor agonist U46619. At the highest concentrations of bET-1 contractions were similar in rings of lean and obese mice in the absence of L-NAME. Inhibition of NO synthesis facilitated responses particularly in obese animals (n=6-9, P<0.01). Exogenous ET-1 contracted potently preparations of all groups starting from 3 nM on; the response to the peptide was augmented by obesity in the absence and presence of L-NAME (each n=6-10, P<0.001). ECE-activity calculated as the ratio of the responses bET/ET-1 was significantly higher in rings from obese mice in the presence of NO at 10 nM (n=5-9, P<0.01) and at 30 nM of the peptides in the presence of L-NAME (n=6-9, P<0.05). Contractions to 5-HT and U46619 were comparable between groups. These experiments demonstrate the high responsiveness of the renal vascular bed to both endogenous and exogenous ET-1, and an increased activity of the endothelin system in obesity, whereas responses to 5-HT_2 and TP receptor activation are unaltered.

P-27

ET-1-Induced Contraction of Renal Afferent Arterioles of Dahl Salt-Sensitive Rats is Impaired by Targeted Modification of a p66 Shc Regulatory Phosphorylation Site

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The adaptor protein p66 Shc, a longevity-associated product of *Shc1* gene, is implicated in the pathogenesis of age-related diseases and regulation of sensitivity to oxidative stress. The ability of p66 Shc to promote age-related diseases requires phosphorylation of serine 36 residue (Ser36). We have shown that Endothelin-1 (ET-1), an important regulator of the renal microcirculation, induces p66 Shc Ser36 phosphorylation and mediates protein-protein interactions of p66 Shc in renal cells. As the known p66 Shc-mediated effects are highly dependent on Ser36 phosphorylation, we specifically modified this amino acid by introducing a knock-in substitution of this amino acid in Dahl salt-sensitive (SS) rats. One cell embryos were extracted from SS rats and mRNA encoding two engineered ZFNs targeting portion of *Shc1* gene encoding p66 Shc isoform were injected into the embryo along with a plasmid template encoding an Ala36 modification. The double strand break caused by microinjection of ZFNs targeting *Shc1* gene stimulated homologous recombination with co-injected template plasmid containing desired mutation. We have established a breeding colony of rats with the Ser36 to Ala36 (S36A) substitution. The absence of Ser36 phosphorylation in response to ET-1 was confirmed by western blotting with phosphospecific antibodies. The juxtamedullary vasculature was isolated for study from genetically modified SS rats and an afferent arteriole was monitored continuously by videomicroscopy. After control diameter measurements, responses to ET-1 (0.001-10 nmol/L) were determined in afferent arterioles. Since preglomerular arterioles isolated from S36A rats exhibit an impaired vascular response to ET-1 when compared with their wild type littermates, p66 Shc is important for ET-1-induced vascular responses in renal vessels.

P-28

Blocking Endothelin-1 Induced Multiple Drug Resistance Permits Effective Inhibition of Activation of Renal Proximal Tubules Exemplified by the PKC alpha-microRNA15a Loop

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In Multiple Drug Resistance (MDR) cellular transport mechanism such as the MDR-1 and the MRP1-5 proteins are induced. Cells become resistant by accelerating the efflux of reagents being reabsorbed. This mechanism could be a major component of therapeutic resistance after proximal tubule activation in chronic proteinuric disease. By qRT-PCR, we identified Endothelin-1 (ET-1)-inducible MDR-transport proteins in normal (RPTEC) and tumorous (CAKI-1) proximal tubule cells. Verapamil and elacridar, a first and third generation MDR-inhibitor, blocked those transport proteins demonstrated by calcein-AM-assay. Fluorinated calcium was retained best in the cells after elacridar treatment of prestimulated proximal tubule cells and shows a functional impact. To further analyse the effect of therapy after blocking MDR we used a previously (von Brandenstein et al., 2010) described regulatory loop, causally connecting the upregulation of microRNA15a with the downregulation of PKC alpha after ET-1-stimulation in proximal tubule cells. Selegilin, an inducer of PKC alpha, downregulates micoRNA15a levels in ET1-stimulated proximal tubule cells. The therapeutic dose of selegilin could be significantly reduced after pre-treatment with elacridar. We conclude: i) blocking the MDR-transport system in proximal renal tubules overcomes chemoresistance after ET-1-stimulation; ii) therapeutic doses of PKC alpha-inducer selegilin counteracting microRNA15a production can be reduced. This approach is a first step towards an effective therapeutic protection of proximal tubules being activated by the endothelin system contributing to proteinuria in chronic renal disease.

P-29

Evidence for Extrarenal Vascular Endothelin-1 in the Maintenance of Sodium Homeostasis

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It has recently been established that a high salt diet leads to sodium storage within the skin of rats and mice. This increase in sodium in the highly vascularized skin results in macrophage infiltration and lymphangiogenesis. While dysfunction in this process has been implicated in salt sensitive hypertension, the mechanisms are poorly understood. Because vascular endothelin-1 (ET-1) is upregulated in response to a high salt diet or hypertonicity, and is a potent chemoattractant, we hypothesized that endothelial derived ET-1 mediates infiltration of immune cells into the skin during chronic high salt intake, thereby allowing sodium clearance from the skin and preventing accumulation. Our data indicate that increasing extracellular concentration of human endothelial cells by 40 mOsm with NaCl, similar to what is seen in in the interstitial space of rats placed on a high salt diet, leads to a 50% increase in ET-1 production, a mechanism likely mediated by TonEBP. Furthermore, in response to one week of high salt diet, skin Na/water ratio was elevated in vascular endothelial cell ET-1 knockout mice compared to wild type (0.112 ± 0.007 vs. 0.096 ± 0.004 mmol/ml). These data suggest a critical role for ET-1 in preventing the accumulation of sodium in the skin during a high salt intake, an emerging mechanism of the body's ability to buffer blood pressure changes in response to increases in sodium intake.

P-30

Endothelin Converting Enzyme Inhibition Attenuates Early Albuminuria and Late Renal Failure in Streptozotocin Induced Diabetic Mice

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BACKGROUND: Recent clinical trials with endothelin receptor antagonist on diabetic nephropathy have been terminated by side effect of fluid retention in spite of their beneficial effect for proteinuria. The alternative approach for interrupting endothelin pathway by endothelin converting enzyme (ECE) inhibition is anticipated. METHODS AND RESULTS: We injected streptozotocin (STZ; 180mg/kg i.p.) once for generating type1 diabetes into ten-weeks old male ECE-1 heterozygous knockout (KO) mice and their wild type (WT) littermates. The analysis for kidney function and morphology has been performed since early stage at 2 month until late stage at ten months after STZ. In early phase, diabetic mice have shown remarkable hyperglycemia, polyuria, renal hypertrophy, and glomerular hypertrophy at the same degree between WT and KO mice. But ECE-1 inhibition suppressed protein urea and albumin urea (WT vs KO: 1011±387 vs 283±69μg/day, $p<0.05$). Along with time passage until late stage, polyuria and glomerular hyperfiltration state made the shift to oligourea. ECE-1 inhibition attenuated the progression of renal failure showing preserved GFR (WT vs KO: 238±48 vs 370±59μl/min) and renal atrophy (WT vs KO: 12.6±0.8 vs 14.8±0.7 mg/mm tibia lengh). Superoxide in whole kidney quantified lucigenin assay has increased in WT mice and suppressed in KO mice (WT vs KO; 112±10 vs 70±6 cpm/mg). Tubular fibrosis by silius red staining has also prevented in KO mice. CONCLUSION: ECE-1 suppression prevents the progression of renal failure by suppressing glomerular barrier damage, oxidative stress, tubular fibrosis in diabetic mice.

P-31

High Salt Diet Attenuates ET-1 Mediated Calcium Signaling Responses in Preglomerular Smooth Muscle Cells from WT and ETB Receptor-Deficient Rats

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High salt diet reduces myogenic reactivity in resistance vessels and autoregulatory responses of juxtamedullary afferent arterioles. High salt diets increase endogenous endothelin levels, and enhance preglomerular ETB receptor expression. Renal autoregulatory responses are modulated by endothelin receptors and endothelin-mediated preglomerular vasoconstriction requires elevation of intracellular calcium concentration. Accordingly, experiments were performed to determine the impact of high dietary salt on the endothelin-mediated calcium signaling responses in freshly isolated preglomerular microvascular smooth muscle cells (PMVSMC's). Kidneys were harvested from wild type (WT) and ETB-deficient (ETBdef) rats fed normal salt (NS, 0.4% NaCl) or high salt (HS, 8% NaCl, 14 days) diets and PMVSMC's were prepared. Calcium signaling responses were studied using fura-2-based photometry. PMVSMC's isolated from WT rats on NS responded to ET-1 (10nM) with a biphasic increase in intracellular calcium from a baseline of 63 ± 6nM to a peak response of 983 ± 141nM ($P<0.05$) and in PMVSMC's isolated from WT rats fed HS, intracellular calcium increased from 56 ± 4nM to 423 ± 95nM or approximately 38% of the NS response ($P<0.05$). In contrast, PMVSMC's isolated from ETBdef rats on NS responded to ET-1 with an increase in intracellular calcium from 49 ± 4nM to 713 ± 152nM ($P<0.05$) whereas cells from HS ETBdef rats increased intracellular calcium from 52 ± 4nM to 418 ± 117nM or approximately 50% of the NS response ($P>0.05$). These data demonstrate that ET-1 signals in PMVSMC's with an elevation of intracellular calcium concentration. In addition, these data demonstrate that increasing dietary salt blunts the calcium response to ET-1.

P-32

Collecting Duct NOS1 Knockout Mice Lack ET-1 Mediated Inhibition of Collecting Duct ENaC

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On a normal salt diet (NSD) flox control and principal cell-specific collecting duct NOS1 knockout (CDNOS1KO) mice display similar blood pressure (BP). On high salt diets, CDNOS1KO mice retain Na with significantly increased BP. Given that ET-1 increases NO in the CD, we hypothesized that CDNOS1KO mice have a dysfunctional renal ET pathway. To test this hypothesis, we measured urinary ET-1 excretion, inner medullary (IM) ET receptor (ETR) expression from mice on a NSD or 7 day high salt diet (7DHS) and the response of ET-1 mediated epithelial sodium channel (ENaC) activity in flox and CDNOS1KO mice. ET-1 excretion was similar between NSD flox and CDNOS1KO mice (0.14 ±0.02 and 0.17 ± 0.06 pg/day, $n = 10$) and significantly increased similarly after 7DHS (0.60 ± 0.1 and 0.60 ± 0.08 pg/day). IM ETR expression was similar between the mice on a NSD (~40% ETA, ~60% ETB receptors) and similarly shifted to ~95% ETB expression on a 7DHS. Basal CD ENaC open probability (Po) was similar (flox: 0.3 ± 0.08 CDNOS1KO: 0.3 ± 0.05). Acute ET-1 treatment significantly reduced ENaC Po from flox mice but not CDNOS1KO mice compared to basal (flox 0.1 ± 0.03 and CDNOS1KO 0.3 ± 0.05, $n = 6$ animals $P < 0.05$). In conclusion, CD NOS1 appears not to regulate renal ET-1 production or ETR expression. However, the mechanism of ET-1 inhibition of CD ENaC is via NOS1. We propose that the salt-dependent increase in BP and Na retention observed in CDNOS1KO mice is mediated by the loss of ET signaling in the CD.

P-33

Increased of Heparanase Expression in Hipoxic Endothelial Cells and Kidney Ischemic-Reperfusion Injury Associates with Endothelin-1 Elevation and eNOS Reduction

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Renal ischemia/reperfusion injury (I/R) is the most frequent cause of acute kidney injury. It had been reported that endothelin-1 deletion from endothelial cells attenuates I/R injury. Heparanase is an enzyme that degrades endothelial surface layer and induce endothelial injury. The association between heparanase and ET-1 in kidney I/R is still unclear. We induced hipoxic condition for 30 minutes in the MS-1 endothelial cells culture using hipoxic bag. We extracted RNA and quantified pre-pro-ET1, heparanase, endothelial Nitrite Oxide Synthase (eNOS) and ICAM-1. To examine heparanase contribution in I/R, we performed kidney I/R injury model in black-six mice (n=7) using renal pedicle clamping for 30 minutes and sacrificed the mice in 1, 3 and 24 hour after operation. Sham-operation procedure (SO, n=5) was used as control. PAS was used to quantify tubular injury score. Serum creatinine was quantified from orbital venous. We did immunostaining for heparanase and double glycocalyx-von Willebrand factor to elucidate contribution of heparanase in the early step of ischemic acute kidney injury. Western-blot was used to analyze eNOS expression.Ischemic induced a significant increase of pre-proET-1 and heparanase mRNA expression, that were associated ICAM-1 elevation and eNOS reduction. In-vivo, we found elevation of heparanase mRNA expression in the early of I/R injury (1,3 and 24 hour). This associated with increase of tubular injury score, creatinine serum level and eNOS reduction. Further analysis, EC derived ET-1 significantly reduced heparanase mRNA ($p<0.05$) expression after kidney I/R injury. In this study, we suggested that heparanase might contribute to the ET-1 effect in inducing endothelial injury in hipoxic and kidney I/R condition.

P-34

Endothelial Cells-Derived Endothelin-1 Exaggerates Kidney Fibrosis Through ETAR Activation in Renal Interstitial Cells

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Kidney fibrosis is a final pathway of Chronic Kidney Disease (CKD) and characterized by myofibroblast formation from renal interstitial cells. Endothelin-1 and its receptors involved in CKD, however clear mechanism in interstitial cells proliferation is still unknown. We performed Unilateral Ureteral Obstruction (UUO) in Vascular Endothelial Endothelin-1 Knock-Out (VEETKO, n=7) and WT mice (n=7), then sacrificed in day 3 and 14. We observed renal fibrosis, myofibroblast area, and capillary number using Sirius Red, α-SMA, and CD31 immunostaining. Double α-SMA and PDGFRβ staining and quantification were done to examine interstitial cells expansion. Renal blood flow was observed and quantified by laser Doppler imaging. Western blot was done to examine α-SMA, PDGFRβ and TGFβ1 expression. Kidney ET-1 system was measured using ELISA and real time PCR. Double α-SMA and ETAR immunostaining was done to elucidate ETAR in myofibroblast cells. We found significantly lower fibrosis, myofibroblast area, and TGFβ1 expression ($p<0.05$) in VEETKO mice compare to WT. Kidney ET1 and pre-pro ET-1 mRNA levels increased after UUO, however significantly lower in VEETKO mice. VEETKO mice also had significantly lower interstitial cells expansion and myofibroblast area compare to WT. EC derived ET-1 deletion also improved renal blood flow and capillary number ($p<0.05$) after UUO. We observed ETAR expression in myofibroblast area and collocollized with PDGFRβ. EC derived ET-1 deletion attenuates kidney fibrosis via preserving capillary, reducing interstitial cells expansion and myofibroblast formation. ETAR from interstitial cells may induce proliferation and myofibroblast formation. Targeting ET-1 and ETAR axis in EC and interstitial cell may give best approach to treat kidney fibrosis.

P-35

Hypoxia Stimulates Glomerular Reactive Oxygen Species Through an Endothelin-1/ET-A Dependent Mechanism

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Sickle cell nephropathy (SCN) is a chronic manifestation of sickle cell disease (SCD). We previously found that reactive oxygen species (ROS) were elevated in glomeruli from SCD mice. Importantly, treatment with ABT-627, a selective ET-A receptor antagonist, reduced glomerular ROS production in SCD mice to levels observed in controls. Because SCN is thought to progress secondary to repeated occlusions in the microvasculature, we hypothesized that hypoxia stimulates the production of glomerular ET-1, causing deleterious effects through the over-production of ROS. To directly test if ET-1 is increased in response to hypoxia in unaffected mice, we exposed vascular endothelial ET-1 knockout (VEET) mice and floxed controls to normoxia or 3 hours hypoxia (8%O2). In response to hypoxia, floxed mice had significant increases in glomerular ET-1 mRNA while there was no response to hypoxia in the VEET mice, indicating hypoxia stimulates endothelial-derived glomerular ET-1 (floxed hypoxia: 2.1±0.2, floxed normoxia: 1.1±0.2, VEET hypoxia: 0.7±0.1, VEET normoxia: 0.8±0.3 fold change, $p<0.01$, n=6/group). To determine the influence of chronically elevated ET-1 on glomerular ROS production, C57BL/6J mice were treated with saline or ET-1 (2 weeks @2pg/kg/day) via miniosmotic pump. Mice treated with ET-1 demonstrated significant increases in stimulated ROS production compared to saline controls (5452±655 vs. 2177±359 luminescence/protein*min, $p<0.01$, n=5-6). These data reveal that hypoxia leads to upregulation of endothelial-derived glomerular ET-1 and that chronic elevations in ET-1, similar to what is seen in SCD, increases ROS production in glomeruli. Taken together, these data identify a novel mechanism by which hypoxia stimulates the upregulation of ET-1 in glomerular endothelial cells and promotes glomerular ROS production.

P-36

Combined Endothelin A Receptor and Renin-Angiotensin System Blockade is Superior to Isolated Renin-Angiotensin System Blockade Against the Progression Renal Damage in 5/6 Nephrectomized Ren-2 Transgenic Hypertensive Rats

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Hypertension plays a critical role in the progression of chronic kidney disease (CKD). Recent studies have shown that besides the inappropriately activated renin-angiotensin system (RAS) also enhanced intrarenal activity of endothelin (ET) system via activation of ET receptors type A (ETA) contributes to the pathophysiology of hypertension and progression of CKD. We therefore evaluated whether addition of selective ETA receptor blockade to the standard RAS blockade will exhibit additional beneficial effects on the progression of CKD. Ren-2 transgenic rats (TGR) underwent 5/6 renal ablation (5/6 NX) serving a model of CKD. A combination of angiotensin-converting enzyme inhibitor (trandolapril, 6 mg/l drinking water) and angiotensin II receptor blocker (losartan, 100 mg/l drinking water) was used. ETA receptor blocker (atrasentan, 5mg.kg-1.day-1) was employed with the combination of RAS blockade. The follow-up period was 44 weeks after 5/6 NX. Following parameters were evaluated: survival rate, systolic blood pressure (SBP), proteinuria and renal glomerular damage. Both therapeutical regimes improved survival rate, however the efficiency of isolated RAS blockade considerably decreased at 36 weeks after 5/6 NX (final survival rate was 65%). The combined RAS and ETA receptor blockade exhibited final survival rate 91%, which was significantly better as compared with isolated RAS inhibition, even if there were no significant differences in SBP among experimental groups. In addition, the RAS and ETA receptor blockade further reduced proteinuria and renal glomerular damage. Our data show that a combined RAS and ETA receptor blockade exhibited additional beneficial effects on the progression of CKD in 5/6 NX TGR as compared with isolated RAS inhibition.

P-37

Endothelial Cell-Derived ET-1 Contributes to the Severity of Septic Kidney Injury

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Septic acute kidney injury (AKI) remains associated with high mortality rate, partially, because of the poor understanding of its (patho)physiology. Endothelin-1 (ET-1), which is produced and is secreted from vascular endothelium, plays important roles on renal hemodynamics via autocrine/paracrine and, possibly, hormonal mechanisms. Previous studies have demonstrated that either selective or non-selective endothelin antagonists attenuate the hemodynamic changes in experimental sepsis models. Thus, we investigated the role of endothelial cell-derived ET-1 on the severity of lipopolysaccharide (LPS)-induced AKI by using endothelial cell-specific ET-1 knock-out mice (VEETKO). The renal microcirculation dynamics was observed by intravital 2-photon laser microscopy. LPS (from E. coli O55:B5, 5mg/kg, i.p.) increased leukocytes attachment in the capillary walls, whereas there was maintained plasma flow in the peritubular capillaries in VEETKO. The urine flow rate was reduced to half in VEETKO compared with that in normal (LPS-untreated) C57B6 mice, while the blood urea nitrogen level was still at normal level in VEETKO (22.8±0.2 mg/dL vs. 52.9±12.7 mg/dL in C57B6) at 24h after LPS injection. Fluid resuscitation (1.5 mL of saline, s.c., at 6 and 14h after LPS) normalized the LPS-induced increase in leukocyte attachment and the decrease in urine flow rate in VEETKO, but the effects were partial in C57B6 mice. These results suggest that VEETKO kidney is less sensitive against endotoxemia than the kidney of C57B6 mice, and that leukocyte attachment and reduction of urine flow in VEETKO may be due to the decrease in blood pressure by endotoxemia in combination with the lack of ET-1.

P-38

Renal Phenotype of Type 1 Diabetic Endothelial Cell Derived ET-1 Deficient Mice

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Endothelin-1 (ET-1) has been shown to have an important role in diabetic nephropathy (DN). Here we investigated the contribution of endothelial cell-derived ET-1 to the changes in renal phenotype of diabetic mice. Therefore, we induced type 1 diabetes in vascular endothelial cell-specific ET-1 knockout (VEETKO) mice and their wild type (WT) littermates by streptozotocin injection (i.p. 50mg/kg/day, five consecutive days). After ten and 22 weeks of diabetes, we observed an increase of food and water intake, urine volume and creatinine clearance in both genotypes compared to non-diabetic mice. The renal cortical expression of ET-1 mRNA was not significantly activated by diabetes. Nevertheless, VEETKO mice showed only about half of ET-1 mRNA expression compared to WT mice. Though, there were no significant differences in the renal function including albumin and protein excretion between the genotypes. Based on hematocrit measurements, neither diabetes nor ET-1 deficiency had an impact on fluid retention. After ten weeks of diabetes, systolic blood pressure measured by tail-cuff method decreased in WT mice. After 22 weeks, heart rate, systolic and diastolic blood pressure were similar between all groups. At baseline the kidneys of VEETKO mice were significant heavier (+15%) compared to WT but this gap was not observed in diabetic condition.In contrast to previously presented data, in this model of type 1 diabetes we are not able to confirm the pivotal role of endothelial cell derived ET in the development of DN.

P-39

Absence of ETA Receptors on Podocytes is Not Antialbuminuric in Diabetic Mice

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BACKGROUND: Endothelin receptor antagonists reduce albuminuria in diabetic patients. However, adverse effects related to fluid retention and attributed to the blocking of the tubular endothelin receptors prevent the use of ERA clinically. The endothelin A (ETA) receptors on podocytes might be implicated in albuminuria during diabetes. We hypothesized that the suppression of the ETA receptors on the podocytes may reduce albuminuria without affecting the tubular functions. METHODS AND RESULTS: To address this question, we generated podocyte specific ETA deficient mice using ETA floxed mice mated with mice expressing the Cre recombinase under the control of the nephrin gene (PodoETAKO mice). We induced type-1 diabetes in seven-week old male PodoETAKO mice and their wild type (WT) littermates by streptozotocin injection (i.p. 50mg/kg/day, five consecutive days). A set of animals were treated with macitentan, a dual ETA/ETB antagonist (25mg/kg/day, orally, mixed with food). The hyperglycemic mice developed glomerular hyperfiltration, renal hypertrophy, reduced serum creatinine levels, albuminuria, tubular injury, glomerular hypertrophy and inflammation. After 20 weeks of diabetes, the absence of ETA receptors on podocytes had no effect on these parameters. Macitentan treatment however reduced albuminuria and restored serum creatinine levels in wild type mice. Interestingly, the effects of macitentan were diminished in PodoETAKO mice. Similarly, macitentan increased podocyte number per glomerulus (WT-1 positive cells) and glomerulus size in WT but not in PodoETAKO mice. Neither ETA deficiency nor macitentan treatment increased water retention measured as free water clearance. CONCLUSION: In contrast to systemic dual ETA/ETB receptors blockade, the suppression of the ETA receptors on podocytes is not antialbuminuric in diabetic mice.

P-40

ET-1 Plasma Levels, Choroidal Thickness and Multifocal Electroretinogram in Retinitis Pigmentosa

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Retinitis Pigmentosa (RP) is an inherited retinal disorder characterized by bone spicul pigment in the retina, attenuated retinal blood vessels and a pale, waxy optic nerve head with early visual field contraction and decrease of t he electroretinogram (ERG). Retinal hemodynamic impairment is still present in early stages of RP and various hypotheses have been advanced as to a cause. We studied with multifocal electroretinogram (mfERG) of the macula and optical coherence tomography (OCT) of the choroid 24 patients, 14 males and 10 females, aged 63-45 yrs. (mean 55±7 yrs.) and affected by simplex RP. The patients had a visual acuity of 0.1 logMAR with a mean defect (MD) of the visual field of -12.32±8.48 dB, a pattern standard deviation index (PSD) of 6.09±4.22 dB and a b-wave electroretinogram (ERG) amplitude of 45.08±8.24 &muV. An increase of endothelin-1 (ET-1) plasma levels was found: 2.143±0.258pg/ml vs. 1.219±0.236 pg/ml in non-RP controls ($p<0.002$). The choroidal thickness was 226.75±76. 37 µ vs. 303.9±39.87 µ ($p<0.002$) in normal controls. The Spearman's correlation test highlighted that the decrease of choroidal thickness ($r=-0.702$; $p<0.023$) and the increase of time latency in the rings 2 ($r=-0.669$; $p<0.034$) and 3 ($r=-0.883$; $p<0.007$) of mfERG was related to the increase of ET-1 plasma levels. It is thought that an increase in ET-1 in our RP could lead to a vasoconstriction in the choroidal vessels and worsening the abiotrophic process of the macular photoreceptors with increase of the conduction implicit time.

PC-1

Clinical Value of Plasma Pentraxin 3 Levels for Predicting Cardiac Troponin Elevation After Percutaneous Coronary Intervention

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Background: Percutaneous coronary intervention (PCI) is often complicated by post-procedural myocardial necrosis as manifested by elevated cardiac troponin. Plasma pentraxin 3 (PTX3) levels are increased in patients with arterial inflammation, especially unstable angina pectoris (AP). The study tested whether plasma PTX3 levels can predict post-PCI cardiac troponin T (TnT) elevation. Methods: We evaluated 94 consecutive patients with AP of normal pre-PCI TnT levels who underwent PCI. Pre-PCI virtual histology-intravascular ultrasound was performed to assess culprit plaque composition. Plasma PTX3 levels and serum hs-CRP levels were measured at pre-PCI. Patients were divided into 2 groups according to the presence (Group I, n=34) or absence (Group II, n=60) of post-PCI TnT elevation >3×the upper limit of normal at 24 hours after PCI. Result: Plasma PTX3 (4.06±2.05ng/ml vs 2.17±1.02ng/ml, P<0.001) and serum hs-CRP levels (0.25±0.03 vs 0.16±0.03, P=0.048) were significantly higher in Group I than in Group II. Plaque burden (80.9±5.3 vs 75.4±10.6%, P=0.047), incidence of positive remodeling (59 vs 25%, P=0.034), and percent necrotic core area (19.0±7.4 vs 14.0±5.9%, P=0.046) were significantly higher in Group I than in Group II. By ROC analysis, plasma PTX3 levels (AUC 0.823) well discriminated prediction for post-PCI cardiac TnT elevation in comparison with serum hs-CRP levels (AUC 0.618). Best predictive values of plasma PTX3 levels were 2.83ng/ml. In the multiple logistic regression analysis, plasma PTX3 levels were most independent predictors of post-PCI cardiac TnT elevation. Conclusion: Plasma PTX3 levels may be a useful marker for predicting post-PCI cardiac TnT elevation, which is associated with inflammatory status of culprit lesions.

PC-2

Quantitative Determination of Diastolic Suction Using with Vector Flow Mapping

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Background: Suction flow can be visualized with color Doppler images. However, characteristics of suction flow have never been quantified. Vector flow mapping (VFM) can be used to assess intraventricular hemodynamics quantitatively. The purpose of the present study was to assess the magnitude and timing of suction flow kinetic energy and to investigate the relationship between left ventricular (LV) function and geometry in heart failure patients.

Methods: We studied 24 subjects with elevated LV filling pressure (eFP group) and 36 normal subjects (normal group) were enrolled. Suction was defined as the apical flow directing to the apex during the period from soon after the ejection to before mitral inflow. Flow kinetic energy was quantified as the sum of the products of blood mass and velocity vector using VFM. We measured magnitude and time to peak suction kinetic energy.

Results: Suction was recognized 12 patients (50%) in eFP group and 36 subjects (100%) in normal group. eFP group showed significantly smaller suction kinetic energy than normal group (2.7±3.8 vs. 5.7±4.4 g/sec/cm2, P<0.01). eFP group with suction showed smaller LV end systolic volume (ESV) (P<0.05), more ellipsoidal geometry (P<0.05), and greater untwisting rate (P<0.01) than those patients without suction. Regression analysis indicated a significant linear relation between suction kinetic energy and LVEF (r=0.48, P<0.01), eccentricity index (r=0.27, P=0.04), and untwisting rate (r=0.03, P=0.04).

Conclusion: Magnitude of the suction flow kinetic energy derived from VFM may allow quantitative assessment of suction flow, which correlates to LV systolic function, geometry, and untwisting mechanics.

PC-3

The Anti-Hypertensive Effect of Radiofrequency Renal Denervation with a Reduction of Renal Tissue Norepinephrine Content in the Spontaneously Hypertensive Rats

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Introduction: The renal denervation (RDN) has been featured as a novel treatment for hypertension. In animal models of hypertension, a surgical RDN by cutting nerves with staining phenol has been reported; however, a radiofrequency (RF) energy has been rarely applied for the RDN. We aimed to evaluate the effects of RF-RDN in the spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Methods: The RF energy was applied to the bilateral renal arteries by using a 2Fr catheter placed through an abdominal incision under anesthesia in 8 SHR and 8 WKY. The sham operation was performed in the other 8 SHR and 8 WKY. The systolic and diastolic blood pressures (BP) and urinary norepinephrine secretion were followed up for 3 months after the operations. The RDN was confirmed by a decrease in renal tissue norepinephrine content as compared to the sham operation. Results: The RF-RDN in the SHR restrained a spontaneous rise in systolic BP (46±12% increase from 158±8 to 230±14 mmHg vs. 21±18% increase from 165±9 to 197±20 mmHg, p=0.012) and diastolic BP (55±27% increase from 117±9 to 179±23 mmHg vs. 28±13% increase from 120±7 to 154±13 mmHg, p=0.035). The systolic and diastolic BP in the WKY, however, were not affected by the RF-RDN. Although the RF-RDN did not affect the urinary norepinephrine secretion, the renal tissue norepinephrine content was decreased by the RF-RDN in the SHR (302±41 vs. 159±44 ng/g kidney, P<0.001) and WKY (203±33 vs. 145±26 ng/g kidney, p=0.010). Conclusion: The RF-RDN demonstrated the anti-hypertensive effect with a reduction of renal tissue norepinephrine content in the SHR.

PC-4

The Case of Pulmonary Veno-Occlusive Disease Succeeded in Administration of Sildenafil by Dose Adjustment

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We report the case of a 49 year-old woman with pulmonary veno-occlusive disease (PVOD) who was available to take a vasodilator, sildenafil, by dose adjustment. In 2001, she complained dyspnea on exertion and was diagnosed as idiopathic pulmonary arterial hypertension; she started receivingberaprost sodium; however, she discontinued it due to decrease of oxygen partial pressure.Based on that history and on chest computed tomographic finding of smoothy thickened interlobular septa, we diagnosed her as PVOD. In 2005, bosentan was administered without any adverse reaction. In November 2012, she complained dyspneamoreworsened; she showed the decrease of six-minute walk distance (6MWD) to 140m and elevation of right ventricular systolic pressure (RVSP) estimated by echocardiography to 89mmHg. Sildenafil was initiated from a dose of 20mg per day and increased to 40mg in addition to bosentan without acute adverse events. Her dyspnea was markedly improved; 6MWD was extended to 225m; and RVSP was diminished to 62mmHg. When thedoseof sildenafil reached to 60mg, she complained breath shortness again; RVSP roseto 85mmHg and 6MWDdiminished to 180m. We reduced the dose to 40mg and her symptom was soon disappeared; 6MWD improved to 250m. Right heart catheterization revealed reduction of pulmonary vascular resistance (from 2057 to 1658 [dyne・s・cm-5]) and mean pulmonary arterial pressure (from 66 to 60 [mmHg]) compared to those of pre-administration of sildenafil. Although it is difficult to predict the unfavorable response of pulmonary vasodilators in patients with PVOD, sildenafil would be an effective and feasible drug if you carefully determine the dosage.

PC-5

Prognostic Significance of Remaining Severe Left Ventricular Diastolic Dysfunction after Cardiac Resynchronizaion Therapy

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Background: CRT (cardiac resynchronization therapy) improves systolic dysfunction and causes left ventricular (LV) reverse remodeling in patients with drug refractory heart failure. Several studies have demonstrated that reverse remodeling predicts better prognosis. However, prognostic impact of LV diastolic dysfunction is unknown. This study sought to clarify the impact of CRT on diastolic function and prognosis.
Methods: In 68 patients who performed CRT, LV diastolic function was determined by Doppler trasmitral flow pattern immediately after CRT, and classified into restrictive filling pattern (RFP) and non-RFP groups. RFP was defined as the ratio early to late peak velocity (E/A) $\geqq$2 and the deceleration time of E $\leqq$160ms. The clinical endpoint comprised time to death from any cause or unplanned hospitalization for a major cardiovascular event. Responders were defined by reduction of LV end-systolic volume >15% at 6months after CRT.
Results: CRT had acute effect on sifting from RFP to non-RFP in 6 patients.
In responders, E/A was decreased from 1.7 to 1.3, however, in non-responders, E/A was increased from 1.8 to 2.2 (P<0.05, vs. responders). RFP immediately after CRT was significantly related to worse prognosis than non-RFP (Log-rank, p=0.04, HR 3.8, 95%CI 1.56-9.12), whereas responder did not represent better prognosis than non-responders.
Conclusions: CRT improves diastolic function in responders. Remaining severe LV diastolic dysfunction after CRT is the strong prognostic predictor.

PC-6

Elevated Blood Pressure in Resting Daytime-Phase in A170/p62-Knockout Mice, a Newly Established Obese Model

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A170, also called p62/ZIP/sequestsome1, is an oxidative stress-inducible protein, which is partly regulated by the transcription factor Nrf2. To examine the role of A170 in vivo, we have created A170-knockout mice (A170-KO). A170-KO exhibited mature-onset obesity (by 1.8-fold body weight [BW] increase at 50-week old) and impaired glucose tolerance. As the gain of BW in A170-KO, the amount of food intake was increased. The restriction of feeding inhibited BW increase and other phenotypes. Although the intraventricular injection of leptin reduced the amount of food intake in wild type (WT), it did not changed in A170-KO. It suggests that A170-KO is a hyperphagia-induced obese model with leptin resistance. Next, we measured daily blood pressure (BP) by a telemetry system. During light resting phase, BP was significantly higher in KO than in WT (systolic BP, 124±1 [mean±SE] vs 113±2 mmHg, n=5, P<0.001), however, BP did not differ between both groups during dark active phase. It suggests that A170-KO exhibited non-dipper type of BP elevation. Heart rate during light resting phase was significantly higher in A170-KO than in WT (562±4 vs 521±3 bpm, P<0.001). Furthermore, total amount of urinary excretion of both adrenalin and noradrenalin was significantly higher in A170-KO than in WT, suggesting that sympathetic nerve activity is augmented in A170-KO.
Conclusion: A170-KO is an obese model, which has characteristics of metabolic syndrome. The cause for BP elevation in resting phase in A170-KO may partly be the increase in sympathetic nerve activity.

PC-7

Relationship between Plasma Klotho Concentration and Physical Activity Level in Middle-Aged and Elderly Women

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Background: Regular exercise is an efficacious therapy for preventing cardiovascular disease and aging. Klotho, an anti-aging protein, has been known to decrease with advancing age. However, it has not been clarified the effect of physical activity on plasma Klotho concentration. The purpose of this study was to investigate the relationship between plasma Klotho concentration and physical activity level in middle-aged and older adults.

Methods: Thirty postmenopausal women (48-67 years) participated in this study. We measured plasma Klotho concentration and physical activity (PA) level. Subjects were divided into inactive lifestyle (inactive) and active lifestyle (active) groups, with the dividing line set at the median value of PA level per day.

Result: PA levels were 225±20 kcal/day in inactive group and 482±21 kcal/day in active group ($P< 0.05$). Plasma Klotho concentration in active group was significantly higher than in inactive group ($P< 0.05$). Plasma Klotho concentration was positively correlated with PA level ($P< 0.05$). After adjusted with age and BMI, there was a significant association between plasma Klotho concentration and physical activity level ($P< 0.05$).

Conclusion: We showed a significant and positive correlation between plasma Klotho concentration and physical activity level in middle-aged and elderly women. These results suggest that regular exercise may increase Klotho production.

PC-8

Relationship between Plasma Asymmetric Dimethylarginine Concentrations and Aerobic Exercise Capacity in Postmenopausal Women

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Background

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide (NO) synthase, an enzyme responsible for generation of NO. Plasma concentration of ADMA increases in elderly people and in postmenopausal women. Elevated ADMA leads endothelial dysfunction and increases risk for cardiovascular disease. Habitual aerobic exercise has a favorable effect on vascular aging. However, the relationship between ADMA and aerobic exercise capacity is unknown. The aim of this study was to determine whether plasma ADMA concentrations are associated with aerobic exercise capacity.

Methods

Thirty healthy, postmenopausal women aged from 50 to 76 years, participated in this study. We measured plasma concentrations of ADMA, and oxygen consumption at ventilatory threshold (VO2VT), an index of aerobic exercise capacity. Plasma ADMA concentrations was measured by enzyme-linked immunosorbent assay. VO2VT was measured during the incremental cycle ergometer exercise with respiratory gas analyzer. Each individual VO2VT was determined using regression analysis of the slopes of CO2 production, O2 uptake, and the minute-ventilation plot. Relationship between plasma ADMA concentrations and VO2VT was analyzed using Pearson's correlation. Stepwise regression analysis was used to identify independent associations of plasma ADMA concentrations.

Results

There was negative correlation between plasma ADMA concentration and VO2VT ($R = -0.532$, $P< 0.01$). In addition, stepwise regression analysis showed that plasma ADMA concentration was significantly associated with VO2VT.

Conclusion

We found that plasma ADMA concentrations were associated with aerobic exercise capacity in postmenopausal women. These results suggest that habitual aerobic exercise may decrease plasma ADMA concentrations.

PC-9

Relationship between Digit Ratio and Idiopathic Pulmonary Hypertension in Japanese Women

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Background: The second to fourth digit (2D:4D) ratio is a biometric marker influenced by testosterone concentrations, and covarying with the sensitivity of the androgen receptor in uterus. Some reports described the 2D:4D ratio linked to disease predisposition among patients with gender-dependent diseases. Furthermore sex hormones are also reported to modulate plasma endothelin levels. Since idiopathic pulmonary arterial hypertension (IPAH) is more prevalent in women, we hypothesized the 2D:4D ratio is linked to disease predisposition reflecting the association with sex steroids.
Methods: 13 female patients with IPAH at Keio University Hospital and 41 unrelated age-matched control women were studied. Digital cameras were used to photograph the right hand of patients and controls. Finger lengths and the 2D:4D ratio were measured by two experienced scorers.
Results: Mean age of the patient group and the control was 43.2±3.5 years for IPAH, and 40.9±1.7 years, respectively. The 2D:4D digit ratio was significantly higher for patients with IPAH than for the control women; 0.975±0.042, 0.940±0.039, P<0.05). The age at the onset of IPAH did not correlate with the ratio.
Conclusions: Female patients with IPAH had a higher 2D:4D digit ratio, suggesting prenatal circulating testosterone is lower than that of healthy subjects. Several studies show that the plasma basal levels of ET-1 were increased in male with hypogonadism. In conclusion, the 2D:4D digit ratio is a useful biomarker for IPAH, and prenatal testosterone levels is a important factor to maintain plasma ET-1 levels and have a protective effect against developing IPAH.
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PC-10

The Role of NPBWR1 on Autonomic Nervous System

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Neuropeptide B/W receptor (NPBWR1) is a G-protein coupled receptor whose ligands, neuropeptide B (NPB) and neuropeptide W (NPW) were identified. Intracerebroventricular administration of NPW to rats was reported to increase arterial blood pressure, heart rate (HR), and plasma catecholamine concentration (Yu et al., 2007). To elucidate the role of NPBWR1 in autonomic functional regulation under stress, we examined the phenotype of NPB/WR1 deficient (*Npbwr-/-*) mice. The urinary catecholamines amount of *Npbwr-/-* mice was increased for 24 hours. To elucidate the role of NPW-NPBWR1 on acute stress, we created a stress model being contacted with intruder C57/BL6J mice for 30 minutes and monitored HR, activity, and body temperature using a telemetry system. In *Npbwr1-/-* mice, recovery to the steady state of the HR after contact with the intruder was significantly slower compared with wild type mice and the increase of HR lasted 4 hours. In 12 week-old *Npbwr-/-* mice, cardiac hypertrophy was increased in comparison with the same-aged wild-type mice. After administration of angiotensin II for 2 weeks, the cardiac hypertrophy of *Npbwr-/-* mice tended to deteriorate as compared with wild-type mice.

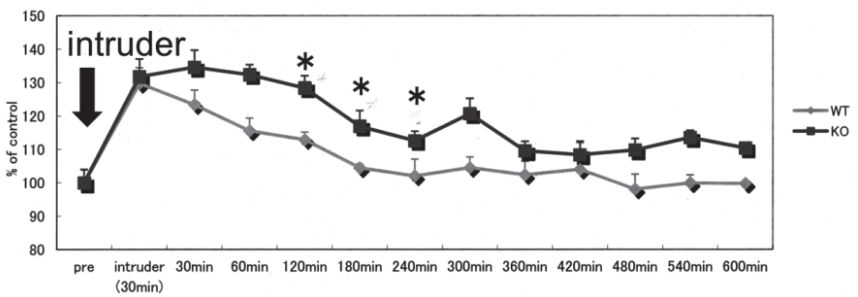

Fig.1 The change of HR (%) after contact to intruder

PC-11

The Hypoxia-Mimetic Agent Cobalt Chloride Induces the Expression of Intrinsic BMP Antagonist Noggin Independently of Endothelin Pathway

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BACKGROUND

Mutations in the bone morphogenetic protein type 2 receptor (BMPR2) are responsible for the majority of cases of heritable pulmonary arterial hypertension (PAH). Low penetrance of BMPR2 mutation in heritable PAH, however, suggests the involvement of second-hit elements in pathogenesis of PAH. We have previously reported that treatment with endothelin-1 induced in vitro increased expression of noggin, an intrinsic bone morphogenetic protein antagonist, in human pulmonary artery smooth muscle cells (PA-SMCs). Moreover, chronic exposure to hypoxia is a well-known inducer of remodeling in pulmonary arteries. However, the potential link between chronic hypoxia exposure and noggin expression has not been elucidated.

AIMS

We hypothesized that hypoxia could induce, in PA-SMCs, the expression of endothelin-1 which could secondarily result in the upregulation of noggin.

METHODS AND RESULTS

Cultured human PA-SMCs were treated during 3, 6, 24, 48 hours with the hypoxia-mimetic agent, cobalt chloride (CoCl2; 100 µM) and gene expressions of preproendothelin-1 (ppET1), endothelin converting enzyme-1 (ECE1) and noggin were then evaluated by QRT-PCR. CoCl2 treatment progressively increased the expressions of ppET1 and noggin, with maximal response after 24 hours and 48 hours of stimulation respectively. Gene expression of ECE1 was not changed. After pretreatment or not with a non selective endothelin receptor antagonist (bosentan), we stimulated PA-SMCs with CoCl2 during 5 hours. Gene expression of noggin significantly increased after CoCl2 treatment and this reaction was not changed by pretreatment with bosentan.

CONCLUSIONS

Noggin, an intrinsic bone morphogenetic protein antagonist, was upregulated by CoCl2, independently of hypoxia-induced endothelin-1 pathway at earlier timing (5 hours).

PC-12

Combination of Polymorphisms in Angiotensin-Converting Enzyme and Estrogen Receptor-Alpha Genes Increases the Risk for Elevation of Arterial Stiffness

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Background: Increased arterial stiffness is an independent risk factor for cardiovascular disease. The -401 T/C and insertion/deletion (I/D) polymorphisms of estorogen receptor-alpha and angiotensin-converting enzyme (ACE) genes are associated with arterial stiffness. We examined the effect of this combination of single-nucleotide polymorphisms on the risk for increased arterial stiffness. **Methods:** Our cross-sectional study comprised 403 middle-aged and older human participants. We determined the genotypes of -401 T/C and I/D single-nucleotide polymorphisms in estorogen receptor-alpha and ACE by TaqMan PCR method. We also measured arterial stiffness by brachial-ankle pulse-wave velocity (baPWV). Subjects were divided into high arterial stiffness and low arterial stiffness groups, with the dividing line set at the median value of baPWV. **Results:** The odds ratio for the presence of high arterial stiffness in individuals having the TT genotype of estorogen receptor-alpha compared with those having the other genotypes (TC and CC) was 2.46. With regard to the I/D polymorphism in ACE, the odds ratio for the presence of high arterial stiffness in individuals having the II genotype of ACE when compared with those having the other genotypes (ID and DD) was 1.99. Interestingly, the odds ratio was 5.31 for individuals having a combination of the TT genotype of estorogen receptor-alpha and II genotype of ACE when compared with those having a combination of TC and CC genotypes of estorogen receptor-alpha and ID and DD genotypes of ACE. **Conclusion:** We revealed that a combination of the TT and II polymorphisms in estorogen receptor-alpha and ACE remarkably increased the risk for elevation of arterial stiffness in middle-aged and older humans.

PC-13

The Impact of RV/LV Volume Ratio on Biventricular Function

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Background: Right ventricular (RV) dilation and dysfunction after corrected tetralogy of Fallot (c-ToF) is associated with their prognosis. In contrast, left ventricular (LV) function has been focused as a novel determinant of prognosis in patients with c-ToF. We aimed to assess RV and LV volume in consideration of interaction with biventricular EF.
Methods: We studied 45 patients with repaired ToF (23 male, 20.8 yrs, range 7-49 yrs). We examined 2-dimmensional and 3-dimmensinoal transthoracic echocardiography. To determine the severity of pulmonary stenosis (PS), we recorded the maximum flow velocity through the pulmonary valve obtained from continuous wave Doppler measurement by 2-dimmensional echocardiography. The pressure gradients were calculated from this velocity using a simplified Bernoulli's equation. RV and LV end diastolic volume index (EDVI, ml/m2), end systolic volume index (ml/m2), stroke volume index (ml/m2) and ejection fractions (EF) were measured with 3-dimensional transthoracic echocardiographic sysytem (RV; Tomtec imaging systems, LV; 4D auto LVQ. GE Vivid E9, Japan).
Results: RVEDVi, LVEDVi and were measured 80.2±22.6 ml/ m2, 53.0±10.1ml/m2. RV / LV EDVI ratio (1.57±0.59) was negatively correlated with RVEF (r=-0.350, p=0.021). In the multivariate stepwise analysis, LVEF was associated with RV / LV EDVI ratio and RVEF (R=0.518). On the other hand, the degree of PS didn't correlate with biventricular volume and function.
Conclusions: LVEF may be affected rather by RV/ LV volume ratio and RVEF in the patients with c-ToF.

PC-14

Immediate Improvement of Pulmonary Hypertension with Out-of-Proportion Physiology After Percutaneous Coronary Intervention for Ischemic Heart Disease

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Introduction: Pulmonary hypertension (PH) due to left heart disease is common, and associated with increased left atrial pressure. In some patients with PH caused by left heart failure, there is entity called 'PH with out of proportion' (further increase of the pulmonary artery pressure evoked by elevated precapillary pulmonary vasoconstriction and vascular remodelling). The treatment of these clinical condition is often difficult. We report a markedly improved case of PH with left ventricular dysfunction caused by coronary artery disease (CAD) which was treated by percutaneous coronary intervention (PCI).
Case presentation: A 68-year-old man was admitted to our hospital for the purpose of detailed examination about CAD. Outpatient examinations including echocardiogram, treadmill exercise test and coronary computed-tomography angiography suggested that he had systolic and diastolic left heart failure because of CAD. Severe pulmonary arterial hypertension without significant valvular disease was also observed by echocardiography and his systolic right ventricular pressure was estimated to be 80mmHg. Coronary angiography showed significant stenosis of right coronary artery (diffuse in mid to distal portion) and left anterior descending artery (proximal portion). Right heart catheterization was performed before percutaneous coronary intervention and revealed severe PH with out-of-proportion physiology. He underwent elective percutaneous coronary intervention for these two vessels. After these interventions, pulmonary wedge pressure, pulmonary artery pressure, and pulmonary vascular resistance were apparently improved, even though in and out valance was almost equal during procedure.
Conclusion: Ischemic left ventricular diastolic dysfunction showed an important impact to the severe pulomonary resistance elevation. Coronary revasculization would be the first line therapy for PH patients with CAD.

PC-15

Diabetes and Obesity Are Significant Risk of Morning Hypertension. From Large Scale Home BP Study: Ibaraki Hypertension Assessment Trial (I-HAT)

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Background: Morning hypertension (HT) has been identified as a major cardiovascular risk, however, the population susceptible to morning hypertension is unknown. This study aimed to clarify the relationship between morning hypertension and diabetes or obesity in large scale population.

Methods and Results: Total of 2,554 outpatients with hypertension at 101 clinics or hospitals were enrolled. Their clinic blood pressures (BPs) and 2-weeks awakening BPs were recorded. The mean office BP> 140/90 mmHg or awakening BP>135/85 mmHg was considered as HT. Subjects were classified into four groups on the basis of office BP and home BP, normal BP, white coat HT, masked HT and sustained HT. The morning BP (mmHg) elevated significantly and progressively in order normal glucose tolerance (134.1 +/- 12.2), impaired glucose tolerance (135.4 +/- 13.1), and diabetic patients (137.5 +/- 11.5) (P<0.0001). Incidence of morning HT also increased significantly and progressively in the same order (53.4%, 55.6%, 66.4%, P<0.0001). Moreover, the morning BP of obese diabetic patients was significantly higher than that of non-obese and non-diabetic patients (138.8 +/- 10.5, 133.1 +/- 11.9, P<0.0001). In addition, the incidence of morning HT in obese diabetic patients was significantly higher than the others (73.0%, 49.9%, P<0.0001).

Conclusion: Morning hypertension is frequent in diabetic or obese patients.

Session 4: Resistant Hypertension

Invited Lecture 3

Treatment-Resistant Hypertension: The Challenges for Drug Treatment

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Resistant hypertension is present in patients who have a blood pressure above target despite treatment with 3 or more drugs, including a diuretic. Poor adherence to treatment is a major factor to explore before making a reliable diagnosis of treatment-resistant hypertension (TRH). Directly observed therapy and ambulatory blood pressure monitoring are important adjuncts to this diagnosis, which is clinically important because people with TRH have poor cardiovascular outcomes. After combining an ACE inhibitor/ARB, calcium antagonist and diuretic, cheap and effective next steps include additional diuretic therapy, a mineralocorticoid antagonist, an alpha-adrenoceptor antagonist or beta-adrenoceptor antagonist. Currently, it is not clear which approach offers the greatest benefit.

More recently, endothelin (ET) receptor antagonists (ETRAs) have undergone clinical trials in this indication; in particular the landmark studies with darusentan (DORADO and DORADO-AC). Although most observers would argue that darusentan was both safe and effective in this indication [1], the drug failed to achieve its predetermined primary endpoint in phase 3 trials, and the drug was not take forward. Together with data from studies with bosentan in primary hypertension, sitaxentan in hypertensive chronic kidney disease, and atrasenten in diabetic nephropathy, these agents seem not only to lower blood pressure, but also to reverse arterial stiffness, endothelial dysfunction and renal dysfunction (in terms of a proteinuria surrogate). On the other hand, ETRAs cause fluid retention and are teratogenic, and the balance of efficacy against safety and cost (now low for what are mostly generic drugs) remains unclear. While there may still be a market for a new agent in TRH, drug therapy will now have to compete with devices such as those used in renal denervation therapy and baroreceptor stimulation therapy.

[1] Webb DJ. DORADO: opportunity postponed: lessons from studies of endothelin receptor antagonists in treatment-resistant hypertension. *Hypertension* 2010:**56**:806-7.

Invited Lecture 4

Impact of Neuromodulation on Pressure Dysregulation -From Hypertension to Hypotension-

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In the human body, all cells, tissues, organs, and systems operate coherently. The presence of well-developed neurohumoral communications among these components of the body is the essential infrastructure that makes coherent functioning possible. Since the design goal of the cardiovascular system is to provide adequate perfusion to peripheries, the normal operation of cardiovascular regulatory system is vital. Recent investigations indicated that the neurohumoral regulatory system plays a central role in the pathogenesis of refractory cardiovascular disease such as hypertension, hypotension and heart failure. The fact that one cannot sustain arterial pressure even for a few seconds in the upright position without baroreflex indicates that pressure stabilization is an essential requirement to maintain homeostasis. Hence the vasomotor center in the brainstem regulates the cardiovascular system mainly through the autonomic nervous system, we may be able to intervene the function of cardiovascular system nonpharmacologically if we can artificially modulate the autonomic nerves.
The case of central baroreflex failure is an archetypal pathophysiology requiring such an intervention. In treating this disease, it is conceivable that one can implement an artificial baroreflex system as a kind of biological proxy capable of emulating the native central baroreflex function of the failing vasomotor center. The artificial baroreflex system consists of a pressure sensor, microprocessor and nerve stimulator for activation of sympathetic efferent nerves. The system operates as an intelligent negative feedback regulator, and has been demonstrated early in the 2000s to be effective in restoring normal baroreflex functioning. The clinical impact of direct manipulation of autonomic functions is very profound, particularly in the treatment of refractory cardiovascular disease. We would like to discuss the principle of closed loop neuromodulation, implementation of the artificial regulators into the cardiovascular systems and renal denervation as well as baroreflex activation in managing refractory hypertension.

Session 5: Neurology, Pain and Stroke

Invited Lecture 5

Targeting Endothelin Axis to Treat Pain

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Endothelin participates in a variety of conditions that cause pain. Local injection of ET-1 produces pain which is independent of its vasoactive properties. Pain due to inflammation, nerve injury and cancer has been studied extensively. Most of the studies indicate that ETA receptor antagonists decrease nociception; and some studies indicate that ETB receptor stimulation may be antinociceptive; while other indicate that ETB receptor antagonism may be antinociceptive. However, the idea of ETB receptor stimulation having antinociceptive effect is gaining ground. It was demonstrated that methylation of ETB receptors which results in transcription silencing is present in painful cancerous tissues and not in normal tissues from the same patients. Preclinical studies with ETA receptor antagonists demonstrated potential for reducing pain, and some clinical phase II studies also showed the benefit of using ETA receptor antagonists in reducing cancer pain. In experiments carried out in mice, with deletion of ETA receptors selectively in nociceptive sensory neurons, while preserving expression in non-neuronal cells and the CNS, it was found that ETA receptors are important in the modulation of cancer pain and act independent of ETA receptor function in tumor cells. However, human phase III studies have failed to demonstrate any benefit in reducing pain of cancer patients. It has been found that ETA receptor antagonism predominantly mediates its antinociceptive action through endogenous opiates. Secretion of beta-endorphin and leu-enkephalin is increased by ETA receptor antagonists. ETA receptor antagonists potentiate morphine and oxycodone antinociception in rats and mice; and tolerance to opioid agonists was reversed by treatment with ETA receptor antagonists. Moreover, ETA receptor antagonists enhanced G-protein coupling to opioid receptors and restored morphine analgesia in tolerant animals. This creates the possibility of combination therapies with opioids and ETA receptor antagonists to manage pain in cancer patients with lower doses of opiates and reduced probability of tolerance development.

O-9

Endothelin B Receptor Agonist, IRL-1620, Enhances Neurovascular Remodeling Following Cerebral Ischemia in Rats

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Endothelin B (ETB) receptor agonist, IRL-1620, has been shown in previous studies, conducted in our lab, to provide significant neuroprotection at both 24 hours and 1 week following permanent cerebral ischemia. It is possible that IRL-1620 may be neuroprotective due to angiogenesis and neurogenesis. However, the effect of IRL-1620 on neurovascular remodeling following cerebral ischemia has not been established. The present study was conducted to determine the effect of IRL-1620 [Suc-[Glu9, Ala11, 15]-Endothelin-1 (8-12)] on astrocytes, neurons, and vascular endothelial cells after the induction of cerebral ischemia. Male Sprague-Dawley rats undergoing permanent middle cerebral artery occlusion (MCAO) received three intravenous injections of either vehicle or IRL-1620 (5 µg/kg) at 2, 4, and 6 hours post occlusion. Brain tissues of animals euthanized at 24 hours or 7 days post occlusion were processed for immunofluorescent labeling of ETB receptors, astrocytes, neurons, and vascular and neuronal growth factors. At 24 hours post occlusion, IRL-1620 treatment increased ETB receptor expression and preserved neuronal numbers in the cortex, striatum and subventricular zone of the ischemic rat brain. IRL-1620 also enhanced the number of blood vessels labeled with vascular endothelial growth factor (VEGF) when compared to vehicle treatment. By 1 week following MCAO, VEGF-positive vessels/30 µm brain slice in the IRL-1620 group numbered 11.33±2.13 versus 4.19±0.79 in the vehicle group ($P<0.01$). Additionally, animals receiving IRL-1620 displayed an increased number of proliferating cells ($P<0.0001$) and cells positively staining for nerve growth factor ($P<0.0001$) in the infarcted brain. Results of the present study indicate that IRL-1620, administered on the day of infarct, is neuroprotective and enhances neurovascular remodeling following cerebral ischemia.

O-10

Endothelin Receptor A as a Modulator of Photoreceptor Signaling

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We have previously demonstrated that the endothelin receptor A (ETRA) is selectively localized in the synaptic terminals of photoreceptors (Torbidoni et al., 2005 and 2006). To test the function of this endothelinergic receptor in visual signaling, we tested the effect of clazosentan, an ETRA inhibitor, in the mouse retina (Balb-c strain).First, we evaluated the effect of clazosentan on activation of c-fos in nuclei of the inner retina after physiological stimulation (0.3 lux at 2 Hz for 60 min). The number of c-fos+ nuclei, reflecting transmission of visual stimuli, was significantly higher in clazosentan treated animals than in controls. Physiological light stimulation also increased immunoreactivity for postsynaptic density protein 95 (PSD95) in photoreceptor terminals of mice receiving clazosentan. After phototoxic exposure (1500 lux for 48 h), PSD95 immunoreactivity was higher in animals treated with clazosentan than in the corresponding controls. By contrast, ETRA immunoreactivity was lower in animals receiving clazosentan than in the controls. Clazosentan treatment also increased photoreceptor attrition induced by toxic light levels. Since ETRA inhibition induced molecular changes of photoreceptor terminals and increased light-driven activation of the inner retina, we may suggest that endothelin, via the A receptor, could modulate transmission of light signaling. Observations under phototoxic conditions further suggest that lack of ETRA signals would increase vulnerability of photoreceptors to light-induced damage.

O-11

Endothelin-1 Treatment Induces Experimental Cerebral Malaria During *Plasmodium Berghei* NK65 Infection

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Cerebral malaria (CM) is a life-threatening complication of *P. falciparum*. Infection of C57BL/6 (B6) mice with *P. berghei* ANKA (PbA) is an experimental CM (ECM) model which recapitulates many aspects of CM. Infection of B6 mice with *P. berghei* NK65 (PbN) does not induce neurological complications, and this is used as a control model. Blockade of endothelin type A (ETA) receptors in PbA-infected B6 mice prevents development of ECM, indicating that ET-1 is involved in the disease pathogenesis. We hypothesize that treatment of mice with PbN infection with exogenous ET-1 can trigger development of ECM. Mice were infected with 10^6 PbN-parasitized red blood cells and treated with either ET-1 or saline. Saline-treated mice did not develop ECM and survived until 12 days post-infection (DPI). ET-1 treated mice died between 4-8 DPI and exhibited signs of ECM. ET-1 treatment had no effect on parasitemia. All infected mice had significantly lower rectal temperature (RT) and body weight (BW) than uninfected controls over the course of infection, and reduction in these parameters was significantly greater in mice treated with ET-1 (>20% weight loss). Uninfected mice treated with ET-1 had a modest reduction in RT but not in BW. Brain histopathology of PbN-infected mice treated with ET-1 demonstrated the presence of petechial hemorrhages throughout the parenchyma, and leukocyte infiltration to the endothelia, 6 DPI, which were not evident in PbN-infected mice treated with saline or in uninfected control mice. These data indicate that ET-1 triggers the development of ECM in PbN-infected C57BL/6 mice.

Session 6: Actual Applications and Future Perspectives of Dual ETA/ETB Antagonists

Invited Lecture 6

Benefits of Dual Endothelin Receptor Antagonists: Mechanisms of Action, Current Studies, and Future Directions

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Over expression of endothelin (ET) and its receptors ET_A and ET_B results in pathological changes including hypertrophy and fibrosis. These changes are hallmarks of conditions such as pulmonary arterial hypertension (PAH). Dual, but not single (ET_A) endothelin receptor antagonists (ERAs), have been shown to improve survival in animal models of pulmonary hypertension and chronic heart failure. Hyperstimulation of ET_B receptors following ET_A receptor blockade by single ERAs leads to increased vascular permeability and fluid retention. These observations support the rationale that dual receptor antagonism may have optimal efficacy and minimized risk of edema.

Macitentan is a novel dual ERA designed for optimized physicochemical properties, favoring sustained receptor binding and enhanced tissue penetration. Macitentan does not increase bile salts, the proposed underlying mechanism for elevated liver enzymes observed with the dual ERA bosentan. Macitentan was studied in the Phase III SERAPHIN trial, the first event-driven, outcome trial in PAH. The study included over 740 patients. Macitentan 10 mg reduced the risk of morbidity and mortality by 45% versus placebo. Moreover, there was no difference in incidence of peripheral edema or liver enzyme elevations between placebo and ma citentan-treated patients.

Current preclinical and clinical research on the ET system highlight the potential for dual ERAs in other diseases. Future directions may include the fields of scleroderma, pulmonary fibrosis, heart diseases and oncology.

Invited Lecture 7

Effects of Bosentan on Digital Ulcers in Patients with Systemic Sclerosis

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Systemic sclerosis (SSc) is a connective tissue disease involving tissue fibrosis and endothelial injury. In 1990's, several studies indicated plasma endothelin (ET)-1 was elevated in patients with SSc, which might contribute to endothelial injuries (i.e.: Raynaud's phenomenon, digital ulcer, pulmonary hypertension, renal dysfunction). Interestingly, ET-1 exerted a fibrotic effect inducing collagen production in skin fibroblasts, suggesting that ET-1 might be involved in both tissue fibrosis and endothelial injury in patients with SSc.
Digital ulcer in patients with SSc results from peripheral vascular damage which related to an excessive vasoconstriction. That may be refractory for the treatment with any prostanoids. The ability of vasodilation in prostanoids could be almost equivalent to that in endothelin receptor antagonist (ERA). However, prostanoids could not exhibit a anti-fibrotic properties, which may be different from the effects of ERA.
In this Session, I am going to show the effects of bosentan on digital ulcers in patients with SSc and to evaluate the inhibitory effects of bosentan on the fibrogenic responses through TGF-β in SSc fibroblasts.

DAY 2

Session 7: Gene Regulation, Molecular and Cellular Biology

Invited Lecture 8

Tissue-Specific and Time-Dependent Regulation of the Endothelin Axis by the Circadian Clock Protein Per1

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Renal collecting duct Endothelin-1 is a critical regulator of the epithelial sodium channel (ENaC) and blood pressure (BP). Recently, we showed that mice lacking the circadian clock protein Per1 exhibited dramatically lower BP compared to wild type mice. Since ET-1 reduces Na+ retention and Per1 represses expression of ET-1 mRNA in the kidney, we hypothesized that elevated renal ET-1 levels contribute to the lower BP in Per1 KO mice. Examination of ET-1 peptide levels in the inner medulla of Per1 KO and WT mice showed that Per1 KO mice expressed higher levels of ET-1. ET-1 peptide levels varied with a circadian pattern that correlated with dipping of BP in wild type mice. To further investigate a role for Per1 in the regulation of the Endothelin axis, ET-1, ETAR and ETBR mRNA expression were measured in lung, heart, liver and the renal inner medulla. Measurements were performed at noon and midnight, representing the peak of murine rest and active phases, respectively. The effect of reduced Per1 expression on levels of the Endothelin axis and the circadian pattern of expression appeared to be tissue specific. For example, in the renal inner medulla, ETAR and ETBR exhibited peaks of expression in opposite circadian phases. In contrast, lung expression of ET-1, ETAR and ETBR did not vary with a circadian pattern, but ET-1 expression was dramatically decreased in Per1 heterozygous mice. Heart and liver also showed distinctive circadian expression of ET-1, ETAR and ETBR. These observations may have important implications for our understanding of the best time of day to deliver Endothelin receptor antagonists.

Invited Lecture 9

Endothelin Signaling in Craniofacial and Cardiac Development

Hiroki Kurihara
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Since a series of gene knockout studies in 1990s, the endothelin system has emerged as a key determinant in embryonic development. We have shown that the endothelin-1 (ET-1)/endothelin type-A receptor (ETAR) pathway acts as a molecular switch that specifies the ventral identity of the pharyngeal arches to form the lower jaw and related structures. Mice deficient in ET-1/ETAR signaling also showed anomalies in the great arteries. The preotic (cranial) and postotic (cardiac) neural crest cells are responsible for these processes as a target cell population. Recently, we found that the cranial neural crest from the preotic region, rather than post-otic 'cardiac' neural crest cells, migrate into the heart and differentiate into coronary artery smooth muscle cells in the proximal region (Nat. Commun. 3: 1267, 2012). Ablation of the preotic neural crest in chick embryos causes abnormalities in coronary septal branch and orifice formation. Appropriate migration and deployment of neural crest cells and subsequent smooth muscle differentiation require multicellular interactions involving ET-1/ETAR signaling possibly through G12/13-mediated mechanisms, whereas ET-1/ETAR signaling is involved in ventral identification of the pharyngeal arches through Gq/11-mediated, Dlx5/6-dependet mechanisms. These findings indicate that the ET-1/ETAR signaling pathway is involved in craniofacial and cardiac development through different trimeric G-proteins.

O-12

Erythropoietin Induced Blood Pressure Rise, Vascular Inflammation and Oxidative Stress in Mice Overexpressing Human Endothelin-1: Improvement by Exercise

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Erythropoietin (EPO) is used to correct anemia in chronic kidney disease (CKD). EPO has been shown to increase blood pressure (BP) in patients and animals with CKD. Plasma endothelin (ET)-1 levels are increased in CKD animals and patients, and enhanced by EPO. EPO-induced BP rise was blunted by ETA receptor blockers. This study was designed to determine whether pre-existing ET-1 overexpression is required for EPO to cause adverse vascular effects, and whether this could be prevented by exercise training. We treated 8-10-week old male mice with endothelial specific ET-1 overexpression (eET-1) with EPO (100 IU/kg, SC, 3 times/week) or not, and subjected or not the mice to swimming exercise training (1 h/d, 6 d/week) for 8 weeks. Wild-type littermate mice were treated or not with EPO as above and maintained sedentary. EPO increased systolic BP by 24 mmHg (P<0.05) in eET-1 mice, and decreased vasodilatory responses to acetylcholine by 25% (P<0.01). EPO enhanced ET-1-induced increase in resistance artery media/lumen by 31% (P<0.05), aortic NADPH oxidase activity by 50% (P<0.05), reactive oxygen species generation by 93% (P<0.001) and monocyte/macrophage infiltration by 160% (P<0.001), and raised plasma ET-1 by 130% (P<0.05). EPO had no effect on wild-type mice. Exercise training prevented all of the above effects of EPO as well as ET-1 (P<0.05). EPO-induced SBP rise and adverse vascular effects are dependent on the pre-existing level of ET-1 expression. Exercise training prevented EPO-induced BP rise and adverse vascular effects in part by inhibiting ET-1 overexpression-induced oxidative stress, inflammation and immune activation.

O-13

Endothelin-1-Induced Oxidative Stress and Inflammatory Cell Infiltration Contribute to High-Fat Diet Induced-Atherosclerosis and Aneurysm Formation in Apolipoprotein E Knockout Mice

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Endothelin (ET)-1 promotes reactive oxygen species (ROS) production and inflammation in the vasculature. ET-1 has been implicated in the pathogenesis of atherosclerosis in both human and animal models. Abdominal aorta aneurysms (AAA) occur in association with atherosclerosis. We hypothesized that ET-1-induced ROS and inflammation contribute to the development of atherosclerosis and increase occurrence of AAA in high-fat diet (HFD)-fed apolipoprotein E knockout (*Apoe*$^{-/-}$) mice. Eight-week-old male transgenic mice overexpressing ET-1 in the endothelium (eET-1), *Apoe*$^{-/-}$, eET-1/*Apoe*$^{-/-}$ and wild-type mice were fed HFD for 8 weeks. eET-1/*Apoe*$^{-/-}$ mice presented 2- and 4-fold fold more atherosclerotic lesions in aortic sinus and ascending aorta, respectively, compared to *Apoe*$^{-/-}$ mice (P<0.05). Aortic aneurysms were observed at suprarenal level in 6 of 15 eET-1/*Apoe*$^{-/-}$ compared to none of 15 *Apoe*$^{-/-}$ mice (P<0.05). ET-1 overexpression increased ROS production >2.6-fold in perivascular fat (PVAT), media and plaques of *Apoe*$^{-/-}$ mice (P<0.05). ET-1 overexpression increased monocyte/macrophage infiltration 5- and 8-fold in PVAT and media of *Apoe*$^{-/-}$ mice, respectively (P<0.05). CD4+ T cell infiltration was observed with greater frequency in PVAT (3/6) and plaques (5/6) in ascending aorta of eET-1/*Apoe*$^{-/-}$ compared to *Apoe*$^{-/-}$ (1/6) mice (P<0.05). Spleen pro-inflammatory Ly-6C^{hi} monocytes were 65% higher in *Apoe*$^{-/-}$ compared to wild-type mice (P<0.05), which was further increase by 26% in eET-1/*Apoe*$^{-/-}$ mice (P<0.05). Stretching and fragmentation of elastin fibers at suprarenal level were detected only in eET-1/*Apoe*$^{-/-}$ mice. These results suggest that ET-1 promotes development of atherosclerotic lesions and AAA by increasing oxidative stress, monocyte/macrophage and T cell infiltration. ET-1-induced alteration in elastin fibers may play an important role in AAA development.

O-14

Flow Regulation of Inner Medullary Collecting Duct Endothelin-1 Production

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Collecting duct (CD)-derived endothelin-1 (ET-1) inhibits Na and water reabsorption; its deficiency causes marked hypertension. CD ET-1 synthesis is enhanced by extracellular fluid volume expansion. In cultured cortical CD (CCD) cells, increased flow (as would occur in tubule fluid during volume expansion) stimulates ET-1 production; this is ENaC-dependent. Since the inner medullary CD (IMCD) is the major site of renal ET-1 synthesis, we examined the effect of flow on IMCD ET-1 production. Mouse IMCD3 cells were subjected to static conditions or flow (2 dyne/cm2 for 2 hr), followed by determination of ET-1 mRNA. Flow increased ET-1 mRNA by 2.2-fold. Absence of perfusate Ca prevented the flow response. BAPTA, W-7, calmidazolium, KN-93, calphostin C and cyclosporin A reduced the ET-1 flow response, indicating that PKC and Ca/calmodulin/calmodulin kinase/calcineurin pathways are essential for the flow response. Amiloride or benzamil did not affect the ET-1 response to flow. Increasing perfusate osmolality to 450 mOsm with NaCl, mannitol or urea elicited a marked flow response (4.4-fold increase in ET-1 mRNA). Removal of cilia with chloral hydrate reduced the flow response, while flow failed to stimulate ET-1 mRNA in mIMCD3 cells deficient in polycystin-2. These data suggest that IMCD ET-1 synthesis is stimulated by tubule fluid flow via increased solute delivery and possibly cilia deformation, which in turn activates PKC- and Ca-dependent pathways. We propose that Na delivery stimulates CCD ET-1, which, in turn, inhibits CCD Na reabsorption, while solute and water delivery stimulate IMCD ET-1, which, in turn, inhibits IMCD water and urea reabsorption.

O-15

Ubiquitin Modification Plays an Important Role in ET-1-Dependent Endothelin Type B Receptor Trafficking

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Two types of endothelin receptors, ET_AR and ET_BR, are internalized upon ET-1 stimulation, but their fates are different after stimulation despite of their sequence homology. To get insights into the mechanisms for different fates of these receptors, we examined stimulation-induced ubiquitination of the receptors. After ET-1 stimulation, ET_BR was ubiquitinated, whereas ET_AR was not. The mutant ET_BR receptor in which all lysine residues in C-terminal (C-tail) were replaced by arginine was not ubiquitinated. After ET-1 treatment, the amount of cell surface ET_BR decreased rapidly, but that of ET_BR mutant was virtually unchanged. In addition, the level of ERK phosphorylation and Ca^{2+} response was enhanced in mutant ET_BR-expressing cells compared to those in wild type ET_BR-expressing cells following ET-1 stimulation. There are 8 lysine residues in ET_BR C-tail for probable ubiquitination: 3 lysine before and 5 lysine after palmitoylation site (PS). The mutant in which 5 lysine residues after PS were replaced with arginine was not ubiquitinated upon ET-1 stimulation, whereas the mutant in which 3 lysine residues before that site were replaced with arginine was ubiquitinated. ET_BR mutants in which either one of 5 lysine residues after PA was left unreplaced were ubiquitinated and internalized following ET-1 stimulation. These results indicate that ubiquitination of either one of lysine residues in ET_BR C-tail is sufficient for ET-dependent internalization.

O-16

Aldosterone Alters Chromatin Structure of the Murine Endothelin-1 Gene

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Aldosterone increases sodium (Na) reabsorption in the renal collecting duct (CD) and systemic blood pressure. We have shown that aldosterone induces transcription of the endothelin-1 (*Edn1*) gene and increases protein (ET-1) levels in the kidney (JBC 2009;294:30087). Since ET-1 inhibits the action of aldosterone on CD Na reabsorption, we hypothesize that ET-1 acts in a negative feedback loop on Na retention. Whereas transcriptional regulation of the *Edn1* gene has been investigated, the importance of epigenetic *Edn1* gene regulation is largely unexplored. Here we examine the effect of aldosterone on chromatin structure of the *Edn1* gene. We used a novel quantitative PCR-based DNaseI hypersensitivity assay of chromatin structure in four regions of the *Edn1* gene known to affect transcription in murine inner medullary collecting duct-3 (IMCD-3) and outer medullary collecting duct1 (OMCD1) cells. The calcium-responsive element (NFAT) (JBC 2010;285:28520) at -1263 bp has low chromatin accessibility in both cell lines under all conditions tested. However, the NFAT element at -1563 bp upstream has low accessibility and is significantly more accessible in IMCD-3 cells, but not OMCD1 cells, exposed to aldosterone. The distal aldosterone hormone response element (HRE2) is highly accessible in both cell lines under all conditions tested. However, aldosterone caused a significant increase in chromatin accessibility of the more proximal promoter including HRE1 in both cell lines. The results extend our previous observations and suggest that aldosterone activation of the mineralocorticoid receptor (MR) results in MR binding at HRE2 to open chromatin structure of specific regions of the *Edn1* gene.

Session 8: Pulmonary Hypertension

Keynote Lecture 3

The Evolving Paradigm of Pulmonary Arterial Hypertension and the Evolving Role of Endothelin-1

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Pulmonary arterial hypertension (PAH) remains a devastating illness, with an average survival that is still less than 5 years. Our understanding of PAH has evolved from considering it a disease of vasoconstriction, to recognizing that disordered cellular proliferation and resulting physical obstruction of the microvascular lumen are the principal causes of the increased pulmonary microvascular resistance. In parallel, insights gained from less frequent types of PAH, such as the heritable form of idiopathic PAH and PAH associated with Hereditary Hemorrhagic Telangiectasia, have highlighted the importance of disordered growth factor signalling in the origin and pathogenesis of the disease, particularly by members of the transforming growth factor β superfamily, including bone morphogenic proteins. Moreover, it has become clear that the endothelial cell is central to the disorder, in terms of abnormal cell signalling, mediators and proliferation. Emergence of apoptosis-resistant endothelial clones and change to a neoplastic phenotype are hallmarks of PAH.

Endothelial-derived mediators normally help maintain vascular heath. Abundant locally-produced prostacyclin and nitric oxide, and low levels of edothelin-1 normally preserve vascular patency. This balance is reversed in PAH, with low levels of prostacyclin and nitric oxide, and excess production of endothelin-1. Therapeutic blockade of endothelin-1 has resulted in clinical benefit for thousands of patients with PAH, who have had improvement in functional capacity and fewer clinical deteriorations. Each generation of endothelin antagonists improves on this track record. However, none of these agents is curative, and the potential reasons will be discussed.

There is tremendous crosstalk between mediator systems in the vascular wall. With regard to endothelin-1, this is an area that requires further exploration, in order to understand the triggers for increased endothelin-1 levels and how to control them in PAH. Recent evidence suggests important interactions between the bone morphogenic protein and endothelin-1 systems, which deserve more study. The ultimate goal would be to suppress endothelin-1 synthesis and eliminate its contribution to the progression of this disorder.

Invited Lecture 10

Balloon Pulmonary Angioplasty as a Treatment Option for Chronic Thromboembolic Pulmonary Hypertension

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Although pulmonary endarterectomy (PEA) can sufficiently decrease pulmonary arterial pressure (PAP) in patients with chronic thromboembolic pulmonary hypertension (CTEPH), not all patients can undergo PEA because of technical limitations. Several drugs such as endothelin receptor antagonist to treat pulmonary hypertension have been used to manage these patients, but sufficient decrease of PAP cannot be achieved. Balloon catheter would easily reach to surgically inaccessible lesions and therefore, we hypothesized that balloon pulmonary angioplasty (BPA) would be an effective treatment option for inoperable patients with CTEPH. We have treated 162 inoperable patients with CTEPH (WHO functional class III or IV despite full medical treatment, mean age 62 years old) with BPA. Mean PAP significantly decreased after BPA (from 44.9 to 24.0 mmHg ($P<0.001$)). Forty patients developed severe reperfusion pulmonary injury after BPA and 5 of them died during hospitalization. Additional 3 patients died during follow up period and 3- year survival rate was 94.4%. Sixty-four out of 132 patients have been followed up for more than one year after BPA. Decrease of mean PAP was maintained (21.9mmHg). Thus, BPA would be an effective therapeutic option in these patients who have otherwise no proven treatment.

O-17

Adipose-Derived Regenerative Cells Therapy Improves Monocrotaline Induced Rat Pulmonary Arterial Hypertension with Suppressing Endothelin-1 Though an Anti-Inflammatory Mechanism

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Introduction Pulmonary arterial hypertension (PAH) is characterized by functional and structural changes in the pulmonary vasculature, and despite the progress of pharmacotherapy, the prognosis of patients with advanced PAH remains poor. Adipose-derived regenerative cells (ADRCs) therapy has recently emerged as a novel therapy for ailments of various organs by promoting cell regeneration at site of pathology. In this study, we investigated the efficacy of ADRCs therapy on PAH using monocrotaline (MCT)-induced PAH rat model, and explored underlying mechanisms. **Methods** Male Wistar rats were divided into the control, MCT, and MCT with ADRCs transfusion (M/A) group. Seven million ADRCs were transfused by intravenous injection at day 7. PAH was evaluated by measuring acceleration time (AT) and deceleration (Dct) of PA flow using echocardiography. At day 28, pathological changes in pulmonary vessels were assessed. The expression of genes associated with PAH was analyzed at day 14 by real time RT-PCR. **Results** Echocardiography showed that ADRCs therapy inhibited the development of PAH at day 28 (AT: MCT 20.4 vs. M/A 24.0, Dct: MCT 1474.0 vs. M/A 898.6, $p<0.005$). By histological analysis, pulmonary vascular remodeling induced by MCT were also inhibited (Vessel wall thickness: MCT 0.44 vs. M/A 0.31, $p<0.001$). MCT treatment increased mRNA levels of Endothelin (ET) receptor-A, ET receptor-B, ET-1 and Transforming growth factor (TGF)-beta in the lung. ADRCs therapy suppressed these increased mRNA ($p<0.05$). **Conclusions** ADRCs therapy inhibited the development of PAH by reversing the changes in ET expression and inflammatory cytokines. These findings suggest that ADRCs therapy may open a novel strategy to treat PAH.

O-18

Role of Bradykinin and Endothelin-Converting Enzyme-1 in Pulmonary Hypertension

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Pulmonary hypertension (PH) is an unremitting disease defined by a progressive increase in pulmonary vascular resistance leading to right-sided heart failure. Using ECE1 knockout mice (ECE1+/-) we demonstrated here that heterozygous deficiency of ECE1 protects mice against PH, RV hypertrophy, and pulmonary vascular remodeling on 3 weeks of exposure to hypoxia. We also observed that chronic hypoxia-induced PH is not only associated with increased levels of systemic and pulmonary endothelin-1, but also associated with diminished level of bradykinin (BK) peptide in lung. Genetic inactivation of ECE1 did not affect ET-1 levels but prevented the degradation of BK in lungs during hypoxia-induced PH. The clinical relevance of the data was indicated by our observation that the level of plasma BK in the pulmonary vein (PV) of patiens with pulmonary hypertension due to atrial septal defect (ASD-PH) is significantly lower than in ASD patients without pulmonary hypertension (ASD-PH: 14.0±7.2ng/mL; ASD-noPH: 24.3±20.8ng/mL; $p<0.05$). Furthermore, plasma BK level in PV has significant correlation with some hemodynamic parameters in this patient group (i.e. the pulmonary cardiac output, the ratio of pulmonary/systemic cardiac output and the systemic vascular resistance). Together, these data show that inhibition of ECE1 is protective against the the development pulmonary hypertension through the preservation of bradykinin action. This study also showed that bradykinin level was diminished in PH and was correlated significantly with some hemodynamic parameters in mice and patients with PH, indicating BK as a promising therapeutical target for PH.

O-19

Long-Term Survival in Japanese Patients with Idiopathic/Heritable Pulmonary Arterial Hypertension

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Background: Idiopathic/heritable pulmonary arterial hypertension (I/HPAH) is reported to have a poor prognosis despite the available therapeutic options. Although there are reports on survival of patients from Western countries, there is a shortage of data from Asia. Methods: A retrospective chart review was performed on 56 patients with I/HPAH. Survival analysis was conducted by using Kaplan-Meier method and differences between parameters measured at baseline and after treatment were tested by the paired t-test. Results: There are 41 females (73%) and 15 males (27%) included in this study. The mean age was 32±17 years old at the time of diagnosis. Mean survival time from diagnosis was 14.5±0.8 years (95% CI, 12.9-16.2 years), with 1-, 2-, 3-, 5- and 10-year survival rates of 100, 96, 96, 96 and 78%. In patients who underwent follow-up right heart catheterization at least 3 months later from the first catheterization at our hospital, WHO functional class improved from 3 to 2 ($P<0.01$), and mean pulmonary arterial pressure was decreased from 63.2±15.0 to 34.8±10.3 mmHg ($P<0.01$). Cardiac index was improved from 2.3±0.8 to 3.5±0.9 L/min/m^2 ($P<0.01$). At follow-up, 98% of patients were on PAH-targeted drugs: prostacyclin analogue (n=52, 93%), endothelin receptor antagonists (n=38, 68%), and phosphodiesterase type 5 inhibitors (n=29, 52%). Forty-two patients (75%) were treated with combination therapy. Conclusions: The study revealed a better survival of Japanese patients with I/HPAH than ever reported. Hemodynamic parameters were significantly improved. It might be caused by the difference of ethnicity or high prescription rates of targeted drugs used to treat I/HPAH.

Lunch Session 3

LS3

Twenty-Five Years of Research Leading to a New Generation of Endothelin Receptor Antagonists

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The discovery of macitentan, a novel dual endothelin receptor antagonist (ERA), is the result of 25 years of research. Academic as well as pharmaceutical company research led to an understanding of the pathophysiological role of endothelin (ET) and the subsequent development of ET pathway inhibitors, including ERAs and endothelin converting enzyme-1 inhibitors. The first non-peptidic ERAs, Ro 46-2005 and bosentan, were effective in animal models of ET-related diseases, prompting clinical development of the dual ERAs bosentan and tezosentan, as well as the ET-A selective ERA clazosentan. In 2001, bosentan was approved as the first oral drug for pulmonary arterial hypertension (PAH); the ET-A selective ERA ambrisentan followed several years later.

Based on the experience gathered from research with ET and ERAs, an extensive research programme was initiated to develop an ERA with improved efficacy and safety. This lead to the discovery of macitentan. Macitentan displays optimised physiochemical properties, and is more efficacious than other ERAs in animal models.

Macitentan was studied in 742 patients in the phase III SERAPHIN trial, the first event-driven outcome trial in PAH. Macitentan significantly reduced morbidity and mortality; the effect of macitentan on patients with or without background PAH therapy was consistent with the overall treatment effect. The secondary endpoint of death due to PAH or hospitalisation for PAH was significantly reduced with macitentan. In addition, the number of PAH-related hospitalisations and inpatient days (10 mg only) per year were significantly lower in macitentan-treated patients. Macitentan was well-tolerated, with similar rates of peripheral oedema and liver enzyme elevation between placebo and macitentan-treated patients.

Lunch Session 4

LS4-1

The Roles of Endothelin Receptor Antagonists and Phosphodiesterase Type 5 Inhibitors in the Management of Skin Ulcers in Systemic Sclerosis

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Systemic sclerosis (SSc) is a multisystem connective tissue disease characterized by autoimmunity, inflammation, vasculopathy and resultant fibrosis of skin and certain internal organs. Although the pathogenesis of SSc still remains unknown, increasing evidence suggests that endothelin-1 plays a critical role in the developmental process of fibrotic and vascular involvement associated with this disease. For instance, circulating endothelin-1 levels are elevated in diffuse cutaneous SSc patients, limited cutaneous SSc patients, and SSc patients at scleroderma renal crisis (SRC) compared with healthy controls and in limited cutaneous SSc patients with pulmonary arterial hypertension (PAH) as compared to those without. Clinically, bosentan, a dual endothelin receptor antagonist (ETRA), has been shown to prevent the development of new digital ulcers in SSc patients by two high quality randomized clinical trials. Given that long-term outcomes of SSc-PAH and SRC patients treated with bosentan are favorable compared with historic cases, this drug potentially has a disease modifying effect on SSc vasculopathy, especially proliferative obliterative vasculopathy.
Phosphodiesterase type 5 (PDE5) inhibitors, another group of drugs acting through cGMP modulation, prevent the reduction of cGMP and smooth muscle cell proliferation in blood vessels as well as induce vasodilation by increasing endogenous nitric oxide levels. A series of studies including a randomized clinical trial demonstrated the efficacy of PDE5 inhibitors for Raynaud's phenomenon and digital ulcers associated with SSc. Furthermore, a combination therapy of ETRAs and PDE5 inhibitors has been shown to be a possible option for digital ulcers refractory to the canonical therapies.
In this seminar, the clinical data regarding the efficacy of ETRAs and PDE5 inhibitors for digital ulcers in SSc will be overviewed. Furthermore, the potential of ETRAs and PDE5 inhibitors as a disease modifying drug for SSc vasculopathy will be discussed using animal models of SSc vasculopathy.

LS4-2

The Challenge to Refractory Pulmonary Hypertension Associated with Scleroderma

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Pulmonary hypertension (PH) is an important and poor prognostic complication of scleroderma (systemic sclerosis: SSc). While pulmonary arterial hypertension (PAH) is a typical example of PHs complicating in patients with SSc (PAH), pulmonary venous hypertension secondary to left-side heart disease (PVH) and PH due to interstitial lung disease (PH-ILD) are also included and their causes are wide-ranging. In addition, it has been found recently that pulmonary veno-occlusive disease (PVOD), which is said to be a rare stage in SSc patients, is often complicated and the pathology of PH associated with systemic scleroderma (SSc-PH) is becoming increasingly complicated.
Thus, in the treatment of SSc-PH, it is important to accurately differentiate its cause at first. While an aggressive multidrug therapy is being practiced more frequently for PAH in Japan in recent years, care should be taken as inadvertent multidrug therapy may conversely aggravate the disease. Whereas, in SSc frequently complicating interstitial pneumonia, administration of prostanoid that worsens ventilation-perfusion mismatch or of ambrisentan that may aggravate interstitial pneumonia should be performed carefully, PDE5 inhibitors can be used relatively safely in patients with SSc-PH. In contrast, the importance of supportive therapies such as domiciliary oxygen therapy and anticoagulation therapy, which is decreasing with the advancement of therapeutic drugs, is still high in the treatment of SSc-PH. As SSc-PAH is said to be associated with poor prognosis compared to idiopathic PAH, expectations for the development of a new drug are particularly large. Therefore, at this seminar, I would like to talk about the drugs under development for PAH focusing particularly on the efficacy in the patients with PAH due to connective tissue diseases such as SSc.

Poster Session 2: Gene Regulation, Molecular and Cellular Biology, Pharmacology, and Pulmonary Hypertension

P-41

Endothelin Regulation by miR-218: A Target in Scarring

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The adult human dermis scars; scarless repair occurs in the oral cavity. Alpha-smooth muscle (a-SMA)-expressing myofibroblasts are resposible for scarring. TGFbeta1 causes myofibroblast differentiation in dermal but not gingival fibroblasts (N=3, p&lt0.05). Gingival fibroblasts express less focal adhesion kinase, display less focal adhesion kinase phosphorylation and do not induce endothelin-1 (ET-1) in response to TGFbeta1 (N=3, p&lt0.05). The induction of ET-1 in response to TGFbeta 1 in dermal fibroblasts does not occur in the absence of FAK or in the presence of the FAK/src inhibitor PP2 (N=3, p&lt0.05). Addition of ET-1 to gingival fibroblasts restores the ability of TGFbeta1 to induce myofibroblast formation (N=3, p&lt0.01). Expression profiling revealed that, compared to gingival fibroblasts, dermal fibroblasts overexpress a variety of miRNAs including miR-218. Addition of miR-218 to gingival fibroblasts results in enhanced focal adhesion kinase expression and phosphorylation, cell spreading, endothelin-1 production and in the ability of TGFbeta to induce myofibroblast formation (N-3, p&lt0.01). Knockdown of miR-218 abolishes the ability of TGFbeta1 to induce myofibroblast formation in dermal fibroblasts. These results strongly suggest that the presence or absence of ET-1 is responsible for myofibroblast formation in fibroblasts. Targeting ET-1 might be a viable approach to prevent scarring.

P-42

miRNA-1 Regulates Endothelin-1 in Diabetes

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MicroRNAs-1 (miR-1) plays important roles in several biological processes. ET-1 is upregulated in chronic diabetic complications. In this study, we investigated the role of miR-1, an ET-1 targeting miRNA, in the endothelial cells (ECs) and in the organs of diabetic animals. PCR array was used to identify alteration of miR expressions in the ECs exposed to glucose. miR-1 expression was validated by TaqMan Real-Time PCR assay. Human retinal ECs (HRECs) exposed to various glucose levels with or without miR-1 mimic transfection as well as tissues from streptozotocin-induced diabetic animals after two months of follow-up, were examined for ET-1 mRNA and protein levels, fibronectin (FN) mRNA and miR-1 expression. Array analyses showed glucose-induced alterations of 125 miRNAs (out of 381) in ECs exposed to 25mM glucose (HG) compared to 5mM glucose. Fifty-one miRNAs were upregulated and 74 were downregulated. HG decreased miR-1 expression and increased ET-1 mRNA and protein levels. miR-1 mimic transfection prevented HG-induced ET-1 upregulation. Furthermore, glucose induced upregulation of FN, which is mediated in part by ET-1, was also prevented by such transfection. Diabetic animals showed decreased miR-1 expression in the retina, heart, and kidneys. In parallel, ET-1 mRNA expressions were increased in these tissues of diabetic animals compared to controls. Furthermore these tissues showed upregulation of FN. These studies indicate a novel glucose-induced molecular mechanism of tissue damage, in which miR-1 regulates ET-1 expressions in diabetes. Identifying such mechanisms may lead to potential RNA based treatment for diabetic complications. Supported by Canadian Diabetes Assn. and Heart and stroke foundation of Canada.

P-43

Endothelin-1 Mediates Downstream Profibrotic Effects by Transforming Growth Factor-Beta 1 in Systemic Sclerosis Skin Fibroblasts

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Systemic sclerosis (SSc) is an autoimmune connective tissue disorder characterized by excess collagen deposition and vascular changes that affect multiple organs. Although transforming growth factorβ1 (TGF-β1) and endothelin-1 (ET-1) are known to be potent fibrotic factors in SSc, the relationship between them are not fully understood. The aim of our study was to examine the effects of TGF-β1 on the fibrogenic phenotype of SSc skin fibroblasts through ET-1 production. Human skin fibroblasts obtained from SSc patients were incubated with TGF-β1 in the presence of SIS3 (an inhibitor of Smad3 phosphorylation). In addition, the effects of ETRA, ETRB and dual ETRA/ETRB antagonist were explored. Expression of ET-1, CTGF and type I collagen was evaluated using ELISA and real time RT-PCR. ETRA and ETRB expressions were assessed by immunohistochemistry. We found that TGF-β1 increased ET-1 mRNA and protein expression and this increase in ET-1 was suppressed by SIS3. Upregulation of COL1A1 and CTGF by TGF-β1 were reduced by an ETRA or ETRB antagonist, and a dual ETRA/ETRB antagonist had an additive inhibitory effect. In conclusion, TGF-β1 produced ET-1 through Smad3 phosphorylation and a dual ETRA/ETRB antagonist decreased COL1A1 and CTGF mRNA levels in fibroblasts. Inhibition of ET-1 signaling may exert anti-fibrotic effects in SSc fibroblasts.

P-44

Effect of Feeding Behavior on Circadian Regulation of Endothelin Expression in Mouse Colon Epithelia

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The function, regulation and gene expression of the endothelin (ET) system in intestine is not well understood. We investigated the dependence on feeding schedule and biological clock of the regulation of ET-1 gene expression in mouse colon. Mice were fed freely, fasted for 48 hours, and re-fed after fasting. Gene expression was analyzed by real-time RT-PCR. ET-1 Gene expression was highest in colon compared with other tissues examined in fasted mice. Fasting increased the amplitude, while maintaining the rhythmicity, of ET-1 gene expression in epithelial colonic tissue. Re-feeding, however, decreased gene expression and suppressed rhythmic oscillation, even though the rhythmicity of Per-1 and Per-2 gene expression remained unchanged. Furthermore, the decrease in ET-1 gene expression induced by re-feeding was blocked by pre-treatment with hexamethonium and atropine. The daily change in ET-1 gene expression and peptide production in colon epithelia, which depends on feeding schedule via autonomic nervous system, is synchronized with peripheral circadian oscillators under conditions of free feeding and fasting but not re-feeding. ET-1 plays important physiological roles, which is dependent on feeding behavior.

P-45

cDNA Cloning and Sequence Analysis of Preproendothelin From Barfin Flounder (Verasper Moseri)

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The presence of endothelin (ET)-like immunoreactivity and the cardiovascular effects of mammalian ET-1 in fish have been reported. To identify ET-related peptides in fish, we screened the cDNA library of the barfin flounder (Verasper moseri) intestine by means of rapid amplification of cDNA ends, and we cloned cDNAs encoding an ET-related peptide. The ET-related sequence of 21 amino acids is similar to the trout ET-1 peptide recently purified from kidney specimens of Oncorhynchus mykiss. The deduced amino acid sequence of pre-proET-1 (PPET-1) comprises 244 amino acids, including a putative signal sequence and mature ET-1, as well as big ET-1 and ET-1-like sequences. This precursor, the first reported PPET-1 sequence, has low homology with the sequences of human, mouse, frog (Xenopus laevis), and zebrafish (Danio rerio) PPET-1.

P-46

Shark Endothelin: cDNA Cloning, Sequence and Evolutional Analysis

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Endothelin (ET)-related receptors homologous to mammalian receptors have been cloned fromfish, indicating that ET-related ligands may be present in lower species. Here wecloned cDNAs encoding preproendothelin (PPET) from the Shark intestinal cDNAlibrary. Shark ET cDNAs encode 200 amino acids, including a 20-amino-acid putativesignal sequence, as well as mature ET, big ET, and ET-like sequences. Thissequences together with other published PPET sequences were used to analyze thephylogenetic relationship among all ET family genes.

P-47

Molecular Cloning and Sequence Analysis of Preproendothelin from Medaka, Oryzias Latipes

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The presence of endothelin (ET)-like immunoreactivity and the cardiovascular effects of mammalian ET-1 in fish have been reported. To identify ET-related peptides in fish, we screened the cDNA library of the medaka (Oryzias latipes) intestine by means of rapid amplification of cDNA ends, and we cloned cDNAs encoding an ET-related peptide. The medaka ET-related sequence of 21 amino acids is similar to the trout ET-1 peptide recently purified from kidney specimens of Oncorhynchus mykiss. The deduced amino acid sequence of pre-proET-1 (PPET-1) comprises 200 amino acids, including a putative signal sequence and mature ET-1, as well as big ET-1 and ET-1-like sequences. This precursor, the first reported PPET-1 sequence, has low homology with the sequences of human, mouse, frog (Xenopus laevis), and zebrafish (Danio rerio) PPET-1.

P-48

The Signaling Pathways Involved in the Synergistic Effect of ET-1 and cAMP on IL-6 Transcription

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We demonstrated previously that ET-1 and cAMP may synergistically induce IL-6 release from adipocytes, mainly through their strong stimulatory effect on IL-6 gene expression. In the present study, we further examined the signaling pathways that may be involved. A luciferase reporter driven by promoter (-1310/+198) of mouse IL-6 gene was transfected into 3T3-L1 adipocytes to monitor IL-6 transcription in response to ET-1 and 8-bromo cAMP, and the effects of various inhibitory agents were tested. Whereas the stimulatory effect of ET-1 alone was inhibited by pertussis toxin (PT), GF109203X, U0126, N-acetylcysteine, salicylate, dominant negative CREB (dn-CREB) and mithramycin A, the stimulatory effect of 8-bromo cAMP was only inhibited by dn-CREB. On the other hand, the synergistic effect of ET-1 and cAMP was suppressed by GF109203X, U0126, salicylate, c-Jun-specific antisense oligonucleotide (AS-cJun) and dn-CREB. PT had a partial inhibitory effect, while NAC and mithramycin A had no influence. Since NF-kB activation by ET-1 is mediated by a PKC&epsilon/ROS cascade, the observation that the synergistic effect of ET-1 and cAMP was inhibited by salicylate but not NAC suggests salicylate inhibited some factor in addition to NF-kB. Indeed, we found that another salicylate target p90 ribosomal S6 Kinase (RSK) was involved. Taken together, the synergistic effect of ET-1 and cAMP on IL-6 gene transcription seems to be mediated by pathways involving PKC, MAPK, CREB, AP-1 and RSK.

P-49

A Novel Mouse Model to Characterize the Mechanisms of Endothelin-1-Induced Vascular Injury

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Background: To develop a model to study of the mechanisms of endothelin-1 (ET-1)-induced vascular injury, we generated an inducible endothelial cell (EC)-restricted human ET-1 (*EDN1*) cDNA overexpressing transgenic mouse. **Methods and Results:** A transgene was engineered that expresses chloramphenicol acetyltransferase (*cat*) and *EDN1* cDNA prior and after Cre-mediated excision, respectively, under the control of cytomegalovirus immediate early enhancer/chicken β-actin promoter (CAG). Co-transfection of Cre expression vector and pCAG-*cat-EDN1* caused 30-fold increase in ET-1 production. Transgenic mice were generated and two of seven founder lines were selected based on their cardiac *cat* expression level, which was twice higher in line C-134 than C-170. Inducible EC-restricted *EDN1* mice were generated crossing CAG-*cat-EDN1* mice with transgenic mice expressing CreER^{T2} under control of EC-specific *Tie2* promoter. To investigate the extent of CreER^{T2} activation by tamoxifen and tissue specificity, *Tie2*-CreER^{T2} mice were crossed with *ROSA*$^{mT\text{-}mG/mT\text{-}mG}$ reporter mice expressing a membrane-targeted tandem dimer tomato (mT) before Cre-mediated excision, and membrane-targeted enhanced green fluorescent protein (mG) after excision. *Tie2*-CreER^{T2}/*cat-EDN1* and *Tie2*-CreER^{T2}/*ROSA*$^{mT\text{-}mG/+}$ mice were treated subcutaneously with 1 mg tamoxifen/day for 5 days and sacrificed 14-16 days later. mG expression was detected in 22±3% of mesenteric artery ECs of *Tie2*-CreER^{T2}/*ROSA*$^{mT\text{-}mG/+}$ mice. Plasma ET-1 levels were similar in vehicle-treated *Tie2*-CreER^{T2}/*cat-EDN1* (1.2±0.1pg/mL, n=4) and wild-type mice (1.1±0.1pg/mL, n=2), demonstrating no leaky expression. Tamoxifen induced 8-fold increase in plasma ET-1 levels in *Tie2*-CreER^{T2}/*cat-EDN1* mice (9.1±0.3pg/mL, n = 4). **Conclusion:** *Tie2*-CreER^{T2}/*cat-EDN1*-inducible EC-restricted *EDN1* overexpressing mice allow the study of ET-1 vascular effects independently of developmental effects.

P-50

Remodeling of Endothelial Function in Atherosclerotic Mice Overexpressing Endothelin-1 Restricted to Endothelium

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In human atherosclerosis, which is associated with elevated plasma and coronary endothelin (ET)-1 levels, ET_A receptor antagonists improved coronary endothelial function. Mice overexpressing ET-1 specifically in the endothelium (eET-1) crossed with apolipoprotein E knockout mice (*Apoe*$^{-/-}$) exhibited exaggerated high-fat diet (HFD)-induced atherosclerosis. Since endothelial dysfunction often precedes atherosclerosis development, we investigated whether endothelium-specific ET-1 overexpression causes endothelial dysfunction in *Apoe*$^{-/-}$ mice. Male 8-week old eET-1, *Apoe*$^{-/-}$, eET-1/*Apoe*$^{-/-}$ and wild-type mice were fed a regular diet or HFD for 8 weeks. Endothelial function was assessed in mesenteric arteries by pressurized myography. In HFD-fed mice, acetylcholine-induced endothelium-dependent relaxation (EDR) was reduced 67% in *Apoe*$^{-/-}$ and 41% in eET-1 compared to wild-type (P<0.05). Surprisingly, EDR was not impaired in eET-1/*Apoe*$^{-/-}$ compared to wild-type. Endothelium-independent relaxation to nitric oxide donor sodium nitroprusside and contractile responses to norepinephrine were unaffected. Similar results were observed in regular diet-fed mice. In the presence of inhibitors of either nitric oxide synthase (NOS)-mediated relaxation, N^{ω}-nitro-L-arginine methyl ester, or endothelium-dependent hyperpolarization (EDH)-mediated relaxation, apamin plus Tram34, EDR was blunted in wild-type (P<0.01), whereas NOS-mediated relaxation was reduced 22% (P<0.05) and EDH-mediated relaxation unaffected in eET-1/*Apoe*$^{-/-}$. However, the concomitant inhibition of NOS- and EDH-mediated relaxation reduced EDR 51% in eET-1/*Apoe*$^{-/-}$ (P<0.05). These results show an interdependence of NOS and EDH pathways in EDR in wild-type mice. ET-1 overexpression induced development of compensatory mechanisms in pre-atherosclerotic arteries of *Apoe*$^{-/-}$ mice, which permits either NOS or EDH pathway to mediate EDR independently. Investigation of mechanisms involved in EDR remodeling in eET-1/*Apoe*$^{-/-}$ mice will allow a better understanding of the ET-1 role in atherosclerosis.

P-51

Negative Regulation of Endothelin Type A Receptor-Operated TRPC6 Channel by Adenylate Cyclase-cAMP-Protein Kinase A Signaling Pathway

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Background: Augmentation of Ca^{2+} entry via transient receptor potential canonical channel 6 (TRPC6) in response to stimulation of endothelin type A receptor (ET_AR) with endothelin-1 is involved in the development of pulmonary arterial hypertension (PAH). For the drug therapy against PAH, G_s protein-coupled prostaglandin I_2 receptor agonists to activate adenylate cyclase (AC)-cAMP-protein kinase A (PKA) signaling pathway are administered. This study examined the role of AC-cAMP-PKA pathway in regulation of receptor-operated Ca^{2+} entry (ROCE) via ET_AR-activated TRPC6.
Methods: In HEK293 cells coexpressing ET_AR and TRPC6, intracellular free Ca^{2+} concentration was monitored by using a fluorescent Ca^{2+} indicator, fura-2/AM. To identify the target site(s) of TRPC6 for phosphorylation by PKA, serine residues at positions 14, 28, and 321, and threonine at 69 were replaced with alanine.
Results: Stimulation of ET_AR induced ROCE in TRPC6-expressing HEK293 cells where store-operated Ca^{2+} channels had been maximally activated by thapsigargin-induced Ca^{2+}-depletion/Ca^{2+}-restoration. The ROCE was inhibited by forskolin and papaverine to activate cAMP-PKA pathway, while it was potentiated by Rp-8-Br-cAMP, a PKA inhibitor. Inhibitory effects of forskolin and papaverine were partially cancelled by replacing Ser^{28} but not Thr^{69} of TRPC6 with alanine. Immunoblotting with Phos-tag biotin to detect phosphorylated proteins demonstrated that wild-type and mutant TRPC6 proteins were phosphorylated under basal condition. In vitro kinase assay with TRPC6 proteins dephosphorylated with a phosphatase pretreatment revealed that PKA can phosphorylate TRPC6 on Ser^{28} and Thr^{69}.
Conclusions: ROCE via ET_AR-activated TRPC6 is negatively regulated by PKA-mediated phosphorylation of TRPC6 at Ser^{28} but not at Thr^{69} in vivo, although PKA can phosphorylate TRPC6 on Ser^{28} and Thr^{69} in vitro.

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microRNA Regulation of Endothelin-1 in an Inner Medullary Collecting Duct Cell Line

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A growing body of evidence suggests that microRNAs (miRNAs) regulate endothelin-1 (Edn1) mRNA bioavailability (Jacobs et. al., 2013). miRNAs are a family of small (18-24 nt), single stranded, noncoding RNAs that are components of the RNA-induced Silencing Complex (RISC). RISC controls gene expression by blocking protein translation or inducing degradation of target mRNAs by binding to the 3' untranslated region (UTR). In humans and other mammals, the Edn1 3' UTR represents over 50% of the total mRNA length and contains long tracts of highly conserved sequence. Alignment of 19 species of class Mammalia yielded greater than 80% sequence identity between any two Edn1 3'UTRs. The level of conservation by itself suggests that there are elements in the 3' UTR that are critical for tight regulation of Edn1 mRNA availability. In silico examination of the murine Edn1 3' UTR revealed two likely miR-709 binding sites at positions 610 and 668 (microRNA.org). mFold (http://mfold.rna.albany.edu) was used to examine the free energy of 70 nucleotides flanking the putative miRNA binding sites in the Edn1 3'UTR. The minimum free energy values were higher than randomly expected (delta G = -13.4 kcal/mol) (Martin et al., 2007), suggesting that these sites are accessible to miRNA-RISC complexes. Inhibiting miR-709 through the use of anti-miRNA inhibitors demonstrated that miR-709 significantly affected Edn1 mRNA levels. Immunoprecipitation of the RISC subunit Argonaute demonstrated a direct interaction between RISC and Edn1 mRNA. The transfection of anti-miR-709 inhibitors prior to the RISC immunoprecipitation blocked RISC-Edn1 mRNA interaction. Aldosterone treatment of cells dramatically changed the amount of Edn1 mRNA targeted by RISC.

P-53

The Relative Contributions of Active Response and Passive Stiffness on the Pharmacological Response of Human Normal and Diseased Coronary Arteries to ET-1

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We have discovered that ET-1 mediated constrictor responses in human coronary artery (CA) *in vitro* are not attenuated in atherosclerotic compared to histologically normal arteries, despite extensive thinning of the medial smooth muscle layer with disease. This observation can be replicated in aorta of mice in which 50% of smooth muscle cells are ablated without subsequent loss of ET-1 response. To further understand these data we have investigated the effect of altered arterial structure (medial thinning, intimal proliferation and calcium/lipid deposition) on the passive mechanical properties of the vessel wall and determined whether this may contribute to the maintained ET-1 response in diseased CA.Passive force-stretch tests were performed to characterize the artery mechanical response and tangent stiffness at physiological pressure of normal and diseased CA rings. Results demonstrated that differences in material and geometry lead to a significant increase in the passive response with disease. However when these structural differences were included in a finite element analysis to predict the effect of plaque morphology, material properties and muscle contractile strain on both the passive and ET-mediated active responses, it was found that the comparable ET-1 responses could only be explained by assigning similar active forces in both the normal and diseased CA rings.This study has identified an appropriate methodology to correlate pharmacological and bio-mechanical models of stress and strain in arteries. However, although the atherosclerotic CA wall exhibits structural remodeling resulting in arterial stiffening this does not account for the ability of the diseased arteries to contract to ET-1 to the same extent as normal arteries.

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Mastocytes Derived or Recombinant Mouse Mast Cell Protease 4 (mMCP-4) Converts Big-ET-1 to ET-1 (1-31)

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The role of mouse mast cell protease 4 (mMCP-4) in the *in vivo* conversion of Big-ET-1 to ET-1, *via* the hydrolysis of the inactive intermediate, ET-1 (1-31), has been recently reported (Houde *et al*., JPET, 2013). Our principal aim was to assess the capacity of mast cell derived or recombinant mMCP-4 to convert Big-ET-1 to ET-1 (1-31) *in vitro*. The enzymatic activity of recombinant mMCP-4 (rmMCP-4) or peritoneal mast cell derived proteins was monitored *via* the hydrolysis of the fluorogenic substrate Suc-Ala-Ala-Pro-Phe-amidomethyl-coumarin whereas conversion of Big-ET-1 to ET-1 (1-31) by mMCP-4, in absence or in presence of a specific chymase inhibitor, TY-51469 (10μM) was semi quantified by HPLC. In addition, the impact of the deletion of the mMCP-4 gene on basal hemodynamic parameters was assessed in WT or mMCP-4 KO mice chronically-instrumented with wireless intra-aortic implanted pressure probes. The rmMCP-4 and purified mast cell proteins from WT mice possessed a TY-51469-sensitive chymase-like activity. HPLC-detected production of ET-1 (1-31) generated from the mMCP-4 (recombinant or mast cell extracted)-dependent hydrolysis of Big-ET-1, was also abolished be TY-51469. Finally, deletion of the mMCP-4 gene did not alter basal hemodynamic measurements (MAP (mmHg) and HR (bpm): 106,5±1,72 and 532,7±25,13 or 107,5±1,23 and 522,8±15,91, in WT or mMCP-4 KO mice, respectively). Our *in vitro* results demonstrate that mast cells-derived mMCP-4 convert Big-ET-1 to ET-1 (1-31). mMCP-4 however plays no significant role on basal hemodynamic parameters in conscious mice. Inhibiting mMCP-4 to reduce endogenous ET-1 production may constitute a valid alternative to the targeting of the endothelin-converting enzyme. (Supported by the Canadian Institute for Health Research).

P-55

Involvement of Endothelin-1 in Adrenal Catecholamine Regulation

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Endothelin-1, a 21-amino acid residue peptide, was first isolated as a potent vasoconstrictor peptide. Endothelin-1 is also known to potentiate adrenal catecholamine secretion. In the present study, we evaluated involvement of endothelin system in the catecholamine release and synthesis in the adrenal gland.Expression of endothelin receptors in the rat adrenal gland was confirmed. Intravenous infusion of endothelin-1 (1.0nmol) increased blood pressure (systolic and diastolic). Endothelin-1 stimulated intracellular calcium changes, resulting in increased nuclear factor of T cell (NFAT) activity and epinephrine release from cultured adrenal medullary cells. Furthermore, endothelin-1 increased catecholamine synthesis and caused hypertrophic changes in the cell size. Our results indicate involvement of the endothelin system in the sympathetic regulation of the adrenal medulla.

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The Anti-Inflammatory Effects of Endothelin-A Receptor Antagonism During Hyperdinamic Sepsis in Rats

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Introduction: The early hyperdynamic phase of sepsis is characterized by imbalance between oxygen delivery (DO_2) and consumption (VO_2) of the cells and microcirculatory insufficiencies. The role of vasoconstriction in this process is complex and controversial, but there are evidences for the activation of endothelin-A (ETA) receptors in the pathomechanism. In this study we evaluated the consequences of selective ETA receptor antagonism in a clinically relevant rat model of sepsis. **Methods:** Groups 1 and 2 of Sprague Dawley rats (n=8 each) were subjected to fecal peritonitis (0.6g/kg autofaeces i.p.), group 3 (n=8) served as sham-operated control (i.p. saline). In group 2, the ETA receptor antagonist ETR-p1/fl peptide (100 nmol/kg, iv) was given 17 hr after the induction of peritonitis. Invasive hemodynamic monitoring was started with regular blood gas analyses between the 16-20 hr of the insult to calculate VO_2-DO_2 values. Xanthine oxidoreductase (XO) activity, superoxide (SOX), and nitric oxide (NO_x) production were determined from small intestine biopsies, whereas myeloperoxidase (MPO) activity was measured from the lung tissue at the end of the experiments. **Results:** The increased XO and MPO activities were accompanied by elevated cardiac output and DO_2, while VO_2 decreased significantly as compared to the control values (576.4 ml/min *vs* 995.1 ml/min). The ETR-p1/fl peptide treatment normalized the DO_2-VO_2 values, increased NO_x and reduced SOX levels, and decreased XO and MPO activities. **Conclusion:** In the hyperdynamic phase of sepsis, the inhibition of the vasoconstrictive ETA receptors maintains cellular oxygen dynamics in the short run, and in parallel reduces the activation level of the inflammatory cascade mechanisms.Grant supports: OTKA K104656;TAMOP-4.2.2.A-11/1/KONV-2012-0035

P-57

The Toxicity of Indoxyl Sulfate to Endothelial Progenitor Cells is Rescued by Niacin

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BACKGROUND: Uremic toxin, indoxyl sulfate (IS), may impair proliferation and function of endothelial cells (ET) as well as endothelial progenitor cells (EPC). Nicotinic acid (Niacin), a lipid lowering drug, has antioxidant effect. METHODS & RESULTS: EPC were isolated from healthy subjects and incubated with 1 mM IS. IS decreased the viability of EPC by 34%, and was restored by 1mM niacin. IS did not induce apoptosis of EPC, but increased autophage and senescence of EPC, which were all restored by adding niacin. The ability of migration and tube formation of EPC were 50% inhibited by IS. Niacin restored the migration of EPC by 40%, but not tube formation. IS significantly increased ROS and heme oxygenase-1 expression, and decreased the expression of eNOS and VEGF. All these adverse effects of IS were antagonized by niacin. CONCLUSION: Niacin had beneficial effects on ET in uremic patients, in addition to lipid-lowering effect, through its restoration of EPC function.

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Endothelin-1 Does Not Alter Macrophage Phenotype

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ET-1 and inflammation are involved in the pathogenesis of hypertension. Macrophages, which are recruited to the inflamed vasculature, express both endothelin-A and endothelin-B receptors. Macrophages can also produce and release ET-1 but the precise effects of ET-1 on macrophage phenotype are unclear. Bone marrow derived macrophages (prepared from C57/BL6 mice) were stimulated for 24 hours with ET-1 (10-1000pg/ml), LPS/INFγ (100ng/ml and 10ng/ml) and IL4/IL13 (10ng/ml). Cytokines (TNFα, IL 6 and IL 10) and ET-1 were measured by ELISA and qRT-PCR and mRNA for iNOS, MCP-1, mannose receptor and Arginase 1 by qRT-PCR. Whereas LPS/INFγ increased production of TNFα, IL6 and IL10 and upregulated markers of classical activation (iNOS and MCP-1) ET-1 did not. IL4/IL13 stimulation upregulated markers of alternative activation (mannose receptor and arginase 1) but ET-1 did not. In addition, ET-1 pre-treatment and co-stimulation did not affect the response to LPS. *In vitro* ET-1 does not activate macrophages to alter phenotype and also does not have a co-stimulating effect with factors known to stimulate classical or alternative activation. This suggests that ET-1 is not pro-inflammatory to macrophages and does not induce an alternative activation.

			ET-1 (pg/ml)			
		Untreated controls	10	1000	LPS (100ng/ml) & INFγ (10ng/ml)	IL4 & IL13 (10ng/ml)
Concentration (pg/ml)	TNFα	210	231	216	2530 *	234
	IL6	39	39	29	548 *	27
	IL10	113	123	121	914 *	139

N= 4 repeated in triplicate, * P<0.001 compared to untreated control

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Endothelin-1 Activates Extracellular Signal-Regulated Kinases 1 and 2 Through Transactivation of Platelet-Derived Growth Factor Receptor in Skeletal Muscle Cells

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Background: Endothelin (ET) system plays a critical role in the development of insulin resistance and type 2 diabetes. However, the molecular mechanism for ET receptor (ETR) signaling activated by ET-1 in skeletal muscle remains to be determined. The purpose of this study was to determine the signaling molecules involved in ET-1-stimulated phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in rat skeletal muscle (L6) cells.Methods: Change in the phosphorylation level of ERK1/2 was analyzed by Western blot experiment. Dominant negative dynamin (K44A) along with monomeric strawberry red fluorescent protein (RFP) was overexpressed in L6 cells using adenovirus-mediated gene transfer. Infection efficiency of the adenovirus was determined by fluorescence of RFP with flow cytometry.Results: ET-1 induced concentration-dependent phosphorylation of ERK1/2 in L6 cells. The phosphorylation of ERK1/2 was abolished by BQ-123 (a selective ET_AR antagonist), YM-254890 (a $G_{\alpha q/11}$ protein inhibitor), PD98059 (a mitogen-activated protein kinase/ERK kinase 1/2 inhibitor), and AG370 (a platelet-derived growth factor receptor (PDGFR) kinase inhibitor). The ERK1/2 phosphorylation in response to ET-1 was inhibited by overexpression of dominant negative dynamin (K44A), which blocks clathrin-mediated endocytosis of cell surface receptor.Conclusions: $G_{q/11}$ protein-coupled ET_AR is involved in the ET-1-induces phosphorylation of ERK1/2 in L6 cells. The ET_AR-mediated phosphorylation of ERK1/2 is dependent on transactivation of PDGFR, which requires dynamin-dependent receptor internalization.

P-60

Endothelin Receptor Transactivates the TGFβ Receptor to Stimulate Proteoglycan Synthesis in Human Vascular Smooth Muscle Cells

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The current paradigm of G protein coupled receptor (GPCR) signalling involves transactivation of protein tyrosine kinase receptors. We utilised human vascular smooth muscle cells (VSMC) to address the question if a GPCR, the endothelin receptor, could transactivate a serine/threonine kinase receptor, specifically the TGF-β receptor, TβRI. Signalling molecules were assessed by Western blotting and proteoglycan synthesis by ^{35}S-sulfate and ^{35}S-Met/Cys incorporation and molecular size by SDS-PAGE. Endothelin-1 treatment led to a time and concentration dependent increase in cytosolic phosphoSmad2C which was blocked by the mixed endothelin receptor antagonist bosentan and the TβRI antagonist SB431542. Endothelin-1 treatment led to a time-dependent increase in nuclear phosphoSmad2C. Endothelin-1 stimulated proteoglycan synthesis was partially blocked by SB431542 and completely inhibited by bosentan. The effect of endothelin to stimulate an increase in glycosaminoglycan size on biglycan was also blocked in a concentration-dependent manner by SB431542. These data extend the current paradigm of GPCR signalling to include the transactivation of the serine kinase receptor for TGF-β (TβRI). This response will be considered in the context of responses to endothelin-1 and the options for therapeutically targeting endothelin-1 broadened to include downstream signalling otherwise associated with TGF-β receptor activation.

P-61

Structure of the Precursor of Salmon, Oncorhynchus Keta, Endothelins and Phylogenetic Analysis

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Endothelin (ET)-related receptors homologous to mammalian receptors have been cloned fromfish, indicating that ET-related ligands may be present in this species. Here wecloned cDNAs encoding preproendothelins (PPETs) from the intestinal cDNAlibrary. Salmon ETs cDNAs encode 200 amino acids, including a 20-amino-acid putativesignal sequence, as well as mature ETs, big ETs, and ET-like sequences. Thissequences together with other published PPET sequences were used to analyze thephylogenetic relationship among all ET family genes.

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Decreased MYPT-1 Phosphorylation at Thr696 and Cdc42 Protein Expression are Associated with Decreased Contractile Responses to ET-1 in Corpora Cavernosa and Internal Pudendal Artery from Goto-Kakizaki Diabetic Rats

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Endothelin-1 (ET-1) plays a crucial role in the development of erectile dysfunction (ED). The Goto-Kakizaki (GK) rat is a non-obese type 2 diabetes mellitus model, which displays ED and increased ET-1 plasma levels. The present study tested the hypothesis that GK rats display increased corpora cavernosa (CC) and internal pudendal artery (IPA) contractions to ET-1 as a contributing mechanism for ED. GK rats demonstrated impaired erectile function represented by decreased ICP/MAP responses after cavernous nerve stimulation. In GK rats contractile responses to ET-1 were decreased in both, CC tissue [Control: 396.00 ± 10 vs GK: 168.00 ± 16.00; Emax, mN/µg of tissue] and in IPA [Control: 25.00 ± 1.75 vs GK: 14.83 ± 1.66; Emax, mN]. Gene expression of prepro-ET-1 [Control: 1.00 vs GK: 0.25 ± 0.04] and ETB receptors [Control: 1.00 vs GK: 0.58 ± 0.09], but not ETA receptors was decreased in CC from GK rats. In GK rats, CC protein expression of ETA receptor [Control: 1.00 vs GK: 4.18 ± 0,58], and phosphorylation of ERK 1/2 [Control: 1.00 vs GK: 1.31 ± 0,09] were increased, whereas ETB receptor expression [Control: 1.00 vs GK: 0.75 ± 0.08], Cdc42 protein expression [Control: 1.00 vs GK: 0.40 ± 0.08] and phosphorylation of MYPT-1 [Control: 1.00 vs GK: 0.36 ± 0.15] were decreased. In conclusion, GK rats display ED and exhibit decreased cavernosal and IPA reactivity to ET-1. Whereas decreased phosphorylation of MYPT-1 and Cdc42 protein expression may account for decreased ET-1 responses, it indicates that ED in GK rats is not associated with augmented CC and IPA reactivity to ET-1.

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Generation of Edn2-iCre Transgenic Mice

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Endothelin-2 (ET-2) is a potent vasoconstrictive peptide. Though similar to ET-1, recent studies suggest that ET-2 may act through distinct pathways, necessitating deeper study. ET-2 may play a role in heart failure, inflammation, macular degeneration, and cancer metastasis. It is transiently expressed and tightly regulated in the periovulatory ovary, where it may aid ovulation by inducing constriction of the follicular wall. Here, we present a transgenic mouse line that expresses iCre (codon-improved Cre recombinase) under the regulation of the promoter of the *endothelin-2* (*edn2*) gene, which was developed as a novel model for characterizing the expression of ET-2 and for conditional deletion of genes in cells where ET-2 is expressed. A vector was generated containing *iCre*, a polyadenylation signal sequence, and an frt-neomycin-resistance-frt cassette. Two homologous regions of the *edn2* gene flanking the ATG start codon were isolated from a BAC (bacterial artificial chromosome) clone and inserted upstream and downstream of the iCre-pA-FNF cassette. Homologous recombination was used to re-insert the cassette into the BAC plasmid. Following purification, the plasmid was inserted into fertilized eggs of C57BL/6 mice through pronuclear injection, and resulting eggs were implanted into pseudopregnant mice. Five Edn2-icre transgene-containing lines of mice were established, and one line was bred with ROSA26 reporter. Offspring were used to localize iCre-expressing cells through X-gal staining. Characterization of the staining pattern revealed that iCre was expressed in granulosa cells of ovulatory follicles, cardiomyocytes, the pituitary, and the liver. We expect this novel mouse model to be a useful tool for future studies on the role of ET-2.

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The Calcitonin Gene-Related Peptide (CGRP) Play Beneficial Roles in Myocardial Ischemia Elicited by Endothelin-1

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Purpose: In addition to the adrenergic and cholinergic nerves, the cardiovascular tissues are also innervated by several peptidergic neurons that mediate nonadrenergic noncholinergic (NANC) functions. Among such neuropeptides, CGRP is released from capsaicin-sensitive sensory neurons in peripheral organs. CGRP is known as one of the most potent endogenous vasodilators in coronary arteries, and appeared to show more potent vasodilating effects on the small-diameter resistant vessels than the large-diameter conduit vessels. The purpose is to study the roles of CGRP in the coronary microcirculation and energy metabolism under ischemic conditions elicited by endothlin-1 (ET-1). Methods: We observed the effects of CGRP on the coronary microcirculation and energy metabolism in isolated beating rat hearts. Microcirculation was observed by an intravital fluorescence videomicroscope system and energy metabolism was evaluated under 31P-magnetic resonance spectroscopy (31P-MRS) after coronary microvessels were pre-contracted with ET-1(30 pmole), a potent intrinsic vasoconstrictor of small arterioles in myocardium. Results: Cumulative application of CGRP (3-1000 pmole) elicited both dose-dependent reduction in total coronary perfusion resistance (TPR) and simultaneous vasodilation of arterioles of 10-40 micrometer in diameter (maximal % relaxation was 62%, n=7). The ED50 value of CGRP was 30 pmole, about 5000-fold smaller than that of nitroglycerin. Administration of CGRP (100pmole) decreased TPR (-34%), increased heart rate (+17%), cardiac dP/dt (+76%), work index (+57%), ATP (+21%) and high-energy phosphates (PCr) (+45%) significantly ($p<0.05$, n=14) in myocardium. Conclusion: CGRP relaxed the coronary microvessels, improved the myocardial high-energy metabolism and enhanced the cardiac contractility in the ischemic myocardium, suggesting that the CGRP may play beneficial roles in myocardial ischemia elicited by ET-1.

P-65

The Role of Endothelin Receptors (ETRA/B) in Fibrocyte Differentiation

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Introduction Scleroderma (SSc) is an autoimmune connective tissue disease of unknown aetiology. Pulmonary involvement including the development of pulmonary arterial hypertension (PAH) is characterised by vascular remodelling, collagen deposition and expression of connective tissue growth factor (CTGF). $CD14^+$ monocytes can differentiate into spindle shaped cells termed 'fibrocytes'. Fibrocytes express haematopoietic and mesenchymal markers including collagen, and amplify inflammatory/immune responses via antigen presentation and chemokine secretion. Fibrocyte differentiation is enhanced by fibrogenic cytokines including PDGF. The role fibrocytes play in promoting PAH in SSc is unknown. **Methods** $CD14^+$ PBMCs were isolated from SSc and healthy donor blood. Fibrocyte differentiation in the presence of MCSF and/or ET-1 was assessed after 14 days. The effect of endothelin receptor (ETR) antagonists (selective/dual) on fibrocyte differentiation (n=6) was investigated. SSc and control fibrocyte secretomes were assessed by ELISA (n=6), and the effects on fibroblast-mediated gel contraction determined. **Results** MCSF and ET-1 alone and in combination induced fibrocyte differentiation ($P<0.05$). SSc fibrocytes exhibited enhanced differentiation from $CD14^+$ PBMCs than healthy control donors in response to MCSF ($P<0.05$), ET-1 ($P<0.05$) and in combination ($P<0.01$). ETR antagonists BQ123 (ETR_A), BQ788 (ETR_B) and Bosentan ($ETR_{A/B}$) inhibited MCSF induced fibrocyte differentiation. CTGF secretion was elevated in SSc compared to control fibrocytes ($P<0.05$) cultured with MCSF. Conditioned media from SSc fibrocytes promoted gel contraction by control pulmonary fibroblasts ($P<0.05$). **Discussion** $CD14^+$ SSc PBMCs readily differentiate into fibrocytes in response to ET-1 and MCSF via ETR_A and ETR_B. Our data suggests fibrocytes contribute to the development of PAH in SSc via a paracrine mechanism modulating the functional activities of resident tissue fibroblasts.

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Improved Relaxations to Acetylcholine in Murine Carotid Arteries with Heterozygous Overexpression of Preproendothelin-1 in the Endothelium

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The endothelium can release both NO and contracting factors (EDCFs). Exogenous endothelin-1 (ET-1) causes ET_B receptor mediated release of NO, but also enhances endothelium-dependent contractions. Besides its propensity to exhibit EDCF-mediated contractions, the murine carotid artery is characterized by high basal and stimulated NO generation. The role of the endothelial endothelin system on endothelium-dependent relaxations in this preparation is unknown. Therefore, a model of endothelium-restricted heterozygous overexpression of ppET-1 was used (TET+/- mice). Relaxations were studied and compared in carotid arteries of 34-36 weeks old TET+/- mice and WT littermates. Experiments were performed, in the presence of meclofenamate to exclude endothelium-dependent contractions, in rings suspended in Halpern-Mulvany myographs. Responses to phenylephrine (1 nM to 30 μM) were similar between genotypes, and the final levels of contraction were not significantly different (57±6% KCl in WT vs. 49±5% KCl in TET+/-). Acetylcholine-induced relaxations were potentiated in TET+/- mice compared to littermate controls (PD_2 8.37±0.05 vs. 8.61±0.06 in TET+/-, n=7-10, P<0.01). By contrast, endothelium-independent relaxations to sodium nitroprusside were not different (n=6-8). In the presence of meclofenamate, TET+/- had no effect on contractions to the calcium ionophore A23187 (n=6-7), but maximal responses to the TP receptor agonist U46619 (0.1 nM to 3 μM) were decreased compared to WT control mice (E_{max} 123.4±3.5% vs. 108.1±2.5% KCl in TET+/-, n=6-9, P<0.01). These results suggest that moderate increases in endothelial ET-1 expression in murine carotid arteries enhance endothelium-dependent, NO-mediated relaxations and reduce smooth muscle responsiveness to TP receptor activation.

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Vascular Pharmacology of Quercetin in Rat

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Quercetin, a kind of flavonoids, exerts the cardiovascular actions. In rat aorta, quercetin (0.1 to 100μM) relaxed the contraction induced by pretreatment with 5μM NE in a concentration-dependent manner. NG-monomethyl-L-arginine acetate (L-NMMA)(100μM), a NO synthesis inhibitor, reduced the quercetin (100μM)-induced vasorelaxation from 97.0 ± 3.7% (n=10, P<0.05) to 78.0 ± 11.6% (n=5, P<0.05). Endothelium removal as well attenuated the vasodilatation. In the presence of both 100μM L-NMMA and 10μM indomethacin, the quercetin-induced vasorelaxation was further attenuated by high K (30mM) or 10μM tetraethylammonium (TEA, KCa channel inhibitor). Nicardipine caused less or no effect on the relaxation. The quercetin-induced vasodilatation was attenuated by 0.3μM apamin (SK channel inhibitor), but not by 30nM charybdotoxin (BK and IK channel blockers). Under KCl-induced vasoconstriction, the quercetin-induced vasorelaxation was attenuated by PK-C inhibitors. Gö6983 (α-, β-, γ-, δ- and ζ-sensitive) produced a stronger relaxing effect than Ro-31-8425 (α-, β-, γ- and ε-sensitive). These results indicate that the vasorelaxation is dependent on the endothelium, and is also exerted by the modulation of SK channel and PK-Cδ. In rat mesenteric artery, the quercetin-induced vasodilatation was in part resistant to both 100μM L-NG-nitro arginine methyl ester (L-NAME) and 100μM indomethacin. The L-NAME- and indomethacin-resistant quercetin-induced vasodilatation was attenuated by TEA (1 mM) and also by 100μM 18α- and 50μM 18β-glychrrhetinic acids (gap junction inhibitors). These results indicate that the vasorelaxation is also dependent on the endothelium and KCa channel, and is further produced by the modulation of the gap junction. Therefore, quercetin vasodilates the vascular smooth muscle mediated by endothelium-dependent and -independent mechanisms.

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Effect of Selective Ablation of Endothelin A Receptor in the Granulosa Cells on the Fertility

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Endothelin-2 (ET-2) is highly expressed in the granulosa cells (GCs) of periovulatory ovary. Previous experiments demonstrated that endothelin receptor antagonization inhibits ovulation in rodents. In the ovary ET-2 is expressed only in the GCs, while endothelin receptors are expressed in a variety of ovarian cell types. This study was designed to determine the significance of the expression of endothelin A receptor (ETA, a protein product of Ednra gene) in the granulosa cells. Two lines of GC-specific Ednra knockout mice were generated by crossbreeding Ednra-flox/flox mice with either Amhr2Cre or Cyp19Cre mice which express Cre recombinase under the anti-Mullerian hormone receptor promoter and the aromatase promoter, respectively. While both transgenic mice express Cre in the granulosa cells, the Cyp19Cre mouse were expected to have a higher rate of Ednra deletion than the Amhr2Cre mouse. Reproductive function of the resulting mice was measured by their fertility and litter size (number of pups per mouse and pups per litter) after breeding the GC-specific Ednra knockout female mice with proven males. Ednra deletion driven by either Amhr2Cre mice (GC-EdnraKO-amhr2) or Cyp19Cre mice (GC-EdnraKO-Cyp19) reduced fertility (57.9% in GC-EdnraKO-Amhr2 and 42.3% in GC-EdnraKO-Cyp19 compared to 76.5% in WT, p=0.22 and p=0.02, respectively). In addition, GC-EdnraKO-Amhr2 and GC-EdnraKO-Cyp19 mice had significantly smaller litter size as well (3.55 ± 0.45 and 4.56 ± 0.53 vs 7.08 ± 0.48 in WT, p<0.01 for each). Taken together, this study demonstrates that ETA in granulosa cells plays an important role for female fertility in mice.

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Physiological and Functional Antagonism of Arterial Endothelin$_A$ Receptor Function

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Long-lasting arterial contractions induced by endothelin-1 (ET-1) are caused by tight binding of ET-1 to ET_A receptors. Slow dissociation of ET-1 from ET_A receptors suggests that i) an endogenous system effectively counteracts an otherwise continuously active contractile system and ii) inhibitors of contractile mechanisms or stimuli of relaxing mechanisms must induce long-lasting effects in order to effectively counteract the ET system. In isolated rat mesenteric resistance arteries, we studied these potentially effective pharmacological approaches. Calcitonin gene-related peptide (CGRP) only slightly reduced arterial sensitivity to ET-1 but terminated ET-1-induced vasospasms by promoting dissociation of ET-1/ET_A receptor complexes. The CGRP effects were mediated by CGRP receptors and not mimicked by stimuli of mediators downstream of $G\alpha_s$-proteins such as adenylate cyclase (AC), but rather were blocked by $G\beta\gamma$-inhibition. Stimuli of AC such as forskolin and isoproterenol did produce a readily reversible relaxation of ET-1-induced vasospasms without affecting the initial contractions. The poorly reversible stimulator (Bay412272) and activator (Bay602770) of soluble guanylate cyclase produced long-lasting relaxations, also without inhibiting the initial ET-1-induced contractions. Rho-kinase inhibition (OH-fasudil) was without effect, TRPC3 or L-VOCC inhibition (Pyr3 and felodipine, respectively) only relaxed ET-1-induced vasospasms and PLC inhibition (U73122) strongly reduced contractile responses to ET-1 and relaxed the vasospasms. These results suggest that long-acting physiological and functional antagonists can effectively relax ET-1-induced vasospasms. Therefore, these systems can provide an alternative for current ET_A antagonists which do not display this beneficial characteristic. This study was performed within the framework of TI Pharma projects T2-301 and T2-108.

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Negative Allosteric Modulation of Endothelin ET_A Receptor Function in Resistance Arteries

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Inhibition of resistance artery responses to endothelins (ETs) might be useful in therapy-resistant hypertension and stroke. ETs cause long-lasting arterial contractions by tight binding to smooth muscle ET_A receptors. Chemically diverse ET-receptor antagonists (ERA) inhibit binding of ET-1 to ET_A and reduce sensitivity to ETs. We investigated negative allosteric modulation by ERA in isolated rat resistance arteries. 1 μM BQ123 (cyclic pentapeptide) reversibly relaxed contractile responses to 32 nM ET-1 to a different extent in arteries from different vascular beds (-0 to -80%) and reduced mesenteric artery (MA) sensitivity to ET-1 more avidly (pK_B 7.6±0.4) than that to ET-2 (pK_B 5.6±0.4). This agonist-dependence was less marked with 100 nM PD-156707 (butenolide; pK_B 8.5±0.3 and 7.9±0.3) and not significant with 1 nM BMS-193884 (biphenyl sulphonamide; pK_B 9.3±0.1 and 9.2±0.1). Effects of high concentrations of BMS-193884 on MA sensitivity to ET-1 and ET-2 were not concentration-dependent. In the presence of 10 nM BMS-193884, i) 1 μM BQ123 had no additional effect but ii) 100 nM PD-156707 resulted in a further significant reduction of MA sensitivity to ET-1. Binding i) a fluorophore to PD-156707 (useful for diagnosis) or ii) an angiotensin AT1-antagonistic moiety to BMS-193884 (dual antagonist PS-433540) impaired ET-antagonism by the pharmacophores. Thus, chemically distinct ERA act differently on resting and agonist-activated ET_A receptors and display system- and agonist-dependence and saturability (pharmacological properties of allosteric modulation). Also, the observations suggest presence of several allosteric binding sites on ET_A receptors, the structure-activity relationships of which can be studied.

P-71

Purification Different Forms of Extracellular Superoxide Dismutase and Their Effects on Anti-Hypertension Through Nitric Oxide Induction in Spontaneous Hypertension Rats

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Extracellular superoxide dismutase (EC-SOD), one member of SOD family, exists outside the cells in mammals. It catalyzes conversion of superoxide anion (O2-) into hydrogen peroxide (H2O2), destroying free radicals upon reactive oxygen species (ROS) production to relieve oxidative stress. In this study, a methylotrophic Pichia pastoris system was used to produce a large-scale of recombinant human EC-SOD by yeast fermentation. After purified by fast protein liquid chromatography (FPLC), three different subtypes of EC-SOD were performed. Furthermore, the spontaneously hypertensive rat (SHR) was used to examine anti-hypertension efficiency among different EC-SOD isoforms through blood pressure measurement and NO release in blood after tail intravenous injection of EC-SOD. Results showed that different subtypes of EC-SOD share similar secondary structure but their activity diverse. After injection of high-dose EC-SOD into SHR, we observed obvious decrease of blood pressure and increase of blood nitric oxide (NO) immediately. After blocking the NO synthesis, EC-SOD lost its ability of blood pressure regulation. Our data provided that enormous antioxidant and antihypertensive activities of human EC-SOD were largely produced by yeast fermentation. It can be applied on the extent of health care to prevent hypertension and also cardiovascular diseases.

P-72

Identification and Characterization of Novel Antihypertensive Peptides Obtained from Fermented Milk

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The fermented milk has antithrombotic, immunomodulatory, antihypertensive and cardiovascular effects. In the present study, the fermented milk and its derived peptide, 19AA, were investigated for the first time. Results demonstrated that the fermented milk had a strong antihypertensive activity in spontaneous hypertension rats (SHRs). Among two fractions derived from the fermented milk, the Fraction A exhibited the best antihypertensive activity. Following reverse-phase high-performance liquid chromatography, a 19AA short peptide was further purified and identified from the Fraction A. The synthetic peptide with a sequence of 19AA showed the strong antihypertensive activity from 1 to 10 hours after oral administration of 1 mg of 19AA/kg of body weight, and the effect of systolic and diastolic blood pressure (SBP and DBP) decreasing was maximal at 44.0 ± 2.1 mmHg and 23.0 ± 2.9 mmHg, respectively, after oral administration. The present study revealed that 19AA showed excellent antihypertensive activity, and its activity is better than that of VPP tripeptide which has been already included in functional foods. In conclusion, the 19AA fermented peptide is the bioactive ingredient with potential benefit in the prevention and treatment of hypertension or other associated disorders.

P-73

Counteracting Effects of Treprostinil and Endothelin (ET-1) Receptor Antagonists (ETRAs) on Endothelin-1, ETB Receptor and ECE-1 Levels in Pulmonary Smooth Muscle Cells (PASMCs) Derived from Patients with Pulmonary Arterial Hypertension (PAH)

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Background: ET-1 levels rise in PAH, correlating with increased pulmonary vascular resistance and mortality. Lung endothelial ETB receptors promote ET-1 clearance, which is improved in PAH patients by epoprostenol (prostacyclin). Whether prostacyclin analogues behaviour similarly is unclear or if smooth muscle ETB receptors regulate ET-1 levels. Methods: Cultured human PASMCs from PAH patients were treated for 24 hrs with treprostinil, an ETRA or a combination. A chemiluminescent ELISA was used to measure ET-1 in the supernatant while ET-1 converting enzyme (ECE-1) and ETB expression were evaluated by Westerns and immunohistochemistry in lung sections. Results: Serum doubled ET-1 levels in HPSMCs, an effect abolished by treprostinil (10-100nM). In contrast, the ERTAs (100nM) bosentan and ambrisentan increased ET-1 levels by 100% while BQ788 (selective ETB antagonist) by 200%. Treprostinil in combination with these ETRAs failed to inhibit serum-induced ET-1 elevation. In contrast, BQ123 (selective ETA antagonist) did not affect the response to serum or treprostinil. ECE-1 protein levels were higher with BQ788 and bosentan compared to serum or treprostinil. Furthermore, ETB expression was down-regulated by all ETRAs, but not by treprostinil. In the smooth muscle layer of PAH lungs, ECE-1 and ETB expression was markedly increased. Conclusion: Treprostinil potently inhibits ET-1 levels in human PASMCs which is counteracted by ETRAs targeting ETB but not ETA receptors. ETB receptors may also regulate ET-1 levels through changes in ECE-1 expression. We postulate that higher concentrations of treprostinil may be required to reach clinical efficacy in PAH when combined with non-specific ETRAs.

P-74

Potential Involvement of Functional Tricuspid Regurgitation in the Diagnostic Error to Assess Pulmonary Arterial Pressure by Doppler Echocardiography

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Background: Transthoracic Doppler echocardiography (DE) is useful for the screening of pulmonary hypertension (PH), which is often treated by endothelin antagonist, although recent studies have suggested that estimation of pulmonary artery pressure (PAP) by DE is frequently inaccurate. This study aimed to examine that functional tricuspid regurgitation (TR) with geometric alterations caused by right ventricular dilatation is involved in the diagnostic error of echocardiography for the assessment of PAP. Methods: We conducted a retrospective cohort study of consecutive 127 patients (male, n=58, mean age of 55y) who received both echocardiography and right heart catheterization (RHC) during the 2-year period from November 2008 to October 2010. We defined PH as mean PAP>25mmHg at rest by RHC and "accurate estimated echocardiographic value" when it remained within 10mmHg of the invasive measurement. Results: A total of 75 patients (59%) were diagnosed to have PH by RHC. When the patients were divided into 3 groups; accurate (n=52), over-estimate (n=63) and under-estimate (n=12), the diagnosis of PH by RHC was 42% in accurate, 68% in over-estimate, and 83% in under-estimate groups (P=0.004). In echocardiography, right ventricular dimension was significantly larger in over-estimate group (accurate, 30.0±5.7mm; over, 35.3±8.6mm; under, 32.8±5.2mm, P=0.002), and the severity of TR was significantly worse in over-estimate group (P<0.0001). Right atrium tended to be larger in both over-estimate and under-estimate groups than accurate group (accurate, 38.8±5.7mm; over, 42.6±8.49mm; under, 42.7±6.2mm, P=0.073). Conclusions: Our results indicate that the accuracy of DE is not enough for PAP evaluation, particularly in patients with PH associated with increased TR grading and enlarged right heart dimension.

P-75

Detection of Developing Pulmonary Vasculopathy with Non- Invasive Cardiopulmonary Exercise Testing

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Since the discovery of ET-1, over-expression of ET-1 has been demonstrated in patients with pulmonary arterial hypertension (PAH). In contrast to chronic thromboembolic pulmonary hypertension (CTEPH), patients with PAH have pulmonary vasculopathy (PV). PV leads to impaired dilatation of affected pulmonary vessels, impeding the increase of cardiac output (CO) and stroke volume (SV) during exercise. Peak O_2 uptake shows CO, and peak O_2-pulse shows SV during cardiopulmonary exercise testing (CPX). To investigate the increase of CO during exercise, we performed CPX in 12 patients with PAH and 7 patients with CTEPH. Predicted peak O_2 uptake (45.5±8.0 vs. 60.6±13.4%, p< 0.01) and predicted peak O_2-pulse (55.6±7.6 vs. 69.1±6.9%, p < 0.01) were significant higher in CTEPH than PAH. Diffusion capacity for carbon monoxide (%DLco: 40.3 ± 13.7 vs. 62.2 ± 13.9%, p< 0.01) was also significant higher in CTEPH than PAH, however there was no correlation between %DLco and peak O_2 uptake or peak O_2-pulse. While, there was no difference in mean pulmonary arterial pressure (mPAP: 31±6.8 vs. 30.1 ± 7.0mmHg, n.s.), cardiac output (CO: 4.1±0.6 vs. 4.4 ± 0.4 L/min, n.s.), and pulmonary vascular resistance (PVR: 5.2 ± 2.0 vs. 5.2 ± 2.8 wood units, n.s.) at rest. Our data indicate that, regardless of hemodynamic, both lower peak O_2 uptake and peak O_2-pulse show PV impeding the increase of CO during exercise. CPX can predict the onset of PAH by detection of PV in early stage.

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Vascular Endothelial Growth Factor (VEGF) and the Control of Endothelin-1 Synthesis by Human Lung Microvascular Endothelial Cells: A Possible Pathway for Pathogenesis

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Introduction: Increased endothelin-1 (ET-1) is a hallmark of pulmonary arterial hypertension (PAH), and contributes to its pathogenesis. The factors controlling ET-1 in PAH are poorly understood. Vascular endothelial growth factor (VEGF) blockade results in PAH-like lesions in animal models, and has caused PAH in humans. The effects of VEGF on ET-1 production by human lung blood microvascular endothelial cells (HMVEC - LBl) are unknown. **Methods:** We exposed HMVEC-LBl (Lonza Inc.) *in-vitro* to human $VEGF_{121}$ (40ng/ml) in serum-free medium for 7 hours, in the absence or presence of the VEGF receptor antagonist, SU5416 (Cayman Chemical, 3 and 10 μM). ET-1 production was measured in the supernatant. Phosphorylation of VEGF receptor 2 (VEGFR2) was measured by western blotting after exposure to VEGF ± SU5416 for 5 and 10 minutes. **Results:** VEGF effectively caused VEGFR2 phosphorylation, which was blocked by SU5416. VEGF decreased ET-1 production by 29%. In the absence of VEGF, SU5146 increased ET-1 production, by 16% at 10 μM, and SU5146 was able to completely abolish the VEGF effect on ET-1 production. **Conclusion:** VEGF may promote vascular health by decreasing ET-1 production in HVMEC-LBl. Blockade of VEGF signalling by SU5416 increases ET-1 levels and may thereby contribute to the pathogenesis of pulmonary hypertension seen with VEGF blockade.

P-77

Effect of Bosentan on Exercise Capacity in Patients with Pulmonary Arterial Hypertension or Inoperable Chronic Thromboembolic Pulmonary Hypertension

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Background: Endothelin receptor antagonists (ERA) improve the prognosis of patients with pulmonary arterial hypertension (PAH). However, only limited data are available on the effect of treatment with the ERA bosentan on exercise capacity assessed with cardiopulmonary exercise testing (CPX) in patients with PAH or inoperable chronic thromboembolic pulmonary hypertension (CTEPH). **Purpose**: To investigate the effect of the oral, dual-ERA bosentan on exercise capacity in patients with PAH or inoperable CTEPH by means of CPX. **Methods**: Fifteen consecutive PAH (mean age, 47 ± 21 years) and 9 consecutive inoperable CTEPH patients (mean age, 49 ± 12 years) with World Health Organization Functional Class II to IV were treated with bosentan. All patients underwent cardiac catheterization, echocardiography, and CPX at baseline. CPX was performed both prior to initiation of bosentan therapy and after 6 months. **Results**: In PAH patients, peak VO_2 significantly increased from 13.8 ± 6.8 mL/kg/min at baseline to 16.8 ± 7.2 mL/kg/min after 6 months ($P < 0.01$). Similarly, VE/VCO_2 slope also significantly decreased from 56.8 ± 22.5 to 48.9 ± 17.5 ($P < 0.05$). However, in CTEPH patients, there were no significant differences in peak VO_2 or VE/VCO_2 slope between the before and after bosentan therapy values ($P = 0.35$, $P = 0.67$, respectively). The medication was well tolerated by all patients, and there was no evidence of drug-related liver dysfunction. **Conclusions**: Bosentan therapy improves exercise capacity in patients with PAH within a relatively short period. However, the effect is not seen in patients with CTEPH.

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Short-Term Drug Interaction of Bosentan and Sildenafil under the Long-Term Use in Patients with Pulmonary Arterial Hypertension

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<BACKGROUND> Bosentan and sildenafil are often administered together for the treatment of PAH. Bosentan is a known inducer of CYP3A4 in chronic use and therefore, the plasma concentration of sildenafil is decreased almost by half when co-administered. In the course of daily life, patients tend to take these medicines at the same time in the morning and the evening. We investigated how the plasma concentration of sildenafil changed when bosentan/sildenafil was taken beforehand with the other. <METHODS> A randomized, Open-label crossover study was conducted in PAH patients of WHO functional class III, who chronically received both bosentan and sildenafil. Patients were randomly assigned to either Pattern 1 or 2, both of which consisted of three phases as follows; phase *S* : patients take sildenafil three hours prior to bosentan, phase *B* : patients take bosentan three hours prior to sildenafil, and phase *C* : patients take sildenafil and bosentan simultaneously (control). We collected blood samples on the last day of each phase and measured the plasma concentration of sildenafil using liquid chromatography-tandem mass spectrometry. <RESULTS> Six patients entered the study. In sildenafil C_{max} , phase *S* was 72.9±40.9 (ng/ml, mean±S.D) and it was significantly lower than phase *C* (P=0.0215). Phase *B* was 99.6±33.9 with no significant difference with phase *C* (P=0.6173). In sildenafil $AUC_{0\text{-}8}$, phase *S* was 108.2±126.4 (hr/ng/ml, mean±S.D) and phase *B* was 240.7±121.8. Neither phase proved significant difference with that of phase *C* (203.5±81.3, P=0.3213 and 0.1999, respectively). <CONCLUSION> It is indicated that there is a short-term drug interaction between bosentan and sildenafil which may be relevant to CYP3A4 metabolism.

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Chronic Treatment with Novel Endothelin Receptor Antagonist Macitentan Improved Severe Pulmonary Arterial Hypertension in Rats

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Rationale: A novel dual ETA/ETB receptor antagonist, macitentan, has been reported to significantly reduce morbidity and mortality in patients with pulmonary arterial hypertension (PAH) (SERAPHIN Study). However, the underlying mechanisms warrants further preclinical investigations. We have established a rat model of PAH, which develops severe pulmonary hypertension (PH) with occlusive pulmonary arteriopathy indistinguishable from that in PAH patients. This study investigated the effects of macitentan on hemodynamics and histopathology in the rat model with established severe PAH. **Method and Results:** Rats received a single subcutaneous injection of 20mg/kg SU5416, a VEGF blocker, and then exposed to 3-week hypoxia (10% O_2) followed by 5-weeks of normoxia. Eight weeks after the SU5416 injection, in comparison with normal rats, all rats developed severe PH (RV systolic pressure: 23±6 vs. 102±15mmHg, n=4 or 5 for each, p<0.001) with RV hypertrophy (the mass ratio of RV to LV pulse septum: 0.23±0.01 vs. 0.77±0.05, p<0.001). Five-week treatment with macitentan (30 mg/kg/day, orally, from week 3 to 8) significantly reduced RV systolic pressure (41±5mmHg, n=6, P<0.05) and hypertrophy (0.36±0.03, n=7, P<0.005) without decreasing cardiac output. Also, macitentan significantly attenuated the medial wall thickness and complex occlusive lesions in PAH rats by histological examination. **Conclusion:** Chronic treatment with macitentan markedly hemodynamically and histopathologically improved PAH in SU5416/hypoxia/normoxia-exposed rats. The improvement of arteriopathy may in part contribute to the beneficial effects of macitentan on PH.

P-80

Ambrisentan and Tadalafil Synergistically Attenuate Chronic Hypoxia-Induced PAH in Rats

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We showed previously that ambrisentan, a selective endothelin type A receptor antagonist, and tadalafil, a PDE5 inhibitor, act synergistically to relax endothelin-constricted pulmonary arteries (Liang et al. Hypertension 2012; 59: 705-11). To confirm these findings in an in-vivo model of PAH, we investigated the effect of ambrisentan and tadalafil in combination on hypoxia-induced PAH in rats. Upon exposure to hypoxia (10% O2), male SD rats were dosed with vehicle, ambrisentan (1 mg/kg, q.d.), tadalafil (10 mg/kg, q.d.) or the combination via oral gavage for three weeks. Three weeks of exposure of rats to hypoxia increased mean pulmonary arterial pressure (mPAP) from 10.8&plusmn0.7 mmHg (normoxic, mean &plusmn SEM, n=8) to 23.9&plusmn1.3 mmHg (hypoxic, n=12, p<0.01 vs normoxic). Teatments with ambrisentan, tadalafil and the combination reduced mPAP to 20.1&plusmn0.8 mmHg (n=12, p<0.05 vs hypoxic), 20.8&plusmn1.2 mmHg (n=11, p<0.05 vs hypoxic) and 15.9&plusmn1.0 mmHg (n=12, p<0.01 vs hypoxic), respectively. Chronic exposure of rats to hypoxia also increased the ratio of right ventricle weight/ left ventricle weight (RV/LV) from 0.326&plusmn0.013 (normoxic, n=8) to 0.602&plusmn0.019 (hypoxic, n=12, p<0.01 vs normoxic). The ratios of RV/LV from hypoxic rats dosed with ambrisentan, tadalafil and the combination were decreased to 0.527&plusmn0.014 (n=12, p<0.05 vs hypoxic), 0.531&plusmn0.016 (n=11, p<0.05 vs hypoxic) and 0.430&plusmn0.017 (p<0.01 vs hypoxic). Consistent with the in-vitro pulmonary artery data, the combination of ambrisentan and tadalafil caused a greater effect than each drug alone or the calculated sum of the individual effects of each drug, suggesting that ambrisentan and tadalafil synergistically attenuate hypoxia-induced PAH in rats.

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Real World Experience in the DETECT Study for Pulmonary Artery Hypertension Associated with Systemic Sclerosis

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Objectives: The currently ongoing DETECT study, a two-stage, prospective, observational, cohort study in systemic scleroderma (SSc) patients to evaluate screening tests and the incidence of pulmonary arterial hypertension (PAH) and pulmonary hypertension, is attempting to refine the screening process in pulmonary artery hypertension associated with SSc. We adopted the same multiple screening tests forced vital capacity [% predicted]/DLCO [% predicted]; current/past telangiectasias; anti-centromere antibody; N-terminal pro-brain natriuretic peptide; uric acid; right axis deviation on electrocardiography) in patients with SSc in our hospital to evaluate its Method Date from 21 SSC patients, who had undergone right heart catheterization from 2009 to 2012 in our hospital, were retrospectively analyzed. We compared the result of DETECT screening system to mean pulmonary artery pressure assessed by right heart catheterization. Results: Seventeen SSc patients (80.9%) were categorized as candidates to referral to right hear catherterization in this study. Overall sensitivity was 100% and specificity was 25%. Conclusion: According to the 2012 American College of Rheumatology Annual Meeting, DETECT algorithm was announced that its sensitivity was 96% and specificity was 48%. Therefore, our results indicated that DETECT algorithm had the possibility to overestimate the risk of PAH in SSc patients.

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Reduced Circulating Endothelin-1 Level in Uncorrected ASD Patients with Severe Pulmonary Hypertension

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There is increased risk of pulmonary hypertension (PH) in patients with Atrial Septal Defect (ASD), although the factors associated have not clearly defined. Endothelin-1 (ET-1), a potent vasoconstrictor derived mainly from pulmonary endothelium, has been reported to be elevated in PH associated with congenital heart defect (CHD). However, studies about CHD-related PH included only small number of ASD-patients and most of them were performed in children. In this study, we aim to measure the circulating ET-1 level in adult patients with uncorrected ASD complicated by severe pulmonary hypertension. Fifty-two newly diagnosed ASD patients were participating in this study, aged 20-79 years-old. Measurements of RVSP, characteristics of ASD, remodeling RV were performed using TTE and TEE. The hemodynamic measurement by echo showed significant correlation with right heart catheterization ($r=0.8; p<0.0001$). Peripheral blood was withdrawn from brachialis vein and circulating ET-1 was measured using ELISA. Severe PH were defined as RVSP>60mmHg. The severe PH group (n=25) was confirmed by larger RA diameter, larger RV diameter, reduced RV systolic function, and higher tricuspid valve gradient as compared to non-severe PH group (n=27) (47.6±1.47vs.41.2±1.24mm; $p<0.01$; 48.6±1.16vs.41.2±1.29mm; $p<0.001$; 21.9±1.01vs.25.9±1.45mm; $p<0.0001$; 92±5.5vs.33.7±1.88mmHg; $p<0.0001$; respectively). There were no differences of age, diameter of the defect, and pulmonary flow ratio in the severe PH group. Interestingly, the circulating plasma ET-1 level was significantly lower in the severe group (6.3±0.48vs. 4.7±0.32pg/dl;$p<0.01$). In conclusion, we reported lower circulating plasma ET-1 in ASD patients with severe PH. The further study should be performed to elucidate the ET-1 level in pulmonary circulation in this disease.

P-83

Current State of Medicine Usage and the Predictor of Mortality in Pulmonary Arterial Hypertension in Japan

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<Background> Endotheline receptor antagonist (ERA) is recommended for treatment of pulmonary arterial hypertension (PAH). However, recommendation is based on reports of monotherapy. <Method> We examined consecutive 112 patients diagnosed with Group 1 and 1' PAHwho visited 14 affiliated hospitals from July 2006 to January 2013. The difference in mortality between monotherapy group and combination therapy group (ERA and other PAH drugs) was compared. <Results> There were 41 idiopathic, 43 collagen tissue disease, 24 congenial heart disease and 4 other types of PAH. Mean age was 52.2 years old, female 66.1%, WHO Functional Class1 5.6%, 2 31.5%, 3 53.9%, 4 9.0%, BNP 128 (49.3-406.5) pg/mL, cardiac index 3.4±1.6 (L/kg/m2) , mean pulmonary arterial pressure 48.2±19.3 mmHg, and tricuspid regurgitation pressure gradient (TRPG) 57.5±25.2 mmHg. Pericardial effusion was observed in 28%. ERA, prostacyclin, PDE-5 inhibitor and epoprostenol were used in 71.8%, 60.9%, 51.8%, and 18.2%, respectively. There were no significant difference in characteristics between the monotherapy group and the combination therapy group, except for frequent use of the combination therapy in idiopathic PAH. Although pericardial effusion, cardiac index, and mean right atrial pressure were predictors for mortality in the combination group, there was no difference in mortality between the monotherapy group and the combination group. <Conclusion> Combination therapy was frequently used in idiopathic PAH. However, difference in mortality was not apparent between monotherapy and combination therapy with other PAH drugs.

P-84

Clinical Effect of Ambrisentan in Pulmonary Hypertension

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Background: A development of the drugs used in pulmonary hypertension (PH) contributes a lot to its clinical improvement. An advent of new endothelin receptor blocker, ambrisentan, has further diversified selection of PH drugs. Purpose: The purpose of this study was to evaluate the efficacy and safety of ambrisentan on PH. Method: Ambrisentan was administered to 26 (50± 19 years old, 7 men, 19 women) patients with PH including 11 patients with chronic thromboembolic PH (CTEPH), 5 with Eisenmenger syndrome, 5 with connective tissue disease (CTD) PH and 3 with idiopathic pulmonary arterial hypertension. Ambrisentan was added to the other PH drugs in all the patients. The patients underwent right-side heart catheterization before and after the administration of ambrisentan with measurement of cardiac output (CO), mean right atrial pressure (mRA), mean pulmonary arterial pressure (mPA), pulmonary vascular resistance (PVR). Brain natriuretic peptide (BNP) was also determined. Results: After administration of ambrisentan (the average follow-up period was 168± 97 days), mPA (36 ± 9 vs 22 ± 6 : $p<0.01$) and PVR (11 ± 6 vs. 7 ± 4 : $p<0.01$) and CO (4.0 ± 1.5 vs. 4.8 ± 1.8: $p<0.05$) improved significantly, but BNP, mRA nor heart rate did not. The most frequent adverse reactions was edema with 6 patients, 3 of which abandoned ambrisentan. Conclusions: Ambrisentan is useful for pulmonary hypertension even if added to other PH drugs.

P-85

Long-Term Advanced Therapy with Bosentan Improves Symptoms and the Time to Clinical Worsening in the Japanese Patients with Inoperable Chronic Thromboembolic Pulmonary Hypertension

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Introduction: Chronic thromboembolic pulmonary hypertension (CTEPH) is a serious devastating disease. It is still challenge to treat some patients who are not eligible for pulmonary endarterectomy. Short-term bosentan or PDE5 inhibitor was significantly improved symptoms, hemodynamics and exercise capacity in such patients. However, the long-term beneficial effect of advanced pulmonary vasodilating drugs is little understood. Therefore, we investigated the long-term effect of advanced therapy in the patients with inoperable CTEPH retrospectively. Methods and Results: All consecutive 7 Japanese patients (5 female, mean age 62.6 ± 6.9 years) treated with bosentan (125-250 mg) for symptomatic inoperable CTEPH were included. The time to clinical worsening (TCW) was examined (mean follow-up period 896 ± 564 days). WHO-FC was significantly improved from 3.1 ± 0.4 to 2.1 ± 0.4 ($p < 0.01$). Pulmonary vascular resistance was significantly decreased from 786.9 ± 300.0 to 352.2 ± 210.7 dynes.sec.cm-5 ($p < 0.05$). Mean pulmonary artery pressure and cardiac index were improved from 47.0 ± 7.6 to 43.3 ± 5.0 mmHg and from 2.18 ± 0.39 to 3.02 ± 0.74 l/min/m2 ($n = 3$, follow-up 651-849 days). Six-minute walk distance was increased from 257.0 ± 151.0 to 369.8 ± 85.7 m ($p = 0.06$, $n = 4$, follow-up 651-931 days). Plasma BNP level was significantly decreased from 1160.0 ± 971.4 to 305.1 ± 285.9 pg/ml ($p < 0.05$). None of them were required hospitalization. Conclusions: Long-term advanced therapy with bosentan improves symptoms, hemodynamics and TCW in CTEPH patients. Advanced therapy is proposed as an essential treatment for the patients with inoperable CTEPH.

P-86

Analysis of ET-1 System in Mild and Severe Pulmonary Arterial Hypertension in Mice

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Background: A recently developed mouse model for PAH combines hypoxia with a VEGF receptor blocker. Herein we aim to describe in details this model. The TGFβ/Smad3 pathway is a pivotal factor regulating the transcription of the endothelin (ET-1) gene. Besides, interleukin-1β (IL-1β) increases expression of ET-1, and ETA receptor and reduces expression of ETB receptor. Methods and Results: We placed three-week old male SV129 mice under hypoxia (O2=10%) and treated them with a vascular endothelial growth factor receptor blocker (SU5416) (SU mice) (subcutaneous injection three times a week, 20mg/kg) for three weeks. Compared to mice under hypoxia alone (H) and control mice (CTRL), these mice developed severe PAH, characterized by increased right ventricular systolic pressure measured in anesthetized mice by subxiphoid approach (SU: 37± 1.7; H: 29± 1.4, CTRL: 22.5± 1 mmHg), right ventricularhypertrophy (Fulton index: SU: 0.55± 0.042; H: 0.4± 0.04, CTRL:0.31± 0.026) and muscularization of precapillary vessels together with proliferation of endothelial cells of small arterioles (PCNA positive on endothelial layer/arterial section= SU: 2.9± 0.25; H: 1± 0.13; CTRL: 0.7± 0.08), which lead to completely occluded arterioles by von Willebrand factor expressing cells.Increased ET-1 mRNA, ETA receptor mRNA, protein expression and immunostaining signals and reduced ETB receptor mRNA expression were observed in SU mice only. This was associated with an increased abundance of phosphorylated Smad3 and a 9-fold increase of IL-1β expression. TGF-β mRNA in H and SU mice was similar. Conclusion: In severe PAH, Smad3 by potentiating TGF-β and IL-1β might disturb the expression of the ET system and may represent therefore potential therapeutic targets.

PC-16

Early Detection of Pulmonary Arterial Hypertension by the Exercise Echocardiography in Patients with Connective Tissue Diseases

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[Objective]
To detect an early stage of pulmonary arterial hypertension (PAH) in patients with connective tissue diseases (CTD) who do not show the significant rise of tricuspid valve pressure gradient (TRPG) by echocardiography in rest, using the exercise echocardiography.
[Patients and Methods]
27 patients with systemic sclerosis (SSc), 13 patients with mixed connective tissue disease (MCTD), 8 patients with systemic lupus erythematosus (SLE), and 30 healthy controls (HC). To these patients, exercise echocardiography was performed. CTD patients whose TRPG in rest was 31mmHg or less were enrolled for this study from September 2010 to June 2012. The patients included were when TRPG on exercise went up by 35mmHg or more from that in rest, right heart catheterization (RHC) was conducted, if the patient's written informed consent was obtained.
[Result]
The average increase of TRPG caused by exercise (delta TRPG) in SSc patients was higher than that in SLE patients and HC (both $p<0.05$). Although, there was no SLE patients whose delta TRPG was 35mmHg or more, 5 SSc patients and 3 MCTD patients showed 35mmHg or more delta TRPG. Among these 8 patients, RHC was carried out for two SSc patients and two MCTD patients who have agreed with implementation of RHC. Three patients out of four were diagnosed as PAH by RHC.
[Conclusion]
In CTD patients, exercise echocardiography is a useful tool to detect early stage of PAH patients who do not show the significant rise of TRPG in rest.

P-87

Why Are Endothelin Antagonists Effective in Pulmonary Arterial Hypertension with Right Ventricular Dysfunction?

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In pulmonary arterial hypertension (PAH), increases in endothelin-1 (ET-1) contribute to elevated pulmonary vascular resistance which ultimately causes death by right ventricular heart failure. ET antagonists are effective in treating PAH but in marked contrast, lack efficacy in treating left ventricular heart failure. The aim of the study was to use radioligand binding assays to quantify the density of ETA and ETB in human heart from patients with PAH and in an established model of PAH, the monocrotaline (MCT) rat. This model recapitulates some of the pathophysiological features of the human condition, including increased in right ventricle systolic pressure and hypertophy. In the right ventricles of PAH hearts, there was a significant increase in the ratio of ETA receptors (n=12) but a decrease in ETB ratio compared with normal hearts. There was no change in ratio in left ventricle. In the MCT rat (n=8), receptor density was also significantly different in the right ventricle compared with vehicle control but with ETA downregulation and ETB upregulation. There was no change in left ventricle. In both human PAH and MCT model, ET receptor density changes in the right ventricle although the ratio was reversed in the rat. We have previously shown that ETA receptors in the failing left ventricle of patients with ischaemic heart disease are also significantly increased. Endothelin is a potent positive inotropic agent. In heat failure, increased receptor density may be an adaptive response to increase beneficial cardiac contractility. In PAH, the main benefit of ET antagonists may be in blocking deleterious vascular effects rather than improving cardiac function.

P-88

Endothelin-1 Induces Down-Regulation of IP Receptor in Pulmonary Artery Smooth Muscle Cells Obtained from Patients with Pulmonary Arterial Hypertension

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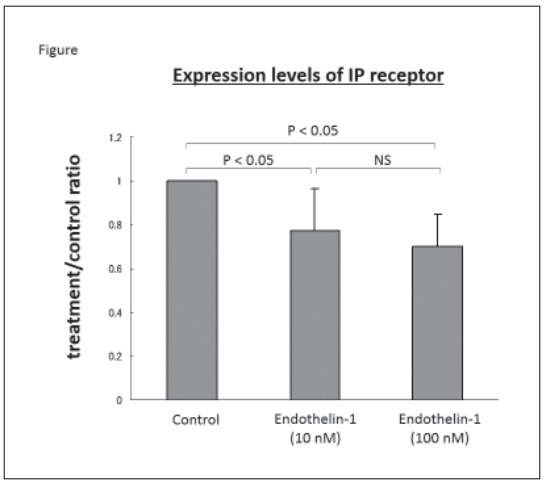
Background: We previously reported that addition of bosentan in pulmonary arterial hypertension (PAH) patients treated with high-dose intravenous prostaglandin I_2 (PGI_2) decreased not only pulmonary artery pressure but also reduced the dosage of PGI_2. IP receptor, a PGI_2 receptor, plays an important role in the treatment of PGI_2. However the relation of IP receptor and endotholin-1 is unknown.Methods: Effects of PGI_2 and endothelin-1 on IP receptor expression was examined by qRT-PCR in pulmonary artery smooth muscle cells (PASMCs) obtained from six patients with PAH.Results: PGI_2 induced time and dose-related down-regulation of IP receptor expression in PAH-PASMCs. Endothelin-1 induced dose-related down-regulation of IP receptor expression in PAH-PASMCs (Figure). Conclusion: Endothelin-1 down-regulated the IP receptor in PAH-PASMCs.



P-89

Efficacy of Oral Triple Upfront Combination Therapy (Long-Acting Prostacyclin Analogue, Endothelin Receptor Antagonist, Phosphodiesterase 5 Inhibitor) in the Patients with Idiopathic /Heritable Pulmonary Arterial Hypertension

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BACKGROUND: The efficacy of oral triple upfront combination therapy for severe idiopathic /heritable pulmonary arterial hypertension (I/HPAH) in long-term has not been established. PATIENTS & METHODS: We retrospectively reviewed three patients in WHO-FC III who received oral triple upfront combination therapy (oral long-acting prostacyclin analogue, endothelin receptor antagonist, phosphodiesterase 5 inhibitor) from 2012 to 2013. RESULTS: After one year of oral triple combination therapy WHO-FC improved from III to II in all three patients and 6 minutes walking test improved from 273±98 to 553±31 ($P<0.05$) and mean pulmonary arterial pressure decreased from 52.7±9.5 to 31±6.2 mmHg ($P=0.05$) and cardiac index increased from 1.5±0.2 to 2.82±0.17 mmHg ($p<0.001$). All three patinets were tolerable with triple upfront combination therapy and had no severe side effect. CONCLUSIONS: Oral triple upfront combination therapy improved symptoms, exercise capacity and hemodynamics for the patients with severe I/HPAH in long-term without severe adverse effect.

P-90

Combination Therapy of Bosentan and Ambrisentan for Portopulmonary Hypertension

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Endothelin receptor antagonists (ERAs) such as bosentan and ambrisentan are principal medicine in the treatment of pulmonary arterial hypertension (PAH). On the other hand, the adverse effects are not uncommon such as liver dysfunction and peripheral edema. These side effects are often intolerable for patients and hinder administration of sufficient amount of ERA. In this report, we present a case of 56-year-old man with liver chirrosis due to non-alcoholic steatohepatitis. He was referred to our hospital complaining progressive dyspnea on effort equivalent to WHO FC III. His mean pulmonary artery pressure (mPAP) was 62 mmHg and peak VO_2 was 10.9 ml/kg/min. He was diagnosed as portopulmonary hypertension (PoPH). Tadalafil, bosentan and beraprost were introduced respectively, and his mPAP ameliorated to 54 mmHg. However, he was intolerant of increasing bosentan more than 125 mg, because of worsening liver dysfunction, while a full dose of ambrisentan was hard to use, as he easily got edematous with various drugs. Finally, we administrated combination of moderate dose of bosentan (125 mg) and ambrisentan (2.5 mg). Three months after administration of both drugs, his mPAP was reduced to 42 mmHg, and peak VO_2 was improved from 14.7 to 17.8 ml/kg/min, with no significant adverse effect of each drugs.To our knowledge, this is the first case report in which combination therapy of bosentan and ambrisentan was practically tried to the patient of PAH, and satisfactory result was obtained. In this report, we will try to discuss the efficacy of combination therapy of bosentan and ambrisentan, in terms of cross-talk of endothelin receptors, based on relevant literatures.

P-91

Experience in Combination Therapy for Portopulmonary Hypertension in the Young with Intravenous Epoprostenol and Endothelin Receptor Antagonists

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Background: Portopulmonary hypertension (PoPH) is an uncommon but devastating complication of liver disease. There is no established medical therapy for PoPH and the condition adversely affects the outcome of liver transplantation. The prognosis of PoPH in the young is still very poor even in the recent era. **Methods:** We retrospectively reviewed seven pediatric patients with PoPH who were treated with different combinations of vasodilators. Expression of endothelin-1 and its receptors in the postmortem lung specimens were analyzed. **Results:** Primary diagnoses for liver disease were congenital biliary atresia in 5, extrahepatic portal vein atresia in one and patent ductus venosus in one. The median age at diagnosis was 14 years old. The onset of PoPH was syncope, dyspnea on exercise and abnormal electrocardiogram. The mean pulmonary arterial pressure was 49 mmHg and pulmonary vascular resistance index was 11.2 Wood units-m2 at the time of diagnosis. Cardiac catheterization revealed no acute response to oxygen, nitric oxide, sildenafil whereas a little response to intravenous epoprostenol (IV-PGI2). IV-PGI2 was administrated in four patients and had chronic effect on hemodynamics. Oral endothelin receptor antagonists (ERA: bosentan or ambrisentan) were administrated in five and there was no evidence of drug-related liver injury. Three patients died and liver transplantation was performed in three. Immunohistochemical staining for the endothelin system revealed increased expression of ET-B receptor in the pulmonary vascular endothelial cells. **Conclusion:** PoPH in the young was diagnosed at moderately severe stage of PH and carried poor prognosis. Combination therapy with IV-PGI2, ERA and PDE5-I may provide a promising therapeutic option for selected patients with PoPH.

PC-17

Peak Systolic Strain at Right Ventricular Free Wall Determined by Two-Dimensional Speckle-Tracking Echocardiography is an Independent Predictor for Pulmonary Hypertension

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Background: Right heart catheterization (RHC) is the invasive but the gold standard examination for assessing pulmonary arterial pressure (PAP). Thus, pulmonary hypertension (PH) is usually predicted using echocardiographic parameters. Regional deformation of the left ventricular (LV) wall detected by two-dimensional speckle-tracking echocardiography is evidently useful for detecting myocardial ischemia, viability and LV function, but its significance of right ventricle (RV) has not been fully elucidated. We investigated the ability of peak systolic strain (PSS) and the post systolic strain index (PSI) of the RV free wall determined by speckle-tracking echocardiography to predict PH.

Methods: Thirty-six images (27 images from patients with PH; nine from patients with connective tissue diseases without PH) obtained by speckle-tracking echocardiography were analysed. PSS and PSI at the basal and mid-RV free wall were calculated and averaged. We investigated the relationship of echocardiographic parameters of pressure/volume overload in RV including RV end-diastolic diameter (RVDd) and the pressure gradient calculated from the velocity of tricuspid valve regurgitation (TRPG) with mean PAP (MPAP) measured by RHC.

Results: PSS, PSI, RVDd and TRPG were significantly correlated with MPAP. Multivariate logistic analysis identified PSS as an independent predictor of MPAP ≥35 mmHg (odds ratio, 1.62; 95% confidence interval 1.02-2.57; p=0.042) and the cut-off value determined from the receiver operating characteristic curve was -20.75% (area under curve, 0.928; 87.5% sensitivity, 87.5% specificity). Furthermore, changes in PSS among patients in whom these parameters were measured at least twice correlated significantly with changes in MPAP (r=0.633, p=0.037).

Conclusions: PSS of the RV free wall might serve as a useful non-invasive indicator of PH.

PC-18

Prognosis of Sleep-Disorder Breathing for Chronic Heart Failure and the Effectiveness of Nocturnal Home Oxygen Therapy and Continuous Positive Airway Pressure

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Background: Sleep-disordered breathing (SDB) has been reported to influence the mortality of patients with chronic heart failure (CHF). However the predictors of lethal cardiac events in CHF patients with SDB remain to be elucidated. Methods: We examined whether the severity of SDB was associated with lethal events in CHF patients, and whether the respiratory therapy for SDB improved the prognosis. Ninety-five patients with stable CHF who had the examination of SDB by overnight polysomonography between August 2000 and November 2008 were enrolled in the present study (62.3±14.5 [SD] years-old, M/F 72/23). SDB events were quantified by the apnea hypopnea index (AHI). All patients with more than 10/h for AHI (n=42) at the initial evaluation, were recommended the respiratory therapy (RT); oxygen therapy (HOT) or continuous positive airway pressure (CPAP). Endpoints were defined as lethal arrhythmic events (sudden death or ventricular tachyarrhythmia) or lethal events (overall death or ventricular tachyarrhythmia). Results: During 29±17 months of follow-up, 18 patients died and 10 ventricular tachyarrhythmias occurred. The multivariate proportional hazard analysis showed that more than or equal to 5AHI was an effective risk factor for both lethal arrhythmic events (P=0.026) and lethal events (P=0.043). Second, RT improved significantly the number of AHI, but did not reduce both lethal arrhythmic and lethal event rates. However, 4 patients who reached less than 5 AHI by RT had neither lethal arrhythmic nor lethal events during follow-up period. Conclusion: SDB could be one of independent predictors of lethal arrhythmic events and lethal events in patients with CHF. Conventional RT might improve the prognosis of these events.

PC-19

Detection of Hydroxyl Radical in Isolated Goto-Kakizaki (GK) Rat Arteries by Trapping with 4-Hydroxybenzoic Acid

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Goto-Kakizaki (GK) rats were established as a model of inbred type 2 diabetes mellitus. We have shown that acetylcholine (ACh)-induced relaxation on aortic ring preparation and ACh-induced NO release from cultured endothelial cells in GK rats were reduced compared to Wistar rats. Furthermore, we have shown that norepinephrine (NE)-induced ATP release from cultured endothelial cells in GK rats were also reduced in GK rats. These differences may be related to vascular endothelial dysfunction in GK rats. Endothelial dysfunction and oxidative stress are the main pathophysiological mechanisms of diabetes mellitus complications, such as cardiovascular disease, renal failure and ischemia-reperfusion injury. In the present study, we investigated the generating of hydroxyl radical from Wistar and GK rat isolated aorta. 4-Hydroxybenzoic acid (4-HBA), a compound structurally very similar to salicylate, was introduced recently as a hydroxyl radical trapping agent. Hydroxylation of 4-HBA by hydroxyl radical yields a single product, 3,4-hydroxybenzoic acid (3,4-DHBA). 3,4-DHBA was quantified by HPLC-electrochemical detection technique. Spontaneous hydroxyl radical generation did not differ from Wistar and GK. NE and ATP significantly increased the hydroxyl radical generation from both arteries. There was no significant difference among the amounts of the hydroxyl radical generation, while NE and ATP did not alter the NO release. Hydroxyl radical generation did not correlate NO release. Moreover, the quantity of mRNA expression of NOS2 and SOD1 was almost the same. These results suggested that hydroxyl radical may be generated from vasculature (arterial smooth muscle and endothelium) and related to vascular dysfunction.

Session 9: Blocking the ETA Receptors: What's New?

Invited Lecture 11

Endothelin Receptor Selectivity in patients with Pulmonary Arterial Hypertension (PAH)

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Considerable controversy that exists among experts in pulmonary vascular biology regarding endothelin A (ETA) and endothelin B (ETB) selectivity of endothelin receptor antagonist (ERA) drugs used to treat pulmonary arterial hypertension (PAH). Some experts believe that dual ETA/ETB blockade is superior due to the effects of ETB on vascular fibrosis, while others have shown evidence for superiority in endothelin-1 clearance with ETA-selective drugs. There are also mixed opinions as to the degree of relative ETA/ETB selectivity conferred by existing ERAs currently used in clinical practice. This lecture will present some of the existing basic science and clinical data that address ETA and/or ETB selectivity, and discuss its relevance to clinical practice.

Invited Lecture 12

Age-Dependent Antihypertensive and Antiproteinuric Effects of ET_A Receptor Blockade in Ren-2 Transgenic Rats

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Ren2-transgenic rats (TGR) with inserted Ren-2 murine gene are a model of angiotensin II-dependent hypertension (with endogenous activation of the renin-angiotensin system) which bear a salt-sensitive component. Their hypertension develops early in life (at 4-5 weeks of age). While heterozygous animals survive hypertension development, hypertension in the homozygous strain is very severe and leads to high mortality. Thus, heterozygous TGR provide a more suitable model of hypertension regarding to clinical studies. Our studies with endothelin (ET) receptor blockade were performed in young (4-weeks-old) rats (prevention study) and adult (50-days-old) rats (regression study) fed either normal (0.45 % NaCl) or high-salt diet (2 % NaCl). Bosentan or atrasentan were given until day 90 of age. High-salt diet was applied not only to induce the transition from benign to malignant phase of hypertension but also to activate ET system. Prevention study in homozygous TGR has demonstrated that both nonselective blockade with bosentan and selective blockade with atrasentan decreased mortality. Proteinuria, glomerulosclerosis, cardiac hypertrophy, as well as left ventricular ET-1 content were partly reduced by bosentan and to a greater extent by atrasentan. However, only atrasentan exerted antihypertensive effects (30 mm Hg). In the regression study (started at time when hypertension has already been developed) atrasentan transiently decreased blood pressure (BP) in homozygous TGR. However, there was no difference in BP between different groups at the end of the study. Interestingly, atrasentan had profound effects on survival. This was accompanied by substantially reduced glomerular podocyte injury, which strongly correlated with the survival. This effect was thus independent of BP. Prevention study in heterozygous TGR confirmed previous results obtained in homozygous animals, i.e. similar effects of nonselective and selective ET blockade on the improvement of survival, cardiac hypertrophy, glomerulosclerosis and ET-1 levels with superior effects of selective ET_A blockade on proteinuria and hypertension. In the regression study in heterozygous TGR, beneficial effects of ET_A blockade were substantiated by the findings of restoration of podocyte structure and reversal of podocyte phenotype changes represented by the expression of CD 10, desmin and vimentin. In the recent study we have analyzed the effects of ET_A blockade on the distinct vasoactive systems contributing to BP maintenance. We have found that BP component dependent on calcium entry through L-type voltage-dependent calcium (L-VDCC) channels was markedly decreased by atrasentan treatment. In addition, vasodilatation through NO, endogenous prostanoids and BK_{Ca} channels (mediated by ET_B receptors) was strongly reduced in atrasentan-treated TGR. Our experiments have demonstrated that BP lowering effect of atrasentan is mainly due to the reduced Ca^{2+} influx via L-VDCC channels. In conclusion, our results have shown that selective ETA blockade is superior in many aspects to the nonselective ET_A/ET_B blockade, this effect being dependent on the preservation of the filtration barrier of the kidney.

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Invited Lecture 13

Dual Endothelin A and Angiotensin Receptor Blockers in the Treatment of Hypertension and Renal Disease

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Pre-clinical studies suggest that combined blockade of ETA and AT1 receptors reduces end-organ injury to a greater extent than either receptor antagonist alone. Towards this end, dual ET/AngII receptor antagonists (DARA) have been developed. The first DARA was reported by Bristol Myers Squibb in 2002 and was based on the observation that ETA antagonists shared the same biphenyl core as several AT1 receptor blockers. A series of these biphenylsulfonamides were reported in 2003 and 2004 and found to be orally active in several rat models of hypertension (AngII, DOCA and SHR). Subsequent synthesis of a 2'-substituted N-3-isoxazolyl biphenylsulfonamide led to a second generation DARA compound with improved pharmacokinetics and enhanced ETA and AT1 receptor potency (Ki of 0.8 nM and 9.3 nM for human AT1 and ETA receptors, respectively). Pharmacopeia acquired the license for the DARA, renaming it PS433540. Based on several Phase I studies, they conducted a single blind Phase IIa study in patients with mild to moderate hypertension (NCT00522925) with a primary endpoint of change in 24-hr systolic BP after 4 weeks of placebo (N=28), 200 mg (N=38) or 500 mg (N=36) daily of drug. The results, reported in 2008, demonstrated no change with placebo, a 12/9 mmHg or 15/10 mmHg drop in SBP/DBP with 200 mg or 500 mg of drug, respectively. There were no significant adverse events. Ligand Pharmaceuticals acquire Pharmacopeia and conducted a Phase IIb trial in a similar group of hypertensive patients (NCT00635232). Patients were treated with daily 200 mg (N=55), 400 mg (N=48) or 800 mg (N=20) PS433540, placebo (N=39), or 300 mg irbesartan (N=44). The primary endpoint was change in office SBP after 12 weeks. The SBP/DBP decreased by 1.8/0.2, 10.7/7.1, 13.2/7.2, 14.2/9.2 and 23.4/14.3 in the placebo, irbesartan, 200, 400 and 800 mg PS433540 groups, respectively. Peripheral edema (all mild to moderate) was noted in 2-3% of patients treated with irbesartan, placebo or 200 mg PS433540, while it occurred in 6.9 and 10.7% of patients receiving 400 and 800 mg PS433540, respectively. More patients on the highest DARA dose had dizziness, flushing and GI symptoms. Ligand licensed the DARA to Retrophin in 2012 who are currently planning a Phase II trial (NCT01613118) with 4 different doses of the now renamed RE-021, as well as the active comparator irbesartan, in 72 patients, aged 8-50 years, with focal segmental glomerulosclerosis. The primary outcome will be a reduction in albumin excretion rate after 6 weeks of treatment. Finally, another DARA has been developed by Torrent Pharmaceuticals, termed TRC120038, a modified biphenylsulfonamide, with an in vitro EC50 of 3 nM and 158 nM for human AT1 and ETA receptors, respectively. Studies in ob-ZSF1 rats showed a greater reduction in mean BP with TRC120038 than with candesartan, as well as reduced renal dysfunction and improved cardiac function. In summary, DARAs are in the early phases of study. Preclinical trials and early clinical trials suggest that this new class of drugs may be well tolerated and could be efficacious in treating cardiorenal diseases where targeting both ETA and AT1 would likely be beneficial.

Invited Lecture 14

Good and Bad News about Single and Dual Biphenyl Endothelin ET_A and Angiotensin AT_1 Receptor Antagonists

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Background: AT_1 antagonists like irbesartan and valsartan (IRB, VAL) and an ET_A antagonist like BMS-193884 (BMS) share a core biphenyl structure. We tested the hypothesis that hybrids of these compounds act as dual ET_A/AT_1 receptor antagonists (DARA). **Methods:** We compared effects of IRB, VAL and BMS with those of structural chimeras of IRB and BMS (PS-433540, DARA1) and of VAL and BMS (ACT-214145, DARA2), all synthesized at Actelion (CH). Contractile responses to angiotensin II (AngII) and endothelins (ET-1 and ET-2) were investigated in isolated rat mesenteric resistance arteries where ET_B-agonists and -antagonists have no effects. **Results:** Presence of IRB, VAL, DARA1 and DARA2 reduced the sensitivity and maximal responses to AngII-induced contraction with comparable apparent affinity (pA_2: 9.5, 9.3, 9.7 and 9.2) while 10 nM BMS was not effective. Presence of IRB (≤3 nM) or Val (≤100 nM) did not modify responses to ET-1(1 - 16 nM). BMS (1 - 30 nM), on the other hand, potently reduced the sensitivity to ET-1 and ET-2 to the same extent (pA_2: 9.2 and 9.2). Notably, this effect of BMS displayed saturability. It did not increase with increasing concentration in the supra-nanomolar range and this was more marked against ET-1 than against ET-2. The antagonistic effect of 10 nM BMS was significantly additive with that of the butenolide ET_A-antagonist PD156707 (100 nM) but not the cyclic pentapeptide ET_A-antagonist BQ123 (1μM). Furthermore, relaxing effects of 10 nM BMS on contractions induced by 16 nM ET-1 or ET-2 (-57±11 and -96±3%) were significantly larger than those predicted by the effect of BMS on the sensitivity to the peptides (-20±10 and -19±7%). These relaxing effects faded rapidly upon washout of both the agonist and antagonist. Finally and in sharp contrast to reported findings on radioligand-receptor binding and on pressor responses induced by big ET-1 in vivo, both DARA1 and DARA2 were≥2.5^{10}Log units less effective in reducing rat mesenteric artery sensitivity to ET-1 and ET-2 than their common BMS core structure (pA_2: 6.4, 6.2 and 9.2 against ET-1 and 6.8, 6.2 and 9.2 against ET-2). **Conclusion:** The good news is that the biphenyl sulfonamide BMS is a negative allosteric modulator of arterial smooth muscle ET_A receptors which unlike BQ123 and PD156707 acts most effectively on agonist-occupied activated receptors. The bad news is that the structure cannot be expended with an AT_1-receptor antagonistic part without profound loss of affinity for ET_A at the tissue level. Also, the results suggest that several allosteric modulatory sites may be present on ET_A receptors.

This research was performed within the frame of TIPharma project T2-301.

Honorary Chair Session

Invited Lecture 15

End o' the Line Revisited

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When endothelin-1was discovered it was hailed as the prototypical endothelium-derived contracting factor (EDRF). However, over the years little evidence emerged convincing this author that the peptide actually contributes to moment-to-moment changes in vascular tone elicited by endothelial cells. This was attributed to the profound inhibitory effect of nitric oxide (NO) on both the production (by the endothelium) and the action (on vascular smooth muscle) of endothelin-1. Hence, at least in the author's mind, endothelin-1 was likely to initiate acute changes in vascular diameter only under extreme conditions of endothelial dysfunction when the NO bioavailability is considerably reduced if not absent. This lecture will survey more recent findings and decide whether this concept should be revised, or maybe not…

Special Guest Session

Invited Lecture 16

Multiple and Integrative Approaches to Cardiovascular Diseases with Stem Cell Technology

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We have been investigating cardiovascular cell differentiation and regeneration using pluripotent stem (ES/iPS) cells. We established a mouse ES/iPS cell differentiation system for cardiovascular cells using Flk1+ cells as common progenitors (**Nature**, 2000; **Circulation**, 2008). Based on this system, we are performing broad researches from basic studies for cell differentiation to applied studies for cardiac regeneration.

I. Basic studies for cell differentiation: we recently succeeded in demonstrating differentiation stage-specific diverse roles of cyclic AMP (cAMP)/Protein kinase A (PKA) signaling during vascular cell differentiation. 1) From pluripotent stage to mesoderm, PKA accelerated differentiation timing with epigenetic silencing of pluripotent genes through methyltransferase G9a (**Cell Stem Cell**, 2012). 2) During endothelial cell (EC) differentiation from mesoderm, PKA increased vascular endothelial growth factor (VEGF) receptor-2 and neuropilin1 expression in vascular progenitors, which enhanced the progenitor sensitivity to VEGF and EC differentiation (**Blood**, 2009). 3) That was mediated by direct transcriptional cascade from cAMP-responsive element binding protein, Etv2 transcription factor, to VEGF receptors (**Stem Cells**, 2012a). 4) Kappa opioid receptor signaling was a novel endogenous inhibitor for PKA and EC differentiation (**Blood**, 2011). 5) cAMP signal induced dual activation of Notch and beta-catenin and induced arterial specification in ECs (**J Cell Biol**, 2010).

II. Chemical biological approach: We found that an immunosuppressant, cyclosporin-A (CSA), showed a novel effect specifically acting on mesoderm cells to drastically increase cardiac progenitors as well as cardiomyocytes (**Biochem Biophys Res Commun**, 2009). Applying this system, we screened small molecules and found several novel compounds enhancing cardiomyocyte differentiation and proliferation. These findings would contribute to discovery for cardiac regenerative drugs and efficient induction of cardiac cells from ES/iPS cells.

III. Cell therapy: We are also examining cell transplantation methods using the cell sheets technology (Shimizu, Curr Pharm Des, 2009) for cardiac regeneration. Combining our cardiovascular differentiation system and cell sheet technology, we set out to reconstitute cardiac tissue re-assembled with defined cardiovascular populations, and examined the effect of the cardiac tissue sheet following transplantation. We observed that cardiac tissue sheet transplantation to rat myocardial infarction model significantly improved systolic function accompanied by neovascularization (**Stem Cells**, 2012b). We extended these strategies to human iPS cells. We recently succeeded in developing efficient cardiomyocyte differentiation and purification methods in human iPS cells (**PLoS One**, 2011a, 2011b). Now we are examining effects of human iPS cell-derived cardiac sheet transplantation in rat and porcine myocardial infarction models.

I would like to show and discuss our studies with stem cell, chemical, and vascular biology.

DAY 3

Session 10: Cardiology, Hypertension, Vascular Disease

Keynote Lecture 4

Endothelin in Myocardial Infarction

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University of Ioannina, Greece

The effects of ET-1 on myocardial necrosis: Myocardial infarction increases plasma ET-1 levels shortly after acute coronary occlusion, causing release from intracellular-storages and *de novo* production. The effects of ET-1 on the ischaemic and necrotic areas in the absence of reperfusion are debated, but ET-1 may play a role in reperfusion injury. In *in vivo* rat-models of ischaemia-reperfusion, ET_A- or dual-(ET_A and ET_B) receptor blockade have been shown to decrease myocardial necrosis and improve left ventricular haemodynamics. Importantly, favourable results were demonstrated in a recent small-scale clinical study, in which short-term, selective ET_A-receptor blockade prior to percutaneous coronary intervention improved myocardial perfusion, decreased infarct size and improved left ventricular function. **ET-1 and sympathetic stimulation**: During acute myocardial infarction, ET-1 increases catecholamine-release from the adrenal glands and modulates norepinephrine-release in sympathetic nerve-endings in the ventricular myocardium. These effects result in local norepinephrine-release via ET_A-receptor activation, whereas the ET_B-receptor appears to exert a protective role by decreasing early sympathetic drive. **Arrhythmogenesis**: ET-1 exerts significant electrophysiologic effects and contributes to ventricular arrhythmogenesis. ET-1 induces spontaneous calcium-transients, leading into early afterdepolarizations, and inhibits the delayed-rectifier potassium-current. In the *in vivo* rat-model of myocardial infarction without reperfusion, lower incidence of ventricular tachyarrhythmias and shorter episode duration were reported after selective ET_A-receptor blockade, resulting in lower arrhythmic-mortality. In the same animal model, dual (ET_A and ET_B) receptor blockade decreased arrhythmogenesis, but this effect was evident only during the later-phase of acute infarction. These results point towards an arrhythmogenic effect of ET-1 during acute infarction, mediated mainly by the ET_A receptor, consisting of increased sympathetic activation and enhanced repolarization inhomogeneity. The restoration of blood-flow after a prolonged ischaemic period causes marked electrophysiological changes and causes a second wave of myocardial necrosis, associated with arrhythmogenesis. Given the possible role of ET-1 in reperfusion injury, the antiarrhythmic potential of ET-receptor blockade during ischaemia-reperfusion has been subject of research for over a decade. However, hitherto studies have produced conflicting results, possibly due to differences in experimental-protocols, including *ex vivo* and *in vivo* models, and the time-window for arrhythmia-recording. Moreover, exogenously administered ET-1 during ischaemia-reperfusion in some studies may even exert antiarrhythmic effects, possibly via a preconditioning effect. **Conclusions**: The effects of ET-1 during myocardial ischaemia and infarction constitute an intriguing topic with potential clinical ramifications. Further research is required on the arrhythmogenic effects of ET-1 during acute myocardial infarction, placing emphasis on those observed during the pre-hospital phase. Since ventricular tachycardia and ventricular fibrillation shortly after acute coronary occlusion account for the vast majority of sudden cardiac death cases, prompt understanding of the underlying pathophysiology may lead to effective preventive therapeutic strategies.

O-20

Endothelin-1 is a Key Candidate to Exert Pathophysiological Effects on Cardiomyocytes Derived from Hypertrophic Cardiomyopathy-Induced Pluripotent Stem Cell

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Background. Despite the accumulating genetic and molecular understandings into hypertrophic cardiomyopathy (HCM), it remains unclear how this condition develops and worsens pathologically and clinically in terms of the genetic-environmental interactions. Thus, establishing human disease models for HCM would help us to evaluate the disease mechanisms and novel therapeutic strategies. Emerging patient-specific induced pluripotent stem cell (iPSC) techniques hold much promise for these tasks. **Hypothesis.** Interactions between genetic backgrounds and environmental factors are involved in the progression of HCM. **Methods.** To clarify candidate disease-promoting environmental factors, iPSCs from unrelated three patients with HCM and three healthy-control subjects were generated. The cardiomyocytes differentiated from the each iPSC line were stimulated by several cardiomyocyte hypertrophy-promoting factors. The HCM pathological phenotypes were examined based on the morphological properties, such as cell size and intracellular myofilament structures, in randomly chosen cardiac troponin-T-positive singled cardiomyocytes. Next, a high-speed video imaging with motion vector prediction algorithm revealed physiological contractile dynamics in the iPSC-derived singled cardiomyocytes. **Results.** Control- and HCM-iPSC-derived cardiomyocytes were similar under the basal condition in pathological features and contractile dynamics. However, only the HCM-iPSC-derived cardiomyocytes showed pathological phenotypes, such as cardiomyocyte hypertrophy and facilitated intracellular myofilament disorganization in the presence of endothelin-1 (ET-1) administration with a dose-dependent manner. Moreover, physiological analyses revealed ET-1-induced contractile dispersion in the self-beating HCM-iPSC-derived cardiomyocytes. Finally, these deleterious effects were rescued by blocking the endothelin receptor type-A. **Conclusions.** Interactions between genetic backgrounds and the environmental factor, ET-1, promoted the pathophysiological phenotypes of HCM in the iPSC-derived cardiomyocytes.

O-21

Endothelin Receptor Antagonists Exacerbate Autoimmune Myocarditis in Mice

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Background: Experimental autoimmune myocarditis (EAM) is a mouse model of inflammatory cardiomyopathy. The amount of endothelin (ET) increases according to the disease progression; however, the pathological role of ET in myocarditis has not been elucidated. **Methods and Results**: EAM was induced by immunization of cardiac myosin peptide with complete Freund's adjuvant on days 0 and 7 in BALB/c mice. ET-A/-B dual receptor antagonist SB209670 was administered by continuous infusion from a subcutaneous pump for 3 weeks. An increase in heart-to-body weight ratio was observed in SB209670-treated mice compared with vehicle-treated mice. The heart pathology in SB209670-treated mice was remarkable for gross inflammatory infiltration, in contrast to the smaller inflammation in the hearts of vehicle-treated mice. We found that ET blockade decreased the number of $Foxp3^+$ regulatory T cells and inhibited the production of immunoregulatory cytokine IL-10 in the heart. ET blockade also inhibited the expression of suppressor of cytokine signaling 3 (SOCS3) that plays a key role in the negative regulation of both Toll-like receptor (TLR)- and cytokine receptor-mediated signaling. EAM is a $CD4^+$ T cell-mediated disease. $CD4^+$ T cells isolated from SB209670-treated EAM mice produced less IL-10 and more inflammatory cytokines IFN-γ and IL-17 than those isolated from vehicle-treated mice. **Conclusions**: ET receptor antagonist exacerbated autoimmune myocarditis in mice. ET may play an important role in the regulation of inflammation in myocarditis.

O-22

Imaging of the Binding of ET-1 and of Linear ET-1 in Rat Mesenteric Resistance Arteries

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In engineered cells, endothelin ET_A- and ET_B- receptors can heterodimerize. We tested whether this is possible in native tissue. Therefore, rat mesenteric resistance arteries were maintained in organ culture for 24 hours to upregulate ETB-mediated contractions in addition to their normal ET_A- mediated constrictions. Thereafter the vessels were cannulated and maintained at constant distending pressure and 37°C under a two photon laser scanning microscope. They were then subsequently exposed to first 100 nM linear ET-1 (ET_B-agonist) tagged with Oregon Green 488 (OG488) and then to 16 nM intact ET-1 (ET_A/ET_B-agonist) tagged with the rhodamine dye TAMRA. After incubation with the labeled ligands, the arterial smooth muscle cells in the tunica media, were efficiently stained and became visible under the two photon microscope. Wrinkling of the autofluorescent internal and external elastic laminae accompanied agonist-induced constriction. TAMRA-ET-1 bound to all smooth muscle cells with a homogeneous cytoplasmic distribution whereas similar staining was observed for labeled linear ET-1 but only on some group of cells. Fluorescence lifetime measurements were employed to probe the interaction of the two receptor subtypes. Fluorescence lifetime of OG488, which acted as a donor, was reduced in the presence of TAMRA, from 2.8 ps to 2.3 ps, which indicates a fluorescence resonant energy transfer (FRET), a phenomenon which can take place only if the receptors are in close proximity (<10 nm). The methodology that is introduced by these preliminary observations may be useful to asses ET-receptor heterodimerization in biopsies from relevant experimental animal models and human patients.

O-23

Sympathetic Endothelin A Receptors Contribute to the Development of Heart Failure

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In preclinical heart failure (HF) models, endothelin receptor A (ETA) antagonists (ETAi) attenuated the disease progression. However, clinical HF trials failed to demonstrate beneficial effects on cardiac function and prognosis. We hypothesized that established HF drugs such as adrenergic receptor blockers interfere with the mechanism of action of ETAi. Here we report, that mice lacking ETA selectively in sympathetic neurons (SN-KO) showed less adverse structural remodeling and cardiac dysfunction in response to pathological pressure overload induced by transverse aortic constriction (TAC). In contrast, mice lacking ETA selectively in cardiomyocytes (CM-KO) were not protected against HF. TAC led to a disturbed sympathetic nerve function as measured by cardiac norepinephrine (NE) tissue levels and [124I]-MIBG PET, which was prevented in SN-KO. In co-cultures of cardiomyocytes (CMs) and sympathetic neurons (SNs), endothelin-1 (ET1) led to a massive NE release and exaggerated CM hypertrophy as compared to CM mono-cultures. ETA-deficient CMs gained a hypertrophic response through wild type SNs but ETA-deficient SNs failed to mediate exaggerated CM hypertrophy. Furthermore, ET1 mediated its effects indirectly via NE in CM-SN co-cultures through adrenergic receptors and histone deacetylases, resulting in activation of the pro-hypertrophic transcription factor MEF2. In conclusion, sympathetic ETA amplifies ET1 effects on CMs through adrenergic neurotransmission. In accordance to our initial hypothesis, anti-adrenergic therapies may blunt potential beneficial effects of ETAi. These findings call for a personalized strategy to identify patients that could benefit from ETAi.

O-24

Mouse Mast Cell Protease-4-Dependent Production of ET-1 (1-31) and of Plaque Progression in a Apolipoprotein E Knock-Out Mouse Model of Spontaneous Atherosclerosis

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The murine homologue to human chymase, the mouse Mast Cell Protease-4 (mMCP-4), activates the pro-forms of angiotensin-II and endothelin-1 (ET-1) in vivo; both peptides being well-established in cardiovascular diseases. In the present study we postulated that mMCP-4-dependent synthesis of ET-1 plays an important role in the development of atherosclerosis in the Apolipoprotein E knock out mouse model (ApoE KO). Peritoneal mast cells were collected from C56BL/6J wild-type (WT), mMCP-4 KO and ApoE KO mice and their granular contents were assayed for the enzymatic processing of the fluorogenic substrate Suc-Val-Val-Pro-Phe-amidomethylcoumarin. WT and ApoE KO derived samples produced a TY-51469 (specific chymase inhibitor) sensitive fluorescence, but not in mMCP-4 KO samples. Via HPLC detection, a TY-51469-sensitive conversion of big ET-1 to ET-1 (1-31) was monitored in mast cells homogenates. Homogenate samples from ApoE KO mice showed significant increases in ET-1 (1-31) production when compared to extracts from WT mice (milliAU × time (s), WT: 763 ± 124; ApoE KO: 1756 ± 339, n = 7; $P = 0.018$). Finally, Sudan IV staining of aortas from 27-30 week old male mice on chow diet showed that the repression of mMCP-4 in APOE KO mice (APOE/mMCP-4 dKO) reduced by 65 % the atherosclerotic lesion area found in ApoE KO mice (ApoE KO: 7.45 ± 1.31 %; ApoE/mMCP-4 dKO: 2.67 ± 0.64 %, n = 9-10, $P = 0.004$). These results suggest an increased contribution role of mMCP-4 in the synthesis of ET-1 in mast cells derived from ApoE KO mice and the pivotal role of that chymase isoform in the development of atherosclerosis. (Funded by CIHR).

Session 11: Clinical Studies Update

Keynote Lecture 5

Endothelin Receptor Antagonists in Clinical Research - Lessions Learned

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Endothelin receptor antagonists are approved for the treatment of pulmonary hypertension. The efforts to approve this class of drugs for renal indications, however, failed so far. Preclinical studies were promising. Transgenic overexpression of ET-1 or ET-2 in rodents causes chronic renal failure in a blood pressure independent manner. Blocking in particular the ETA receptor was effective in the treatment of rodent models of renal failure such as rats with 5/6 nephrectomy, L-NAME induced renal failure and diabetic nephropathy. On the other hand, various animal studies indicate that blocking the renal tubular ETA and ETB receptor may cause water and salt retention partially mediated via the epithelial sodium transporter (ENaC) in tubular cells. Endothelin receptor antagonists were successfully tested clinically in renal indications in phase 2 trials for the treatment of diabetic nephropathy. They showed efficacy in terms of reducing albumin excretion in patients with diabetic nephropathy on top of guideline based background therapy (angiotensin II receptor blockade). However, these promising results could not be translated to successful phase III trials so far. The spectrum of serious adverse events was similar to other phase III trials using endothelin receptor antagonists. We will discuss potential underlying reasons for these failures and what could be done in the future. The lessons learned in renal indications are also important for other potential indications of endothelin receptor antagonists like cancer and heart failure.

Invited Lecture 17

Endothelin Receptor Antagonism Versus Combined ECE/NEP Inhibition in Patients with Type 2 Diabetes and Nephropathy

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Endothelin receptor antagonists have been used in clinical trials to test their renoprotection properties in patients with type 2 diabetes who do not invariably respond to angiotensin II blockers. Thus, combined treatment with ET-1 antagonists on top of angiotensin II blockers could represent an effective tool to reduce blood pressure and ameliorating renal function. Here we tested the effect of combined treatment with daglutril, a combined endothelin-converting-enzyme and neutral endopeptidase (MME) inhibitor on top of losartan (100mg/day) in patients with type 2 diabetes. The randomised, crossover trial was held in two hospitals in Italy. Eligibility criteria were: age 18 years or older, urinary albumin excretion 20-999µg/min, systolic blood pressure (BP) less than 140mmHg, and diastolic BP less than 90mmHg. Patients were randomly assigned (1:1) with a computer-generated randomized sequence to receive either daglutril (300mg/day) then placebo for 8 weeks each or vice versa, with a 4-week washout period. The primary endpoint was 24-h urinary albumin excretion in the intention-to-treat population. Secondary endpoints were median office and ambulatory (24 h, daytime, and night-time) BP, renal haemodynamics and sieving function, and metabolic and laboratory test results. This study is registered with ClinicalTrials.gov, number NCT00160225.
58 patients were screened, of whom 45 were enrolled (22 assigned to daglutril then placebo, 23 to placebo then daglutril) and 42 (20 vs 22) were included in the primary analysis. Daglutril did not significantly affect 24-h urinary albumin excretion compared with placebo (p=0.559). 34 patients had complete 24-h BP readings; compared with placebo, daglutril significantly reduced ambulatory systolic (p=0.0013), diastolic (p=0.015), pulse (p=0.019), and mean (p=0.003) BP, as well as all night-time BP readings and daytime systolic, pulse, and mean BP, but not diastolic BP. Compared with placebo, daglutril also significantly reduced office systolic BP (p=0.028), but not diastolic, pulse, or mean BP, and increase big endothelin serum concentration. Three patients taking placebo and six patients taking daglutril had mild treatment-related adverse events - the most common was facial or peripheral oedema (in four patients taking daglutril).
Our data suggest that daglutril improved control of BP in hypertensive patients with type 2 diabetes and nephropathy particularly as for systolic hypertension, that in patients with diabetes and renal involvement is often resistant to treatment. The treatment effect of daglutril was larger during night time, which is of major clinical relevance, since night time hypertension is a strong cardiovascular risk factor in this patient population. We also found that daglutril on top of full dose losartan had a very good safety profile.

Invited Lecture 18

The Selective Type A Endothelin Antagonist Atrasentan Reduces Residual Albuminuria in Patients with Type 2 Diabetes and Nephropathy

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INTRODUCTION AND AIMS: Patients with type 2 diabetes and albuminuria have high cardiorenal morbidity and mortality. We evaluated whether atrasentan, a selective endothelin receptor A antagonist, could reduce albuminuria in use with renin angiotensin system inhibitors (RASi).
METHODS: 211 subjects with type 2 diabetes, macroalbuminuria, and eGFR between 30-75 ml/min/1.73 m^2, were enrolled in 2 parallel, multinational, double-blind, randomized, placebo-controlled studies. Subjects were randomized to atrasentan 0.75, 1.25 mg QD or placebo for 12 wks after a run-in period that maximized the RASi dose.
RESULTS: Mean UACR values (placebo, 0.75, 1.25 mg) at baseline: 671, 878, and 826 mg/g; wk 2: 696 (NS), 573 and 515 mg/g, ($p<0.001$); and wk 12: 797 (NS), 521 and 470 mg/g ($p<0.001$). More than 30% albuminuria reduction was observed in 51 and 55% of subjects receiving atrasentan. eGFR (49±13, 48±15, and 51±14 ml/min at baseline) did not change significantly compared with placebo. Mean LDL-C changed by+2±3, -13±2 and -13±2mg/dl ($p<0.001$). Similar changes were observed in triglyceride levels. No differences were noted in rate of peripheral edema or heart failure. At week 2, body weight increased (0.7 and 1.2 kg), but weight was not significantly different from baseline at week 12 for the 0.75 mg group. After stopping atrasentan for 30 days, all of the parameters described above returned to pretreatment values.
CONCLUSIONS: Chronic administration of atrasentan reduces albuminuria in patients who are receiving maximum RAS inhibition without incurring volume-related adverse events.

Invited Lecture 19

A Placebo-Controlled Study of Ambrisentan in Subjects with Idiopathic Pulmonary Fibrosis (ARTEMIS-IPF)

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Rationale: Idiopathic pulmonary fibrosis (IPF) is characterized by the formation and proliferation of fibroblast foci. Endothelin-1 induces lung fibroblast proliferation and contractile activity via the endothelin A (ETA) receptor. Data from preclinical models suggests that endothelin blockade can attenuate pulmonary fibrosis. This study tested the hypothesis that ambrisentan, an endothelin type A receptor specific antagonist approved for the treatment of pulmonary arterial hypertension, reduces disease progression in subjects with idiopathic pulmonary fibrosis (IPF).
Methods: This was a randomized (2:1), double-blind, placebo-controlled, event-driven trial enrolling subjects with IPF. Randomization was stratified by presence of pulmonary hypertension at baseline, determined by a right heart catheterization, and IPF diagnosis by surgical lung biopsy. The primary endpoint was time to IPF disease progression, defined as all-cause mortality, adjudicated respiratory hospitalization, or a categorical decrease in lung function defined as a 10% decrease in forced vital capacity [FVC] with a 5% decrease in the diffusion capacity for carbon monoxide [DLCO] or a 15 % decrease in DLCO with a 5% decrease in FVC.
Results: At 75% (492 subjects) of the intended total enrollment and after a mean exposure of 34 weeks to the study drug, the Data Safety Monitoring Board terminated the study due to a low likelihood of demonstrating efficacy. From 136 clinical sites, 329 and 163 subjects were randomized to receive ambrisentan or placebo respectively. At baseline, 36 (11%) and 18 (11%) subjects had pulmonary hypertension (PH) with a mPAP >25 mmHg and wedge pressure < 15 mmHg in the ambrisentan and placebo groups respectively. Ambrisentan treated subjects had more primary endpoint events (90 [27.4%] versus 28 [17.2%], and a 1.74 fold increase in risk of meeting the primary endpoint (95% confidence interval [CI] 1.14-2.66, p=0.010). Evaluation of the primary endpoint components indicated that the number of deaths (hazard ratio [HR] 2.08, 95% CI 0.75-5.76, p=0.100) and subjects with a categorical decrease in lung function (HR 1.53, 95% CI 0.84-2.78, p=0.109) were not statistically significantly different between the groups. However, ambrisentan treated subjects had more respiratory hospitalizations (44 [13%] versus 9 [6%]), and a 2.59 fold increase in risk of experiencing a respiratory hospitalization (95% CI 1.14-5.89, p=0.007). Stepwise Cox multivariate analysis revealed that after adjustment for baseline IPF severity, the risk for primary events was reduced and the p-value was >0.1 (HR 1.42, 95% CI 0.85-2.05, p=0.108). Although the risk for respiratory hospitalization was also reduced, the p-value remained <0.05 (HR 2.11, 95% CI 1.03-4.33, p=0.042). Presence of PH at baseline did not affect these point estimates significantly. The secondary endpoints (FVC, DLCO, 6MWD, TDI and QoL) at 48 weeks and the incidence of liver toxicity were not statistically significantly different between the groups.
Conclusions: Ambrisentan was ineffective in reducing disease progression in IPF and was associated with an increased risk of respiratory hospitalizations. While there is no evidence of treatment benefit, all analyses need to be interpreted cautiously as ARTEMIS-IPF was terminated early for lack of efficacy.
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O-25

Impact of Short Term Endothelin A Receptor Blockade on Plasma Markers for Remodeling and Neutrophil Activation in Patients with ST Elevation Acute Coronary Syndrome

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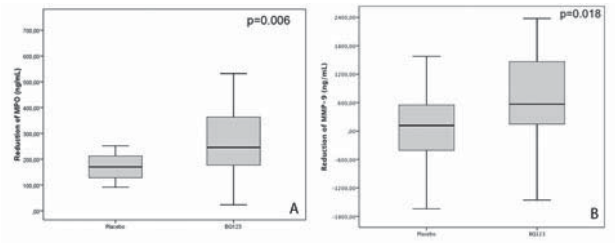
Background: Endothelin (ET) is a pro-fibrotic vasoconstrictor and a mediator of microvascular dysfunction and cardiac remodeling. Animal studies investigating ET receptor blockade in acute myocardial infarction led to conflicting results regarding ventricular remodeling. In-vitro, ET-A receptor blockade decreases neutrophil activation. Methods: Patients with posterior-wall STE-ACS were treated with BQ-123, a selective ET-A receptor antagonist as previously described (n=54). Peripheral blood samples were drawn at baseline, 24 hours and 30 days after PCI. Myeloperoxidase (MPO), matrix metalloproteinase 9 (MMP-9) and the procollagen III N-terminal propeptide (PIIINP), were measured in plasma using commercially available assays. Results: Patients randomized to BQ-123 demonstrated a greater reduction of MPO levels from baseline to 24 hours compared to placebo-treated patients ((177ng/mL reduction for BQ-123 versus 108ng/mL for placebo, p=0.006), Figure 1a). In addition, we observed a significantly greater reduction of MMP-9 levels in patients treated with study drug (568ng/mL versus 117ng/mL, p=0.018, Figure 1b).There was no significant difference in PIIINP values. Conclusion: Short-term administration of BQ-123 reduces MPO and MMP-9 plasma levels in patients with STE-ACS. In trials with larger patient numbers this may translate into improved ventricular remodeling at six months.



Lunch Session 5

LS5

Heart Failure in PAH: Focus on the Right Ventricle

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While the abnormal pulmonary circulation in pulmonary arterial hypertension (PAH) has been the focus of diagnostic, treatment, and prognostic implications for this devastating disease, the focus on right ventricular physiology has become increasingly important. Important observations have led to a better understanding of the role of the right ventricle (RV) in PAH. The RV is both embryologically and morphologically different from the left ventricle (LV). Differences include unique myocardial fiber alignment (mostly longitudinal) which affects contractility, a crescentic cavity shape, and a much lower myocardial mass. Due to these differences, applying our understanding of LV mechanics and flow-volume relationships to the RV leads to errors in assumptions and incorrect modeling. In PAH, characterization of RV performance using newer imaging techniques, and better understanding of fluid mechanics and pulmonary vessel compliance as they relate to RV performance has led to better incorporation of the RV into prognostic equations, and may serve to be useful in optimizing endpoints in clinical trials.

Lunch Session 6

LS6

How to Treat the Patients with Hypertension for the Prevention of Heart Failure -The Back and Forth Strategy between Basic Science and Clinical Medicine-

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Since heart failure (HF) is a common feature of cardiovascular diseases, we have focused on the pathophysiology of HF. In our epidemiological study, either hypertension or diabetes mellitus is linked to both cardiac diastolic dysfunction and cardiovascular events, and recent studies have revealed the importance of the renin-angiotensin-aldosterone system (RAAS) in patients with hypertension: we clinically use either angiotensin converting enzyme inhibitors (ACEI) or angiotensin II type 1 receptor (ARB) to block RAAS. However, ordinary ARBs are not superior to either Ca channel blockers (CCB) or ACEI because 1) some of CCBs increase NO production, and 2) CCBs decrease blood pressure more than ARB. Therefore, we need to test stronger ARBs such as azilsartan to mediate novel cardioprotection. Since we further need to discover the novel unknown factors of HF, we newly apply the scenario of "Back and Forth Methodology between Clinical Medicine and Basic Science". Namely, we firstly target the clinical observation, and extend such clinical hints to basic sciences, and returns the fruitful results to the clinical medicine. To do this, we employ 1) basic investigation of the effective clinical treatments, 2) the use of animal models, 3) the pedigrees of cardiovascular diseases, 4) the clinical samples, and 5) the clinical data records, culminating in large-scale clinical trials (LSCT). However, the results of LSCT do not necessarily contribute to personalized practice. To overcome this deficiency, we tried to constitute the equation or law that formulates the personalized clinical outcomes. Taken together, we should consistently work for the cardiovascular diseases and provide effective treatments in patients with cardiovascular diseases.

Poster Session 3: Hypertension, Vascular Diseases, Cardiology, Clinical Studies, Metabolism

P-92

A Potential Role of Endothelins in Rheumatic Mitral Stenotic Valves

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Background: The genesis of rheumatic mitral valve stenosis has been correlated with the action of endothelin subtype 1 (ET-1) and its receptors (ETRA and ETRB). We aim to analyze, through real time PCR (polymerase chain reaction), the gene expression of ET-1 in rheumatic mitral valves. Methods: This is a randomized and experimental study. We collected ten mitral valves in two hospitals of Aracaju, Brazil. Each valve has suffered fragmentation, originating three segments that were subjected to extraction of total RNA. Then, each sample of total RNA was quantified by spectrophotometry. Through reverse transcriptase reaction, the total cDNA was obtained of each sample and then, it was performed the technique of amplification of target fragment by real time PCR with the quantitation of each sample. Data were tabulated and analyzed by CFX96 Real Time System (BIORAD), and the calculations of relative expression were performed by use of the Delta Ct. Results: The mean concentrations of nucleic acid (total RNA), and cDNA were respectively 27.21 ± 30.26 ng / ul and 609.4 ± 80.60 ng / ul. Mean values of absorbance at 260 and 280nm were respectively 0.67 ± 0.74 UA (A260) and 0.33 ± 0.36 UA (A280). The proportion A260/A280 was 1.91 ± 0.20. Of the seven samples collected, we observed the expression of ET-1 in all of them. Quantitatively, the average gene expression relative to ET-1 was 62.85 ± 25.63%. Conclusions: The expression of ET-1 was expected, since this peptide is related to vasoconstriction and inflammatory processes present in chronic rheumatic valve disease.

P-93

Blood Pressure Independent Downregulation of Plasma Endothelin-1 Levels in a Lavage-Induced Surfactant Depleted Rabbit ARDS Model: Effects of Various Respiratory Maneuvers on Endothelin-1 Levels

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Endothelin-1 (ET-1) is a mediator of vascular inflammation, cell proliferation, and fibrosis, and is, in addition, a potent vasoconstrictor. Previously, treatment with ET-1 antagonists was shown to reduce pulmonary vascular leak and inflammation in several models of lung injuries as well as in experimental acute respiratory distress syndrome (ARDS). The current study used an experimental model of lavage-induced surfactant depleted ARDS, to investigate the circulatory and pulmonary levels of ET-1. In addition, we also tested the effects of open endotracheal suctioning (OES) (a known inducer of alveolar de-recruitment) and the post OES hyperinflation (HI) (performed to recover the alveolar de-recruitment using bagging) on ET-1 levels. Briefly, 18 Japanese White Rabbits were anesthetized and intubated. Normal saline was instillated into the lung and washed mildly. After instillation, rabbits were ventilated at definite settings; total OES and HI duration was for 3 h and performed every 15 minutes from the beginning of the protocol. Circulatory levels of ET-1 were found to have decreased from baseline (3.26±1.01) to after lavage (1.82±1.59, p=0.003), without any significant change in mean blood pressure (baseline 112±13.8; after lavage 113±12.5, p=0.848). In contrast, pulmonary ET-1 levels were almost unchanged irrespective of the induction of lavage-induced lung injury from baseline. It must be noted that, in lung injury state, PaO_2 was significantly decreased, having a parallel relation with ET-1. Either OES or HI failed to recover the down-regulated circulatory ET-1 level. For now, we cannot rule out the mechanism of differential pattern of circulatory ET-1 levels observed in the current model compared to other ARDS models.

P-94

Upregulated Pulmonary Endothelin-1 in Acute Lung Injury is Not Normalized Through Landiolol Hydrochloride Treatment, an Ultra-Short-Acting β-Blocker, in a Rat Model of Endotoxemia

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Molecular mechanisms of sepsis-associated acute lung injury (ALI) are poorly defined. Endothelin (ET)-1, a potent vasoconstrictor that has been implicated in the pathogenesis of sepsis. We recently demonstrated that ET-1 plays important role in development of ALI in a rat model of sepsis. As an extension of recent study, in this investigation we investigated whether landiolol hydrochloride, an ultra-short-acting β-blocker, can play important role in ameliorating LPS-induced ALI through the normalization of ET-1. Male Wistar rats at 8 weeks of age were administered with either saline or lipopolysaccharide (LPS) for three hours and some LPS-administered rats were continuously treated with landiolol for three hours. The features of acute lung injury were observed at sepsis model. At 3h after LPS administration, both circulatory and pulmonary TNF-α level increased and PaO2 was significantly decreased LPS administration. LPS induced a time-dependent expression of ET-1 in the lungs compared to control, peaking and increasing by 3 fold at 6 h after induction of endotoxemia, whereas levels of ET (B) receptor, which has vasodilating effects, were remarkably down regulated time-dependently. We conclude that time-dependent increase of ET-1 and ET (A) receptor with the down regulation of ET (B) receptor may play a role in the pathogenesis of acute lung injury in endotoxemia. Finally, treatment of LPS-administered rats with landiolol for three hours failed to normalize the upregulated pulmonary ET-1 and TNF-α levels. Our another study found landiolol can ameliorate ALI in LPS-induced sepsis model. These data taken together, led us to conclude that landiolol mediated ALI improvement in sepsis does not involve pulmonary ET system.

P-95

Blockade of TRPC6 is a Novel Therapeutic Approach Against Pathological Cardiac Remodeling

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Background; Expression of transient receptor potential subfamily C (TRPC) 6, receptor-operated Ca^{2+} channels, is increased in hypertrophic and failing hearts. TRPC6 has been shown to be a positive regulator of calcineurin-NFAT signaling that drives pathological cardiac remodeling. In this study we examined the effect of TRPC inhibition on the pathological cardiac hypertrophy. Methods and Results; In cultured neonatal rat ventricular myocytes, overexpression of TRPC6 increased basal and ET-1 induced NFAT-dependent RCAN1 promoter activity. BTP2, a selective TRPC channel blocker, significantly and dose-dependently inhibited activation of the RCAN1 promoter, and attenuated hypertrophic response of cultured cardiac myocytes. Knocking-down of TRPC6 and 3 using siRNAs significantly inhibited ET-1- or Ang II-induced increases in Ca^{2+} oscillation, and knocking down either TRPC6 or 3 had a similar effect. In model mice lacking GC-A, which is a common receptor for atrial and brain natriuretic peptides, the expression of TRPC6 and RCAN1 was increased and BTP2 significantly attenuated the cardiac hypertrophy observed in GC-A KO mice without affecting blood pressure. BTP2 also inhibited AngII-induced cardiac hypertrophy in mice. Compatible with the notion that TRPC6 and 3 form heteromultimeric cation channels, Pyrazole-3, a selective TRPC3 blocker, which can inhibit the ion channel activity of TRPC3/6 hetero-complex, also significantly inhibited Ang-II induced cardiac hypertrophy in mice. Conclusions; Blockade of TRPC6 could be an novel therapeutic strategy for preventing pathological cardiac remodeling.

P-96

Effects of Landiolol Hydrochloride, an Ultra-Short-Acting β-Blocker, on Cardiac Endothelin System in a Rat Model of Endotoxemia: A Possible Relevance with Cardiac Functional Compensatory Events at the Early Phase of Sepsis

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Landiolol, an ultra-short-acting and highly cardioselective beta-1 blocker, has become useful for various medical problems. Recent studies have demonstrated that co-treatment with landiolol protects against acute lung injury and cardiac dysfunction in a rat model of lipopolysaccharide (LPS)-induced systemic inflammation which was associated with a significant reduction in serum levels of the inflammation mediator HMGB-1 and histological lung damage. Endothelin (ET)-1, a potent vasoconstrictor, has been implicated in the pathogenesis of sepsis and sepsis-induced multiple organ dysfunction syndrome. In the current study, we investigated whether landiolol hydrochloride, can play an important role in ameliorating the LPS-induced altered cardiac ET system in a rat model of endotoxemia. Male Wistar rats at 8 weeks of age were administered LPS for three hours and some LPS-administered rats were continuously treated with landiolol for three hours. At 3h after LPS administration, circulatory TNF-alpha level was highly increased. Blood lactate concentration and percentage of fractional shortening of heart has also significantly increased after LPS administration. In addition, LPS induced a significant upregulated expression of various components of ET-1 system in the cardiac tissues compared to control. Finally, treatment of LPS-administered rats with landiolol for three hours potentially normalized the increased blood lactate level, cardiac functional compensatory events without an effect on plasma TNF-alpha and ET-1 levels. Most strikingly, landiolol treatment has greatly normalized the various components of ET-1 system in endotoxemic heart. These data taken together, led us to conclude that landiolol may be cardio protective in endotoxemia normalizing the vasoactive peptide like endothelin without altering the circulatory level of potential inflammatory cytokine like TNF-alpha.

P-97

Inhibitory Effect of Eicosapentaenoic Acid on Cardiomyocyte in Endothelin Induced Hypertrophy Via PPAR-α

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Growing body of evidences state the cardiovascular benefit of fish oil including eicosapentaenoic acid (EPA) in humans and experimental animals, but the effect of EPA on endothelin (ET)-1-induced cardiomyocyte hypertrophy is unknown. Previous study demonstrated that peroxisomal proliferator-activated receptor (PPAR)-α ligand (fenofibrate) prevents ET-1-induced cardiomyocyte hypertrophy. Though EPA is a ligand of PPAR-α, there was no study linking relationship between EPA and PPAR-α in the field of cardiomyocyte hypertrophy. The present study investigated whether ET-1-induced cardiomyocyte hypertrophy could be prevented by EPA pre-treatment with possible mechanistic insights. At day 4 of culture, neonatal rat cardiomyocytes were divided into three groups: control, ET-1 (0.1nM) treated and EPA-pre-treated (10μM) ET-1 groups. 2-fold increase in cardiomyocyte surface area, 1.8-fold increase in total protein synthesis rate and an enhanced α-actinin expression in cardiomyocyte were observed after ET-1 administration and these changes were greatly prevented by EPA pre-treatment. ET-1-induced hypertrophied cardiomyocytes showed increases in ANP and BNP mRNA expression, which were also suppressed by EPA pre-treatment. Pre-treatment of EPA could also attenuate phosphorylated JNK (an important component of MAPK cascade), c-Jun (downstream molecules of JNK) in ET-1-induced hypertrophied cardiomyocytes. PPAR-α expression and PPAR-PPRE binding activity was suppressed in ET-1 administered cardiomyocyte and this suppression was improved by EPA treatment. In conclusion, the present study showed that ET-1 could induce significant cardiomyocyte hypertrophy with hypertrophic markers upregulation, and that this remodeling was effectively prevented by EPA-pre-administration through the upregulation of PPAR-α and the suppression of phosphorylated JNK, and c-Jun.

P-98

Higher Circulatory Level of Endothelin-1 in Hypertensive Subjects Screened Through a Cross-Sectional Study in Rural Bangladeshi Women

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Objective: Endothelins are powerful vasoconstrictor peptides that also play numerous other functions in many different organs. Endothelin-1 (ET-1) is the most abundant and important of this family of peptides in blood vessels. ET-1, a potential marker of endothelial dysfunction has been shown in hypertensive subjects. No study yet has investigated the circulatory level of ET-1 in a country from South Asia. The present study assessed circulating levels of ET-1 in subjects with or without hypertension and further examined their association with clinical and metabolic parameters.Methods and results: A total of 1802 rural Bangladeshi women with mean age of 44.16 years were studied using a cross-sectional survey. The prevalence of hypertension was 31.78%. Endothelin-1 levels were significantly higher in hypertensive than non-hypertensive subjects (hypertensive vs non-hypertensive: 4.16±0.32 vs. 3.00±0.08 pg/ml, $p<0.001$). After adjusting for age, ET-1 had significant positive associations with diastolic blood pressure (DBP) ($\beta=0.039$, $p=0.013$) and systolic blood pressure (SBP) ($\beta=0.020$, $p=0.006$). Unlike blood pressures, other variables including insulin, fasting blood glucose, triglycerides, high-density lipoprotein cholesterol, body mass index, waist circumference and vascular endothelial growth factor were not associated with ET-1. Stepwise multiple regression analysis, after adjusting for age and all other potential variables revealed that SBP and DBP were independent determinants of ET-1.Conclusions: The correlation of ET-1 needs further investigations to define the clinical utility and predictive value of serum ET-1 levels in hypertension for South Asian population. Higher concentration of ET-1 suggests endothelial dysfunction already in mild forms of hypertension without further risk factors or cardiovascular complications in this apparently healthy population.

P-99

Inverse Correlation between Systemic Endothelin-1 Level and Pulmonary Artery Pressure in Adult Patients with Uncorrected Atrial Septal Defect

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Patients with ASD have increased pulmonary blood flow and may cause increase in pulmonary arterial pressure. Endothelin-1 (ET-1) mainly produced by pulmonary vascular endothelium and increased plasma ET-1 level has been reported in patients with left-to-right shunt. ASD is the most common congenital shunting in adult. However, no study addressed specifically for ASD and has evaluated the role of ET-1 in this congenital shunting. Therefore, we aim to correlate the peripheral ET-1 level with pulmonary arterial pressure in adult patients with uncorrected ASD. From July 2012-April 2013 we enrolled 55 ASD patients; mean age 34.5 years-old. Confirmation of ASD and the measurement for pulmonary arterial pressure (mPAP), right ventricular systolic pressure (RVSP), and pulmonary flow ratio (Qp/Qs) were performed using TTE dan TEE. These measurements were previously confirmed with right heart cathetherization and showed positive correlation ($r=0.5$; $p<0.0001$ and $r=0.8$; $p<0.0001$ respectively). Peripheral blood was withdrawn from brachial vein. Forty (72%) patients have left-to-right and 28% with right-to-left shunting. Mean mPAP was 40.1±14.9mmHg; mean circulating ET-1 was 5.6±2.1 pg/dl. Unexpectedly, the correlation between circulating level of ET-1 and mPAP were significantly inversed ($r=-0.452$;$p<0.01$), and with RVSP was also significantly negative ($r=-0.405$;$p<0.01$). Accordingly, the reduced circulating ET-1 level might be explain by the decrease in Qp/Qs ($r=0.310$;$p<0.05$). However, no differences of ET-1 were found between LtoR vs, RtoL shunts (5.7±0.36vs.5.3±0.52 pg/dl; NS). As conclusion, we observed inversed relationship between circulating ET-1 and mPAP that might partially be explained by the decreased in pulmonary flow. Further study to elucidate whether pulmonary derived ET-1 may play more roles in this disease is needed.

P-100

Synchrotron Radiation Pulmonary Micro-Angiography to Visualize Pulmonary Artery Micro-Vasculature for Measurement of Pulmonary Arterial Flow Velocity in a High Pulmonary Flow Rat Model

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Background: Increased pulmonary blood flow (PBF) and shear stress may provoke irreversible vascular remodeling. Visualization of the micro-vasculature and the measurement of PBF velocity would provide insights regarding the correlation between high PBF and vascular remodeling. In this study, we aimed to establish a method for utilizing synchrotron radiation pulmonary micro-angiography (SRPA) and measure the PBF velocity in a high PBF rat model. Method: SRPA was performed at the Photon Factory of the High Energy Accelerator Research Organization (Tsukuba, Japan). Synchrotron radiation was converted to monochromatic X-rays by 13° reflection on a silicon crystal. High-sensitivity HARP (High-gain Avalanche Rushing Amorphous Photoconductor) detector camera with a fiber-optic plate provided by NHK Science and Technology Research Laboratories and Hamamatsu Photonics was used as an image receiver. As a high PBF rat model, a fistula between the abdominal aorta and IVC was created. After 8 weeks, SRPA was performed by transvenous infusion of contrast medium. The dynamic changes of density at the pulmonary artery (PA) were measured by a density measurement software (Gray-val; Library Inc.Japan). The PBF velocity was calculated by the transit time of contrast medium in PA. Result: The high spatial and density resolution was achieved by SRPA. The minimum identified vascular diameter was 100μm. The velocity of PA flow in high PBF rats was significantly increased compared with control (2.3±8.5 vs. 46.1±4.3 mm/sec). Conclusion: These results demonstrate the effectiveness of SRPA for visualizing the flow distribution in micro-vasculature and measure PBF velocity in a high PBF rat model.This newly developed technology may help investigate the mechanism of vascular remodeling associated with high PBF and shear stress.

P-101

Effects of Closed vs. Open Repeated Endotracheal Suctioning During Mechanical Ventilation on the Pulmonary and Circulatory Levels of Endothelin-1 in a Lavage Induced Surfactant Depleted Rabbit ARDS Model

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Despite the beneficial roles, endotracheal suctioning is known to accelerate lung injury during mechanical ventilation. More recently, a growing body of evidence demonstrates discretely the difference of open endotracheal suctioning (OES) and closed endotracheal suctioning (CES) on the respiratory and hemodynamic parameters in acute respiratory distress syndrome (ARDS). Endothelin-1 (ET-1), a mediator of vascular inflammation, cell proliferation, and fibrosis in addition to being a potent vasoconstrictor has been potentially implicated in the pathogenesis of ARDS. We investigated the effects of repeated OES vs. CES during mechanical ventilation on circulatory and pulmonary levels of ET-1 in ARDS. Briefly, 18 Japanese White Rabbits were anesthetized and intubated with a 3.5-mm endotracheal tube. Normal saline was instilled into lung and washed mildly. After instillation, rabbits were ventilated at definite setting; OES and CES duration was for 6 hours and performed every 30 minutes. At circulatory level, either OES or CES did not alter plasma ET-1 level compared to the ET-1 level in ARDS before the initiation of endotracheal suctioning (OES 4.7 ± 1.3 CES 4.8 ± 1.5, p = .839). In contrast, pulmonary ET-1 level was significantly higher in CS group compared to the the OES group after 6 hours of repeated suctioning in lavage-induced ARDS (OES 27.2 ± 2.2 CES 29.7 ± 3.3, p = .05). This change in pulmonary ET-1 level could maintain a parallel relation with Pa02 level. The current observation for the first time reported the involvement of vasoactive peptide like ET-1 underlying the pulmonary changes of closed suctioning during mechanical ventilation in a lavage induced ARDS model.

P-102

Localized Effect of Vascular Aging on NADPH Oxidase-Mediated Contractions to Endothelin

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Renal hemodynamics have important effects on blood pressure. Renal arteries are highly sensitive to endothelin-1 (ET-1)-induced contractions, which partly depend on NADPH oxidase (Nox)-mediated superoxide generation. Aging is associated with increased superoxide production, but whether this affects Nox-mediated vascular reactivity to ET-1 is unknown. We studied the effect of aging on Nox-mediated contractions to ET-1 (0.1nmol/L-100nmol/L) in isolated rings of renal arteries and aortas from young and old C57BL6 mice (4 and 24 months of age). Responses were obtained in the presence and absence of the Nox-selective inhibitor gp91ds-tat (3µmol/L) and calculated relative to KCl (60mmol/L)-induced contractions. The nitric oxide synthase inhibitor L-NAME (300µmol/L) was used throughout the study to exclude differential effects of nitric oxide bioavailability between vascular beds. When compared to the aorta, maximal contractions to ET-1 in renal arteries were 6-fold greater in young (102±4% vs. 18±4%, P<0.01) and 23-fold greater in old animals (92±8% vs. 4±1%, P<0.01). Aging did not affect ET-1-induced contractions in renal arteries, which were inhibited by gp91ds-tat in both young and old animals (2-fold, P<0.01). In the aorta of young animals, contractions to ET-1 were equally reduced by Nox inhibition and by vascular aging (4-fold, P<0.05). In old aortas, Nox inhibition had no further effect on responses to ET-1. In conclusion, aging reduces contractions to ET-1 in the aorta by abolishing the contribution of Nox. In contrast, the renal artery appears to be resistant to aging-induced changes of Nox-dependent responses to ET-1. These findings indicate a specific, localized role of Nox in functional vascular aging that determines ET-1-dependent regulation of arterial tone.

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Selective Endothelin (ET)-A Receptor Antagonist and Dual ET-A/B Receptor Antagonist are Effective in Preventing the Decrease in VEGF Signaling and Inadequate Coronary Collateral Development in the Diabetic Hearts

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Inadequate collateral formation in the heart in diabetic (DM) animals and patients was considered to partly caused by decrease in vascular endothelial growth factor (VEGF), the key angiogenic factor. The present study was designed to investigate the therapeutic implication to improve the defect in VEGF signaling in DM hearts by ET antagonists. We investigated whether ET-A/B dual receptor antagonist (SB209670, 1 mg/kg/day) and ET-A receptor antagonist (TA-0201, 1 mg/kg/day) would reverse the downregulated VEGF signaling in early streptozotocin (STZ)-induced diabetic hearts with no obvious cardiac dysfunction in echocardiography. Male Sprague-Dawley rats were administered citrate saline (vehicle) or STZ (65 mg/kg IP). One week after the injection, animals were separated into those receiving SB209670, TA-0201or vehicle by osmotic mini pump for 2 weeks. Cardiac ET-1 level was significantly increased in DM than in non-DM rats and SB209670 treatment greatly reversed the higher ET-1 levels in DM heart than those by the TA-0201 treatment. VEGF level in DM heart was significantly decreased than in non-DM rats, SB209670 and TA-0201 treatments completely prevented this VEGF downregulation in DM hearts. The dual ET-A/B receptor blocker was more effective in reversing the alterations in phosphorylated Akt and eNOS, the two important downstreams of VEGF angiogenic signaling, in DM heart than the ETA-receptor antagonist. In conclusion, ET antagonism is effective in preventing the downregulation of VEGF in diabetic heart, and the dual ET-A/B receptor antagonist and the selective ETA receptor antagonist were effective in ameliorating the decreased VEGF signaling and inadequate coronary collateral development in diabetic hearts.

P-104

Effect of Sitaxentan on Plasma Biomarkers of Proendothelin-1 Synthesis in Patients with Chronic Kidney Disease

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Background: Endothelin-1 (ET-1) is implicated in the development and progression of chronic kidney disease (CKD). It has also been linked to increased cardiovascular risk in patients with CKD. Here we evaluated plasma levels of endothelin-like domain peptide (ELDP; preproET-1[93-166]) and CT-proET-1 (preproET-1[169-212]) in patients with proteinuric CKD after treatment with sitaxentan. Changes in proET-1 peptides were compared with various parameters of cardiovascular and renal function. **Methods and Results:** Twenty-seven patients with proteinuric CKD receiving recommended renoprotective treatment were randomised to 6 weeks of placebo, sitaxentan (100 mg once daily), and nifedipine long-acting (30 mg once daily), in a double-blind, three-way crossover study design. Renal and cardiovascular function, and plasma biomarkers were measured at baseline, week 3, and week 6 of each treatment period. ELDP and CT-proET-1 were measured by specific sandwich ELISAs. Sitaxentan treatment resulted in significant increases in ELDP and CT-proET-1 at both 3 and 6 weeks compared to baseline (mean increases % ± sem at 3 and 6 weeks: ELDP +17.5 ± 3.2, +15.0 ± 3.5; CT-proET-1 +12.9 ± 2.0, +14.4 ± 2.6; P<0.001 for all values). Placebo and nifedipine had no effect on plasma ELDP and CT-proET-1. After 6 weeks sitaxentan plasma ELDP and CT-proET-1 were negatively correlated with 24 h urinary Na^+ excretion (p= 0.015, r^2= 0.2149; p= 0.013, r^2= 0.2227, respectively). **Conclusions:** Increases in proET-1 biomarkers after sitaxentan indicate that ET_A antagonists block a negative feedback effect of ET-1 on *EDN1* gene expression that is mediated via ET_A receptors. The inverse relationship between plasma proET-1 biomarkers and urinary Na^+ excretion after sitaxentan requires further investigation.

P-105

Aging Selectively Impairs Contractions to Endothelin-1 But Not to Angiotensin II in Murine Carotid Arteries

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Aging is a main risk factor for carotid artery disease and stroke. The constrictor peptides endothelin-1 and angiotensin II are important modifiers of age-induced vascular diseases, partly through altered activity of NADPH oxidase (Nox)-derived superoxide and constrictor prostanoids. Whether aging affects Nox- or prostanoid-mediated constrictor responses to endothelin-1 or angiotensin II in carotid arteries is unknown. We studied contractions to endothelin-1 (0.1-100 nmol/L) and angiotensin II (100 nmol/L) in isolated common carotid artery rings from young and old C57BL6 mice (4 and 24 months of age). The nitric oxide synthase inhibitor L-NAME (300 µmol/L) was used throughout the study to exclude endothelin ET_B receptor- or angiotensin AT_2 receptor-mediated nitric oxide release during contractions, which are given relative to KCl (60 mmol/L)-induced responses. Some arteries were additionally incubated with the Nox-specific inhibitor gp91ds-tat (3 µmol/L) or the thromboxane-prostanoid receptor antagonist SQ 29'548 (1 µmol/L) for 30 minutes. Aging markedly reduced maximal responses (E_{max}) and the sensitivity (pD_2) to endothelin-1 (E_{max} 8.9±1.8% vs. 26.2±3.8%, 3-fold, P<0.001; pD_2 8.02±0.07 vs. 8.54±0.02, P<0.01). Inhibition of Nox activity blunted endothelin-1-indcued contractions by 42% and 63% in young and old animals, respectively (P<0.05), while the thromboxane-prostanoid receptor antagonist had no effect in either group. Contractions to angiotensin II were weak in young and old animals (3.6±0.4% vs. 3.1±0.2%, P=n.s.) and unaffected by gp91ds-tat or SQ 29'548. In summary, aging selectively impairs contractions to endothelin-1 in carotid arteries, which depend on Nox activity, but not on constrictor prostanoids. Endothelin-1 but not angiotensin II seems to be involved in functional aging of carotid arteries.

PC-20

Inflammatory State Before Catheter Ablation is Associated with Recurrence of Atrial Fibrillation in Patients with Persistent Atrial Fibrillation after Catheter Ablation

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Introduction
Inflammation is thought to be one of the major factors in the progression of arrhythmogenic atrial remodeling and that promote atrial fibrillation (AF). The aim of this study was to investigate the effects of inflammatory state before and immediately after catheter ablation on clinical outcomes after catheter ablation of persistent AF.
Methods
We investigated 176 patients with long-standing persistent AF (sustained AF duration; 1 to 20 years, with a mean of 3.4±3.8 years) undergoing catheter ablation. The high-sensitivity C-reactive protein (hs-CRP) level was measured as an inflammatory marker before and immediately after the catheter ablation. Patients were divided into two groups according to the hs-CRP level in the baseline: high hs-CRP group (n=84, >0.075mg/dl) and low hs-CRP group (n=92, <0.075 mg/dl).
Results
Catheter ablation was successfully performed in all patients. After 12-month follow-up, 53.4% of the patients had AF recurrence. The hs-CRP level before catheter ablation was significantly associated with AF recurrence (p=0.024), however, neither the hs-CRP level immediately after catheter ablation nor the increment of hs-CRP after catheter ablation was not associated with AF recurrence. Multivariate Cox regression analysis revealed that longer duration of AF (P<0.001), larger left atrial diameter (p=0.049), and higher hs-CRP level (p=0.033) were significantly associated with AF recurrence. In Kaplan-Meier AF free curves, there is a significant difference in AF free rates between low hs-CRP group (57% at 1 year) and high hs-CRP group (35% at 1 year)(p=0.007).
Conclusions
The increased hs-CRP level reflecting an inflammatory state before catheter ablation may is one of the important predictors of recurrence of AF after catheter ablation in patients with long-standing persistent AF.

P-106

Pericardial Resistance Artery Contractile Responses to Endothelins

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Because the parietal pericardium is opened during cardio-thoracic surgeries, the tissue might be used for translational research. We investigated whether i) contractile resistance-sized arteries can be isolated from the parietal pericardium and ii) whether their arterial smooth muscle is responsive to endothelin (ET). Tissue was harvested from pigs i) in a local slaughterhouse (N = 8) or ii) at the end of experimental surgery under a cocktail of anaesthetics and analgesics (N = 7) and stored overnight. Arterial segments were microdissected from the tissue and studied in wire myographs. Arterial lumen diameter at a distending pressure of 100 mmHg was 251±24 µm. Contraction in response to 32 mM K^+ averaged 3.9±0.9 N/m. Responses to ET-receptor stimulation were investigated after desensitization of sensorimotor nerves and irreversible blockade of α-adrenergic receptors and in the continuous presence of inhibitors of NO synthases, cyclooxygenases and small- and intermediate-conductance Ca^{2+}-activated K^+ channels. These pharmacological tools were applied to concentrate on arterial smooth muscle responses. Sarafotoxin 6c (ET_B-agonist) caused potent and strong contractions (EC_{50} 42 pM, E_{max} 4.1±0.3 N/m). Thereafter and in the presence of 30 nM A-192621 (ET_B-antagonist) the sensitivity and maximal contractile response to ET-1 averaged 1.3 nM and 8.5±2.4 N/m, respectively. 100 nM PD-156707 (ET_A-antagonist) reduced the sensitivity and maximal response to ET-1 in these conditions. Differences between arteries from both sources were not statistically significant. We conclude that resistance arteries from the parietal pericardium of patients undergoing cardio-thoracic surgery may be useful for translational studies of arterial smooth muscle responses to ET_A- and ET_B-receptor stimulation and blockade.

P-107

Regional Differences in the Effect of Hypoxia on Endothelin-1-Induced Contraction in Rat Arteries

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Acute arterial occlusion due to an embolus or a thrombus causes hypoxia in the vascular bed, usually resulting in critical injury. Hypoxia affects more or less vascular function, but the response to low oxygen differs in individual vascular beds. The present study examined the influence of hypoxia on endothelin-1 (ET-1)-induced contraction in isolated rat carotid and mesenteric arteries. Although the addition of ET-1 (10^{-10} to 10^{-8} M) produced a dose-dependent contraction either in carotid or mesenteric arteries, the response to ET-1 was significantly attenuated by hypoxia in carotid, but not in mesenteric, arteries. The impaired contraction to ET-1 in carotid arteries was also observed in endothelium-denudated preparations or in the presence of an endothelin type B (ET_B) receptor antagonist (BQ-788, 10^{-6} M). Meanwhile, ET-1-induced contraction of carotid arteries in the presence of an endothelin type A (ET_A) receptor antagonist (BQ-123, 10^{-6} M) was not affected by hypoxia. Incidentally, ET-1-induced contraction was largely inhibited by antagonism of ET_A receptors either in carotid or mesenteric arteries. In addition, IRL-1620 ($<10^{-7}$ M), a selective ET_B receptor agonist, did not cause any contraction in both arteries. Although a crucial feature of the response to hypoxia is to produce reactive oxygen species like superoxide, the treatment with superoxide dismutase (200 U/mL) did not affect the influence of hypoxia on ET-1-induced contraction in both arteries. These findings suggest that although ET-1 induces contraction through ET_A receptors either in carotid or mesenteric arteries, hypoxia impairs this pathway only in carotid arteries. Furthermore, extracellular superoxide seems not to be a causal factor responsible for this regional difference.

P-108

Urinary ET-1 Excretion after Exposure to Radio-Contrast Media in Diabetic Patient and Patients with Preexisting Impaired Renal Function

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Preclinical studies indicate that the renal Endothelin system is involved in the pathogenesis of acute renal failure. Contrast media induced nephropathy (CIN) is one type of AKI and is associated with increased morbidity and mortality. We analyzed 273 patients with either diabetes or preexisting impaired kidney function getting intra-arterial contrast media (CM) for coronary angiograms. Blood was taken for determination of cystatin C before and 24 hours after exposure to intra-arterial CM. Acute kidney injury was defined as increase of baseline cystatin C of at least 25%. Urinary ET-1 and neutrophil gelatinase-associated lipocalin (N-GAL) concentrations were analyzed in spot urine 24 h after CM exposure. 27 patients out of the 273 patients developed CIN. Urinary ET-1 (r=-0.168, p=0.006) and N-GAL (r=-0.173, p=0.004) concentrations were inversely correlated with the change of cystatin C 24 hours after CM exposure. Urinary ET-1 concentrations in spot urine of patients without CIN were 0.659 pg/ml and 0.379 pg/ml in patients with CINI respectively (p=0.004). Urinary N-GAL concentrations in spot urine of patients without CIN were 19.92ng/ml and 8.61 ng/ml in patients with CIN, respectively (p=0.002). In conclusion, the magnitude of alterations in urinary ET-1 excretion is comparable to alterations of the reference biomarker N-GAL. In contrast to other forms of AKI, urinary concentrations of both biomarkers are reduced indicating potentially differences in the underlying pathways leading to kidney damage after CM exposure as compared to other forms of AKI.

P-109

A Predictor to Indicate the Necessity of Measurement of Endothelin and to Give the Calcium Antagonist Medicine in Outpatients

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Purpose: This study aimed to examine a predictor to indicate the necessity of measurement of the endothelin and to give the calcium antagonist medicine of dihydropyridine in outpatients. Method: The investigation was performed in during August 25,2005 to September 19,2009. The data of patient profile and diagnosis, blood pressure, blood tests, and current medication were collected after obtaining approval of the ethics committee for clinical study from 3 hospitals. Result: The effective number of patients for analysis was 376; mean age 59.2±10.4 year. The average clinical values were as follows:weight,64.4±12.8; BMI,24.8±4.0; SBP,129.8±17.0mmHg; DBP,78.3±10.5mmHg; FPG, 119.0±52.9mg/dl. Final stepwise multiple regression analysis indicated predictor y comprising 4 explained independent variables; age, β.493; weight, β.274; FPG, β.162; SBP, β.158.The Breslow χ^2test using the Kaplan-Meier command yielded a significant χ^2 10.44 of y both DM and HT in IHD. The F-test indicated a significance F7.50 for y with calcium antagonist medicine as compared to y without calcium antagonist medicine. $P<0.01$. Conclusion: This study developed the estimator y as a significant predictor to give the calcium antagonist medicine.

P-110

Assessment of Circulatory Endothelin-1 Level Among Pre- and Post-Menopausal Rural Women in Bangladesh: Result from a Population-Based Study

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BACKGROUND: Prevalence of non-communicable diseases are a challenging problems among menopausal women specially in a least developed country like Bangladesh, where majority of women suffering from at least one chronic diseases after menopausal age. The main objective of this study was to determine the circulatory level of endothelin (ET)-1 in Bangladeshi pre- and post-menopausal women living in the rural setting and its association with various cardiometabolic risk factors.METHODS: This study is based on a community based cross-sectional survey among 1802 rural women aged >=15 years. Plasma level of ET-1 was measured by ELIZA. Logistic regression was used to estimate the association between circulatory ET-1 level and cardiomatabolic risk factors.RESULTS: ET-1 levels were significantly higher in post-menopausal subjects (post-menopause vs. pre-menopause: 3.92±0.28 vs. 3.28±0.14 pg/ml, p=0.043). In multivariable analyses, we found that ET-1 had significant positive associations with only plasma cholesterol level (beta =0.005, p=0.012) even after adjusting for age. Metabolic syndrome was presented in 25.6% respondents and it was more prevalent among post-menopausal (39.3%) as compared to pre-menopausal (16.8%) women. Prevalence of high blood pressure, elevated fasting blood glucose, and high triglyceride were significantly higher in post-menopausal women than pre-menopausal women (P < 0.05). Mean values of systolic blood pressure, plasma levels of triglyceride, HDL, cholesterol and vascular endothelial growth factor were significantly higher in post-menopause group compared to pre-menopause group.CONCLUSIONS: The higher level of circulatory ET-1 may be associated with higher prevalence of metabolic syndrome and other cardiometabolic risk factors among post-menopausal women in many developing countries like Bangladesh and proper intervention strategies should be warranted.

P-111

Is Excessive Blood Pressure Elevation During Resistance Exercise a Risk Factor for Arterial Stiffening?

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We previously demonstrated that plasma endothelin-1 (ET-1) concentration is related to resistance exercise-induced arterial stiffening. Additionally, a previous study pointed out that a transient increase in plasma ET-1 concentration after resistance exercise was correlated with blood pressure increase during the exercise. Exaggerated systolic blood pressure (SBP) response to exercise is an independent predictor for future cardiovascular disease. Hence, we hypothesized that excessive elevation in SBP during resistance exercise increases arterial stiffness via ET-1 secretion. The aim of this study was to investigate the association of arterial stiffness with SBP during resistance exercise. After measurements of resting hemodynamic parameters, forty-one middle-aged and older subjects performed leg press, a representative resistance exercise, at 20%, 40% and 60% of their one-repetition maximum. Average SBP during resistance exercise, age and resting heart rate (HR) were entered as independent predictors of aortic pulse wave velocity (PWV, a traditional index of arterial stiffness) into the stepwise regression analysis of sex, antihypertensive medication use, body mass index, blood test results, resting blood pressure, daily physical activity and diastolic blood pressure and HR during the exercise (r^2 = 0.634, P < 0.001). In subgroup analyses according to average SBP during the exercises, aortic PWV was higher in the high SBP group than the low SBP group independent of resting SBP (P < 0.001). We concluded that SBP during resistance exercise is independently correlated with arterial stiffness. The following steps are to elucidate whether excessive blood pressure elevation during resistance exercise increases arterials stiffness by prospective study and to investigate its relationship to ET-1 system.

P-112

A Study of Endothelins and Endothelin Receptors in Rheumatic Mitral Valves

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Introduction: Rheumatic Fever represents a serious public health problem in Brazil, with thousands of new cases each year. It is an inflammatory and autoimmune disease, which occurs in response to infection by streptococcus A group. The aim of this study was to evaluate the immunolabeling for ET-1,ET-A and ET-B receptors in rheumatic mitral valves. Methods: This study focused in quantitative immunoreactivity of ten mitral valves which were collected at a hospital in Aracaju, SE, Brazil. The quantitative analysis of the immunocytochemistry area of each receptor in relation to the total area of each slide was performed by ImageJ software. Statistical analysis was performed using measures of central tendency and standard deviation. In inferential analysis, we used the Pearson partial correlation (R), with significance level of < 0.05.Results: In 10 samples, immunohistochemical expression for ET-1 and for its receptors was observed in eight and seven samples, respectively. In quantitative analysis, it was observed that the average area with expression of ET-1 was 18.21±14.96%. For ETrA and ETrB, the mean expressed areas were respectively 15.06±13.13% and 9.20±11.09%. The correlation between the expression of both endothelin receptors were strongly positive (R: 0.74, p: 0.02), but the correlation between ET-1 and its receptors were negative for both ETrA (R: -0.37, p: 0.25), and ETrB (R: -0.14, p: 0.39).Conclusion: The strong positive correlation between endothelin receptors (ETrA more demonstrated than ETrB) suggests that both have a role in the pathophysiology of rheumatic mitral stenosis.

P-113

Complement C5a Antagonism is Associated with Reduced Big-Endothelin Level after Experimental Cardiac Tamponade

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Objective: Cardiac tamponade is a severe clinical syndrome most often caused by high-energy thoracic injuries. Following tamponade, the release of vasoconstrictive mediators contributes to circulatory redistribution, leading to peripheral macro- and microcirculatory complications including gastrointestinal hypoperfusion. As a consequence of hypoxia the complement system is activated and anaphylatoxin C5a may be produced. Our aim was to investigate the modulator effects of complement C5a antagonist (C5aA) treatment on the endothelin system and the accompanying circulatory and inflammatory changes in a large animal model of experimental cardiac tamponade. **Methods:** In anaesthetized, ventilated and thoracotomized minipigs (n=7) tamponade was induced for 60 min by intrapericardial fluid administration, meanwhile the mean arterial pressure (MAP) was reduced to 40-45mmHg. Group 2 was treated with C5aA (AcPepA, Nagoya, Japan) at the 45th min of tamponade (4mg/kg iv; n=6), while group 3 (n=6) served as sham-operated control. Macrohemodynamics were monitored for 240 min, whole blood superoxide production, plasma HMGB-1 and big-endothelin (big-ET) levels, small intestinal myeloperoxidase (MPO) activity were measured. Average red blood cell velocity (a-RBCV) in the small intestinal mucosa was determined by intravital orthogonal polarization imaging (OPS) technique. **Results:** After tamponade plasma levels of big-ET were increased together with superoxide production, HMGB-1 levels and MPO activities. The C5aA treatment normalized the macrohemodynamics, and besides the a-RBCV was increased, SOX, HMGB-1, MPO and big-ET levels were reduced. **Conclusion:** These results demonstrate the possible connections between the activation of complement- and endothelin systems, and the potential for C5aA to decrease the potentially harmful inflammatory consequences of experimental cardiogenic shock. Grant supports: OTKA-K104656; TAMOP-4.2.2A-11/1/KONV-2012-0035; TAMOP-4.2.2.A-11/1/KONV-2012-0073; TET-JP-16/09

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Endothelin A Receptor Blockade and Long Term Outcome in Patients with ST Elevation Acute Coronary Syndrome

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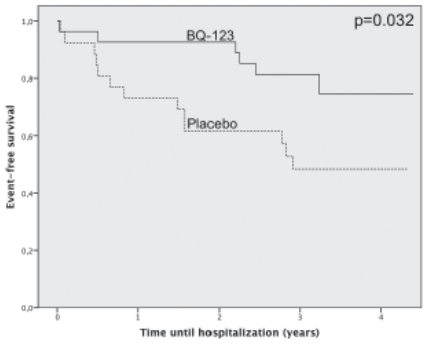
Background: ST-Elevation Acute Coronary Syndrome (STE-ACS) is characterized by thrombotic coronary occlusion compromising blood flow at the epicardial and microvascular levels. Coronary thrombi are a source of large amounts of Endothelin-1 (ET-1), a pro-fibrotic vasoconstrictor and a mediator of microvascular dysfunction and cardiac remodeling. Methods: Patients with posterior-wall STE-ACS were randomly assigned to intravenous BQ-123 or placebo as described elsewhere (n=54). During a three-year follow-up period, patients were followed and kept on optimal medical treatment by an investigator who was blinded to the acute treatment allocation. Results: During the median follow-up period of 3.3 years (IQR 2.9-3.7), no deaths occurred. The reasons for rehospitalisation (n=19) were unplanned coronary revascularization (n=10, 52%), worsening angina (n=3, 17%), hypertensive urgency (n=2, 11%), as well as stroke (n=1), dyspnoea (n=1), ventricular tachycardia (n=1) and cerebrovascular disease (n=1). We observed a longer event-free survival in patients randomized to receive BQ-123 compared with patients randomized to placebo (3.8 years (95% CI: 3.3-4.2) for BQ-123 versus 2.8 years (2.1-3.4) for placebo, p=0.032, Figure 1). Conclusion: Short-term administration of BQ-123 in patients undergoing primary PCI for STE-ACS leads to a longer cardiovascular event-free survival.



PC-21

Clinical Features between Heart Failure and Sleep Disordered Breathing

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Introduction: Little has been known about clinical background of the patients with heart failure (HF) and sleep disordered breathing (SDB). The aim of this study was to elucidate the relationship between HF and SDB.

Methods: 1121 patients who admitted to our institute with the diagnosis of HF between 2006 and 2012 was enrolled. SDB was defined >5/hour of apnea-hypopnea index (AHI).Obstructive sleep apnea (OSA) group and central sleep apnea (CSA) group were defined based on the data of type III sleep monitor (Morpheus).

Results: Among 1121 patients 328 (29%) was underwent screening of type III sleep monitor. In the 328 patients, 275 (84%) patients showed SDB. Among these 275 SDB patients, 135 (41%) were OSA, and 140 (43%) were CSA. AHI was significantly higher (OSA: 22.5±16.2, CSA: 29.8±14.9, P<0.05) and ejection fraction (EF) was significantly lower (OSA: 40.1±17.1 %, CSA: 33.5±14.1 %, P<0.05) in CSA group between two groups. Among 140 CSA patients, 80 (57%) were patients with heart failure with reduced ejection fraction (HFREF) and among 135 OSA patients, 60 (44%) were patients with HFREF.

Conclusions: SDB was highly associated with HF and the clinical features between OSA and CSA with HF were different. CSA patients were associated with lower EF and higher AHI then OSA patients. This study suggested that SDB was one of an important target of treatment HF and to treat HF according to these clinical subsets of SDB was clinically required in the future.

P-115

Selective Deletion of Endothelin B Receptors from Vascular Smooth Muscle Does Not Inhibit Neointimal Lesion Formation

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Pharmacological inhibition and genetic deletion (Murakoshi et al., 2002; Kirkby et al., 2012) suggest that endothelin (ET) A-selective antagonists are preferable to mixed ETA/B antagonists for prevention of neointimal lesion formation. ETB receptors expressed in smooth muscle cells may, however, contribute to lesion development. It was proposed that ETB deletion from smooth muscle (SM) would reduce lesion formation following arterial injury. Methods: Mice bearing a floxed ETB gene or expressing cre-recombinase under the SM22 promoter were crossed to produce SM-selective ETB deletion. SMETB knockout mice were identified by genotyping and backcrossed to C57Bl/6J (4-6 generations). Functional confirmation of ET deletion was determined by exposing trachea, and mesenteric artery and vein, to sarafotoxin 6c in a myograph. Femoral injury was performed in adult, male SMETB knockout mice and littermate controls and arteries were harvested 33 days later for structural analysis. Results: SMETB knockout reduced (~55%), but did not abolish, ETB-mediated contraction in trachea. In contrast, S6c-mediated contraction in mesenteric veins (130+46% KPSS, n=4), and in mesenteric arteries cultured for 24h (72+24% KPSS, n=4), was abolished by SMETB deletion (5.1+3.4% KPSS and 0% KPSS, respectively). Femoral artery injury produced large, neointimal lesions (47.4+10.6%; n=7) but SMETB knockout did not alter lesion size (42.2+4.5%; n=9; P=0.64). Conclusions: Stimulation of ETB receptors in SM does not influence neointimal lesion formation. This supports the suggestion that ETA-selective antagonists are preferable to non-selective antagonists for prevention of neointimal proliferation. Funded by the BHF (project grant and CoRE).Murakoshi et al., (2002) Circulation 106:15Kirkby et al., (2012) Cardiovasc Res, 95, 19

P-116

Neointimal Lesion Formation Does Not Induce Endothelin (ET) B-Mediated Contraction in Murine Femoral Arteries

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Incubation of arteries ex vivo induces ETB-mediated contraction (Adner et al., 1998), possibly via transcriptional mechanisms (Skovsted et al., 2012). ETB receptors are also expressed in neointimal lesions (Azuma et al., 1994). It was proposed that ETB-mediated contraction would be induced by neointimal lesion formation. Methods: Femoral arteries from adult, male C57Bl/6J mice (n=6) were harvested 36+/-2 days after ligation. Isolated mesenteric and femoral veins and arteries from uninjured mice were cultured (DMEM; 37oC; 5% CO2; 5 days) before analysis in a myograph. Contractile function was assessed using phenylephrine (10-9-3x10-5M), endothelin-1 (10-11-10-7M) and sarafotoxin 6c (10-11-10-7M). Relaxant function was assessed using endothelium-dependent (acetylcholine; 10-9-3x10-5M) and independent (sodium nitroprusside; 10-9-3x10-5M) agents after contraction with phenylephrine. Results: Freshly isolated mesenteric veins contracted in response to S6c whereas mesenteric arteries and femoral veins did not. Some (4/ 10) femoral arteries produced small S6c-induced contractions (21.86+/-3.72 % KPSS, n=4). Incubation induced ETB-mediated contraction in mesenteric, but not in femoral, arteries. Arterial ligation had little effect on contractile or relaxant function of murine femoral arteries and did not induce a contractile response to S6c. Conclusions: Neointimal lesion formation did not induce S6c-mediated contraction in mouse femoral arteries, possibly because ETB receptor activity cannot be induced in this artery. These data do not support the need for mixed ETA/ETB antagonists for inhibition of neointimal lesion formation. Supported by the BHF (Project grant and CoRE).Adner et al., (1998) Acta Physiol Scand, 163, 121Azuma et al., (1994) Am J Physiol, 267, H2259Skovsted et al., (2012) Life Sci, 91, 593

P-117

Smooth Muscle Specific Disruption of the Endothelin A Receptor in Mice Reduces Arterial Pressure and Affects Vascular Development

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The role of vascular smooth muscle endothelin A receptors (ETA) in development and normal physiology remains incompletely understood. To address this, mice were generated with smooth muscle-specific knockout (KO) of ETA. Mice were homozygous for loxP-flanked exons 6-8 of the ETA gene (floxed) or were also hemizygous for a transgene expressing Cre recombinase under control of the smooth muscle-specific SM22 promoter (KO mice). Genotyping at 17 days postnatal yielded a 5:1 ratio of floxed: KO mice. Smooth muscle actin staining of embryos at day E9.5 revealed increased tortuosity in dorsal aortae. Mice surviving to weaning developed and bred normally. ETA KO mice aged 2-3 months manifested EDNRA gene recombination in all organs tested. Aortas from KO mice had a >90% reduction in ETA mRNA content, but no differences between genotypes in ET-1 or ETB mRNA levels. Addition of 0.01-100 nM ET-1 to isolated femoral arteries from floxed, but not KO, mice dose-dependently decreased vessel diameter (up to 80% reduction in the presence of ETB blockade). Intravenous infusion of ET-1 into floxed, but not KO, mice acutely increased mean arterial pressure (MAP) (by ~10mmHg). Telemetric analysis revealed decreased MAP in KO mice (by ~7-10mmHg); this MAP reduction was evident on normal and high salt diets. In conclusion, ETA is important for vascular development and is involved in the maintenance of arterial pressure under physiological conditions.

P-118

Plasma Endothelin-1 Level is a Predictor of 10-Year Mortality in a General Population the Tanushimaru Study

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BACKGROUND: Endothelin-1 (ET-1) is a potent vasoconstrictor and an elevated plasma level is a prognostic marker in patients with cardiovascular diseases and/or malignancies. We hypothesized that an elevated plasma level might be a prognostic marker even in subjects without apparent cardiovascular disease or malignancy at baseline. METHODS AND RESULTS: We measured plasma ET-1 levels in 1,440 healthy subjects over 40 years of age (580 men, 860 women) who were periodically followed for 10 years. The follow-up rate was 96.8%. Baseline plasma ET-1 levels were categorized into quartiles. Baseline plasma ET-1 levels were significantly associated with age, blood pressure, high-density lipoprotein-cholesterol, renal function, uric acid and all-cause death, but not with cardiovascular or cancer death. Kaplan-Meier curves demonstrated that all-cause mortality was significantly higher in the highest quartile of ET-1 than in the lowest quartile. Cox proportional hazards regression analysis demonstrated that ET-1 was an independent predictor of all-cause death [hazard ratio: 1.11, 95% confidence interval (CI) 1.01-1.23 per 1 pg/ml difference]. The hazard ratio of all-cause death in the highest quartile of plasma ET-1 (>5.9 pg/ml) vs. the lowest quartile after adjusting for confounding factors was 1.54 (95% CI 1.09-2.20). CONCLUSIONS: The plasma ET-1 level may be a predictor of all-cause death in a healthy population.

P-119

Potential Association Between Circulatory Level of Endothelin-1 and Metabolic Syndrome in Bangladeshi Rural Women: A Population-Based Cross-Sectional Study

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BACKGROUND: Metabolic alterations and endothelial dysfunction are interrelated processes in type 2 diabetes and metabolic syndrome (MetS) that often develop in parallel. In this study we assessed the association of vasoactive peptide, endothelin-1 (ET-1) with MetS conducted in a study in rural Bangladeshi women. DESIGN AND METHODS: Plasma level of ET-1 was measured by ELIZA and MetS was defined according to the criteria of NCEP-ATP III. Logistic regression was used to examine the association between circulatory ET-1 level and MetS and its components. RESULTS: A total of 1485 rural Bangladeshi women aged >15 years were studied using a population based cross-sectional survey. The prevalence rate of MetS was 25.05% (NCEP ATP III). Mean values of BMI, waist circumference, blood pressure (SBP, DBP), plasma level of fasting glucose, triglyceride, HDL, cholesterol, insulin and vascular endothelial growth factor were significantly higher in MetS group compared to non-MetS group. ET-1 levels were significantly increased in MetS subjects (MetS vs. non-MetS: 4.32±0.24 vs. 3.41±0.18, p=0.003). In multivariable analyses, we found that ET-1 had significant positive associations with DBP (beta =0.051, p=0.001) and SBP (beta =0.028, p<0.001) even after adjusting for age. We also found that mean plasma levels of ET-1 increased in direct proportion to levels of MetS components. CONCLUSIONS: We here demonstrate for the first time that in Bangladeshi rural women, plasma level of ET-1 is related to MetS and its components, suggesting a possible role of ET-1 as a surrogate biomarker for the disease and its complications. This is the first study assessing ET-1 in Mets subjects from a South Asian country.

P-120

Dual Endothelin Antagonism from Early Diabetic Stage is Effective in Preventing Various Diabetic Complications Through Both Improving Organ Microcirculation and Restoration of Altered VEGF Signaling

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Purpose: Diabetes Mellitus causes both macrovascular and microvascular complications. Several treatments effectively prevent macrovascular complications such as stroke and AMI. Because current treatments are insufficient to prevent diabetic microvascular complications such as diabetic retinopathy and nephropathy, erectile dysfunction and cardiomyopathy, we aimed to study a possibility of endothelin antagonism on these microvascular complications. Vascular endothelial growth factor (VEGF) was reported to cause a central trigger leading to the structural and functional changes in diabetic complications, we also assessed restoration of VEGF signaling by endothelin antagonism. Since both ET-A and ET-B receptors in vascular smooth muscles are involved in the microvascular contraction/proliferation, we applied the ET-A/B dual antagonist SB209670 to rats. Methods: Male Sprague-Dawley rats were administered citrate saline (vehicle) or streptozotocxin (65 mg/kg IP). One week after injection, rats were separated into those receiving SB209670 or vehicle by osmotic mini pump for 2 weeks. Results: We found reduction of VEGF signaling with capillary density in diabetic heart and penile tissues, whereas found overexpression of VEGF signaling in diabetic retina and kidney. SB209670 administration was effective in preventing development and progression of various diabetic complications through modification of respective microcirculation and restoration of VEGF signaling; SB209670 prevents diabetic cardiomyopathy through restoration of VEGF reduction, prevents erectile dysfunction through restoration of VEGF reduction, prevents the development of diabetic retinopathy and nephropathy through restoration of VEGF overexpression. Conclusion: Dual endothelin antagonism by SB209670 is effective in preventing various diabetic microvascular complications such diabetic retinopathy and nephropathy, erectile dysfunction and cardiomyopathy through both improving organ microcirculation and the restoration of altered VEGF signaling.

PC-22

Lifestyle Modification Induces Decreased Central Blood Pressure and Increased Serum Testosterone Concentration in Overweight and Obese Men

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Background: Increase in central blood pressure (cBP) is a risk factor for cardiovascular desiase. cBP is higher in the obese individuals than the age-matched normal-weight humans. We previously demonstrated that lifestyle modification decreases cBP in obese men. However, the mechanism underlying lifestyle modification-induced decrease in cBP has not been elucidated. Testosterone is a kind of androgens and known to have cardioprotective effect. The aim of the present study was to examine whether testosterone is involved in the mechanism underlying the decrease in cBP with lifestyle modification. Methods: Thirteen overweight and obese men (age: 50±2 yrs, BMI: 30±1kg/m2) completed a 12-week lifestyle modification program (well-balanced 1680 kcal/day diet and brisk walking for 40-60 min/day, 3 days/week). Before and after the program, we measured cBP and serum testosterone level in all participants. Results: After the program, a significant weight loss was observed (-12.7±1.3kg, $P< 0.05$). cBP significantly decreased and serum testosterone level significantly increased after the program (both $P< 0.05$). Moreover, there was a significant relationship between the change in cBP and that in serum testosterone level ($r = -0.63$, $P< 0.05$). Conclusions: After the 12-week lifestyle modification, cBP decreased and serum testosterone level increased in the overweight and obese men. We also demonstrated that there was a negative relationship between the change in cBP and that in serum testosterone level. These findings suggest that increased serum testosterone level may partly contribute to decrease in central blood pressure in overweight and obese men.

PC-23

Characterization of the Binding of [^{125}I]GLP-1(9-36) Amide, the Major Metabolite of the Insulin Secretagogue, Glucagon-like Peptide 1 (GLP-1) and Function of the Unlabelled Peptide in Murine Aorta

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Glucagon-like peptide 1 (GLP-1) is an insulin secretagogue synthesized in the intestine and released in response to meal ingestion and efficiently lowers blood glucose in type 2 diabetic patients. GLP-1(7-36) has a very short half-life and is rapidly metabolized by dipeptidyl peptidase IV to the major metabolite GLP-1(9-36)-amide, often though to be inactive. Inhibitors of this enzyme are widely used to treat diabetes. However, it is unclear whether GLP-1(9-36) mediates functional activity via specific receptors. Following pre-incubation of fresh frozen section of cryostat sections (Bregma~-2.5) from adult mouse brains, binding assays were carried out using increasing concentration of either [^{125}I]GLP-1(7-36) or [^{125}I]GLP-1(9-36) for 90 min at room temperature. Non-specific binding was defined by 10 µM of the GLP-1(7-36) and GLP-1(9-36). In functional experiments, mouse aortae were mounted in wire myographs and the effect of increasing concentrations of each peptide measured.

Ligand binding (mouse brain, n=4)	K_D (nM)	B_{MAX} (fmol/mg)	nH
[^{125}I]GLP-1(7-36)	1.29 ± 0.26	57.0 ± 14.5	0.96 ± 0.06
[^{125}I]GLP-1(9-36)	0.214 ± 0.08	2.69 ± 0.74	1.06 ± 0.05

Vasoconstrictor Assay (mouse aorta, n=3)	pD_2 (nM)	E_{MAX} %KCl maximum	
GLP-1(7-36)	7.69±0.24	35±8%	-
GLP-1(9-36)	7.57±0.64	25±7%	-

In the mouse brain both labelled peptides bound with a single high sub-nanomolar affinity, with Hill slopes close to unity. The density of receptors was an order of magnitude lower for [^{125}I]GLP-1(9-36). In functional experiments both peptides had similar potencies. These results suggest GLP-1(9-36) has functional activity at the GLP1 receptor.

P-121

Dual Endothelin Receptor Antagonism with Bosentan Reverses Established Vascular Remodeling in Diabetic Rats: Relevance to Glycemic Control

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Aim: We have shown that diabetes alters structural and functional properties of the cerebrovasculature in part by the activation of the endothelin (ET) system in a glucose-dependent manner. Here, we tested the hypothesis that established diabetes-induced vascular dysfunction and remodeling could be reversed by glycemic control or dual ET-1receptor antagonism.Methods: Studies were performed in non-obese type-2 diabetic Goto-Kakizaki (GK) rats. GK treated with vehicle, metformin (300mg/kg/day) or dual ET-receptor antagonist Bosentan (100mg/kg/day) after onset of remodeling from 18 to 22-week by oral gavage. Additional groups included vehicle-treated 10 or 18-week GK rats. Blood glucose and mean arterial blood pressure (MAP) were monitored weekly. At termination, middle cerebral artery (MCA) lumen diameter, media thickness (MT), media: lumen (M:L) ratio, cross-sectional area (CSA) and myogenic-tone were measured using pressurized arteriograph (n = 8-14/group). Results: GK MAP was 102, 105 and 119 for vehicle, metformin and bosentan, respectively. 18 and 22-week diabetic GK rats displayed increased M:L ratio and CSA, but decreased lumen diameter and myogenic tone compared to 10-week animals. Glycemic control with metformin significantly improved blood glucose and partially reversed vascular remodeling by decreasing the MT, M:L ratio and CSA. Myogenic tone was improved only at lower pressures. Bosentan improved the MT and M:L ratio and did not affect CSA. Bosentan showed a significant improvement in MCA myogenic-tone over the pressure range despite elevated MAP. Conclusions: Glycemic control or ET-1 antagonism can partially reverse diabetes-induced cerebrovascular remodeling and dysfunction. These results strongly suggest that either approach offers a therapeutic benefit and combination treatments need to be tested.

P-122

Bosentan Restores Impaired Cerebrovascular Relaxation in Diabetes

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AIMS: Up-regulation of the endothelin (ET) system in type-2 diabetes leads to increased contraction and decreased relaxation in basilar artery. We showed that 1) ET receptor antagonism prevents diabetes-mediated cerebrovascular dysfunction, and 2) Glycemic control prevents activation of the ET system in diabetes. The goal of the current study was to determine whether and to what extent glycemic control or ET-receptor antagonism reverses established cerebrovascular dysfunction in diabetes. METHODS: Non-obese type-2 diabetic Goto-Kakizaki (GK) rats were administered either vehicle, metformin (300mg/kg/day) or dual ET-receptor antagonist bosentan (100mg/kg) for 4 weeks starting at 18-weeks after established cerebrovascular dysfunction (n=6-8/group). Blood glucose and blood pressure were monitored weekly. At termination, basilar arteries were collected and cumulative dose-response curves to ET-1 (1-500 nM), 5-HT (1-1000 nM) and acetylcholine (Ach, 1 nM-5 μM) were studied by wire myograph. RESULTS: Only metformin decreased blood glucose. MAP was 102, 105 and 119 for vehicle, metformin and bosentan, respectively. The magnitude of basilar artery constriction in response to KCl was similar among groups. Interestingly, constriction to ET-1 and 5-HT (Area under curve, AUC) was greater in treated animals as compared to vehicle-treated GK rats; however, there was no difference in Rmax or EC50. Both bosentan and metformin improved sensitivity to Ach and only bosentan increased relaxation (Rmax and AUC) despite elevated blood pressure. CONCLUSION: These results suggest that augmented contractile response to vasoactive agents is not improved by glycemic control or ET-receptor antagonism but ET-receptor antagonism is effective in improving relaxation response even if started after established cerebrovascular dysfunction and offers therapeutic potential.

P-123

ETA Receptor Antagonists in the Treatment of Diabetic Ketoacidosis

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In patients with type I Diabetes Mellitus poor management casues a drastic rise in glucose levels resulting in diabetic ketoacidosis (DKA). About 1% of DKA episodes can be complicated by cerebral edema. ET and its receptors are involved in the regulation cerebral blood flow. We studied the effect of ETA receptor antagonists in a rat model of DKA. DKA was produced by streptozotocin (150 mg/kg, ip). Group 1: Control (non-diabetic) animals administered citrate. Animals that developed DKA were divided in five additional groups. Group II: DKA animals without treatment; Group III: DKA animals given saline; Group IV: DKA animals given saline and insulin 1.5u/kg/hr; Group V: DKA animals given saline, insulin 1.5 u/kg/hr and BMS-182874 (9mg/kg) and Group VI: DKA animals given saline, insulin 1.5 u/kg/hr and BQ123 (1mg/kg). Blood glucose and ketones markedly increased by day 4 in DKA rats. Saline/insulin treatment in DKA rats increased the plasma and brain ET-1 levels which were not affected by BMS-182874 or BQ123 treatment. There no change in the expression of ETB receptors in the brain, however, ETA receptor expression increased in DKA rats and was not altered following treatment with insulin, BMS-182874 or BQ123. Animals in insulin/saline group showed a significant increase (160%) in cerebral blood perfusion compared to base line. This increase in cerebral perfusion was attenuated by BQ123 or BMS-182874. Treatment with BQ123 also improved blood pH and ketones in DKA rats. It can be concluded that ETA receptor antagonists maybe of therapeutic use in the management of DKA and its complications.

P-124

Endothelin Antagonism and Diabetic Erectile Dysfunction: Changes in VEGF and NO in Type I Diabetic Penis and Effects of Endothelin Antagonism

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About 50% of male diabetic patients causes erectile dysfunction (ED), which is considered as the vascular and neuropathic complications. Vascular endothelial growth factor (VEGF) has been extensively documented for its pathogenic significance in different complications of diabetes and we reported that VEGF signaling is greatly diminished in penis in a rat model of type II diabetes. The present study used 3 weeks duration of streptozotocin (STZ)-induced diabetic (DM) rat model to assess the VEGF expression with NO system in penile tissue and concomitantly the effects of endothelin antagonism has been studied on these changes. Male Sprague-Dawley rats were administered citrate saline vehicle or STZ (65mg/kg IP). One week after the injection, animals were separated into those receiving endothelin-A/B (ET-A/B) dual receptor antagonist (SB209670, 1mg/kg/day), endothelin-A (ET-A) receptor antagonist (TA-0201, 1mg/kg/day) or saline for 2 weeks by osmotic mini pump. The local ET-1 level in DM penis was higher by 20% than non-MD rats. A 30% decrease in VEGF expression in penile tissue was seen in DM rats. Penile NO and eNOS level was decreased in DM rats; greatly restored by ET-A receptor antagonist while unchanged by ET-A/B dual antagonist. iNOS was not significantly changed in penile tissues among non-DM, DM and ET-A antagonist treated groups. Thus, we conclude that (1) VEGF and pAkt were downregulated in type 1 DM penis, and that (2) the ET-A antagonist was potentially effective in reversing the decreased NO and eNOS levels in DM penis than the those by ET-A/B dual antagonist.

PC-24

An Endogenous Blocker of Oxidized LDL

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Aim: To elucidate the pathophysiological significance of the endogenous oxidized LDL (oxLDL) blocker, which was originally described as an endothelium-derived secreted protein Del-1 (developmental endothelial locus-1).
Background: oxLDL can potentiate the induction of foam cell-formation and inflammatory responses, the processes which are believed to be integral to atherogenesis. However, no endogenous proteins which interfere with oxLDL binding to its receptors have been identified.
Methods: Interaction between oxLDL and Del-1 was examined in cell-free system by ELISA. Inhibition of binding and action of oxLDL was examined with CHO cells expressing LOX-1 and with COS-7 cells transfected with scavenger receptors. Cultured human umbilical vein endothelial cells (HUVEC) and THP-1 were also used to analyze the inhibitory effects of Del-1 on oxLDL action.
Results: We found that Del-1 selectively bound to oxLDL, but not to native LDL. Del-1 inhibited the uptake of DiI-labeled oxLDL (DiI-oxLDL) by LOX-1 expressed in COS-7, but did not inhibit DiI-labeled native LDL uptake by LDL receptor. Del-1 inhibited DiI-oxLDL binding to other oxLDL receptors as well, such as SR-A and CD36. In addition, Del-1 inhibited DiI-oxLDL uptake by HUVEC and THP-1-derived macrophages. Site-directed mutagenesis revealed that two arginine residues in Del-1 were crucial for the binding of oxLDL. Furthermore, Del-1 suppressed oxLDL-induced signal transduction in LOX-1-expressing CHO cells. In HUVEC, Del-1 also suppressed oxLDL-induced signaling and endothelin-1 secretion. Thus, we demonstrated that Del-1 is an endogenous protein which protects cells from oxLDL actions.
Conclusion: We identified, for the first time, an endogenous oxLDL blocker which may play a regulatory role in interfering progression of atherosclerosis.

P-125

Selective Endothelin ETA and Dual ETA/ETB Receptor Blockade Improves Endothelial Function in Patients with Type 2 Diabetes and Coronary Artery Disease

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Purpose: Endothelin-1 contributes to endothelial dysfunction in patients with atherosclerosis and type 2 diabetes. In healthy arteries the ET_A receptor mediates the main part of the vasoconstriction induced by endothelin-1 while the ET_B receptor mediates vasodilatation. The ET_B receptor expression is upregulated in atherosclerosis and may thereby contribute to the vasoconstrictor tone and development of endothelial dysfunction. The aim of the present study was to compare the effects of selective ET_A and dual ET_AA/ET_B blockade on endothelial function in patients with type 2 diabetes and coronary artery disease. Methods: Twelve patients were included in this cross-over study with blinded evaluation. Forearm blood endothelium-dependent and endothelium-independent vasodilatation was assessed by venous occlusion plethysmography during intra-arterial infusions of serotonin and nitroprusside, respectively, before and after 60 minutes of intra-arterial infusion of either the selective ET_A antagonist BQ123 or the combination of BQ123 and the ET_B antagonist BQ788. Changes between the two treatments were compared using 2-way analysis of variance.Results: Dual ET_A/ET_B receptor blockade increased baseline forearm blood flow by 30±14% ($P<0.01$) whereas selective ET_A blockade did not (14±8%). Both selective ET_A blockade and dual ET_A/ET_B blockade induced a 2-fold increase in endothelium-dependent vasodilatation ($P<0.001$). The improvement in endothelium-dependent vasodilatation did not differ between the two treatment strategies. Both treatments improved the endothelium-independent vasodilatation. Conclusions: Both selective ET_A and dual ET_A/ET_B improve endothelial function in patients with type 2 diabetes and coronary artery disease. Addition of ET_B to ET_A receptor blockade increases basal blood flow but does not additionally improve endothelial function.

PC-25

2 Years Follow-Up in Oxidative Stress Levels in Patients with Acute Coronary Syndrome: Insights from the Assessment of Lipophilic vs. Hydrophilic Statin Therapy in Acute Myocardial Infarction (ALPS-AMI) Study

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Background: Statins reduce the incidence of cardiovascular events in patients with acute myocardial infarction (AMI). Although all statins are equally effective in secondary prevention, there might be certain differences in the effects of lipophilic and hydrophilic statins and its association with oxidative stress levels in AMI patients remain unclear. Therefore, we investigated oxidative stress levels in AMI patients with lipophilic atorvastatin or hydrophilic pravastatin. Methods and results: The study population included a prospective, randomized, open-label, study in AMI patients within ALPS-AMI study. Patients that have undergone successful percutaneous coronary intervention will be randomly allocated to receive either atorvastatin or pravastatin with the treatment goal of lowering their low-density lipoprotein-cholesterol level below 100 mg/dl for 2 years. Diacron-reactive oxygen metabolite (dROM) and biological antioxidant potential (BAP) levels were measured in AMI patients with lipophilic (atorvastatin, n = 38) and hydrophilic (pravastatin, n = 36) statin therapy on admission (dROM: 395.5±37.5 vs. 392.1±35.6 Carratelli units (U. Carr) (p = 0.37), BAP: 2756.3±75.2 vs. 2849.1±71.6 mmol/l (p = 0.39), respectively) and 6, 12, 24 months (24 months: dROM: 374.5±41.5 vs. 381.1±37.6 U. Carr (p = 0.28), BAP: 2957.3±135.9 vs. 2941.5±121.3 mmol/l (p = 0.48), respectively). Conclusion: This is the first clinical trial to compare the effects of lipophilic and hydrophilic statin therapy on oxidative stress levels and no difference was noted in oxidative stress levels between lipophilic and hydrophilic statin therapy in patients with AMI during the follow-up period.

P-126

Endothelin-Dependent Vasoconstrictor Activity in Metabolically Healthy and Unhealthy Obese Patients

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Obesity is associated with higher risk of premature death due to metabolic and cardiovascular abnormalities. One third of obese individuals, however, has a "metabolically healthy" phenotype and it is debated whether this status carries lower cardiovascular risk than its metabolically unhealthy counterpart. Given the role of the endothelin (ET)-1 system in the development of obesity-related vascular dysfunction, we investigated whether differences exist in ET-1-dependent vascoconstrictor activity between the divergent obesity subphenotypes. To this end, we compared vasodilator responses (strain-gauge plethysmography) to intra-arterial infusion of the selective ETA receptor blocker BQ-123 (10 nmol/min for 60 min) in healthy subjects (n=31) and obese patients (n=38); obese patients were divided in two subgroups according to the absence (n=11) or presence (n=27) of any of the glucose or lipid abnormalities characteristic of the metabolic syndrome (MetS; ATP III criteria). The vasodilator response to BQ-123 was greater in obese than in lean subjects (P<0.001), whereas no difference was observed in the response to BQ-123 between metabolically healthy and unhealthy obese patients (P>0.05). Similarly, BQ-123-induced vasodilation was related to the number of MetS component in the whole population (P=0.008), but not within the obese group (P>0.05). In the whole population, body mass index and mean arterial pressure were significant determinants of the response to BQ-123 (r=0.46 and r=0.42, respectively; both P<0.001), whereas no correlation was observed between BQ-123-induced vasodilation and either plasma glucose, triglycerides or HDL-cholesterol levels (all P>0.05). In conclusion, ET-1-dependent vasoconstrictor tone is higher in obese patients than in their lean counterpart, but this defect is not influenced by the presence obesity-related metabolic abnormalities.

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Epigallocatechin Gallate Attenuates ET-1-Induced Contraction in Carotid and Thoracic Aorta from Type 2 Diabetic OLETF Rat

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There is a growing body of evidence suggested that epigallocatechin gallate (EGCG), a major catechin isolated from green tea, has several beneficial effects such as anti-oxidant and anti-inflammatory activities. However, whether treatment with EGCG could suppress the ET-1-induced contraction in large arteries from type 2 diabetic rats is unknown. We hypothesized that long-term treatment with EGCG would attenuate the ET-1-induced contractions in type 2 diabetic arteries. To test this hypothesis, Otsuka Long-Evans Tokushima fatty (OLETF) rats (43 weeks old) were treated with EGCG (200 mg/kg/day for 2 months, p.o.) and contractile/relaxant responses to ET-1, phenylephrine (PE), acetylcholine (ACh) and sodium nitroprusside (SNP) in the presence and absence of endothelium were measured in common carotid artery (CA) and thoracic aorta (TA) from EGCG-treated and -untreated OLETF rats and control Long-Evans Tokushima Otsuka (LETO) rats. In OLETF rats, EGCG attenuated sensitivity to ET-1 in CA [pD_2, EGCG-treated: 7.95±0.07 vs. -untreated 8.15±0.06 ($p<0.05$)] and TA [pD_2, EGCG-treated: 7.98±0.08 vs. -untreated 8.24±0.06 ($p<0.05$)] compared to untreated groups. However, EGCG did not alter PE-induced contractions in both arteries from OLETF rats. In the endothelium-denuded arteries, EGCG did not affect ET-1- and PE-induced contractions in both OLETF and LETO groups. ACh-induced relaxations were increased by EGCG treatment in CA and TA from OLETF group. SNP-induced relaxations were similar between EGCG-treated and -untreated groups. Our data suggest that within the timescale investigated here, EGCG attenuates ET-1-induced contractions in large arteries from type 2 diabetic rats and one of the mechanisms may be attributable to normalizing endothelial function.

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Association between Endothelin-A Receptor Gene Polymorphisms in Locus rs10305936 and Primary Nephrotic Syndrome in Children

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Background and objective: previous studies have described an association between kidney diseases and endothelin. Primary nephrotic syndrome (NS) is the common renal disease in children. The aim of this study was to determine whether polymorphisms in the endothelin-A receptor gene might be associated with the morbidity and the steroid response of primary nephrotic syndrome in children. METHODS: 53 children with primary nephrotic syndrome were as case group, the case group were subdivided into steroid resistance NS group and non- steroid resistance NS group; hypertention group and non-hypertention group. 50 healthy children were as control group, All subjects were genotyped for endothelin-A receptor polymorphisms in locus rs10305936 (in exon 8 of the EDNRA) by using the polymerase chain reaction and direct gene sequence test technique. RESULTS: (1) There is an adenine insertion in locus rs10305936. The frequencies of A homozygote, A heterozygote and no insertion genotype on case group and control group were 7.5%, 81.1%, 11.3% and 0%, 98.0%, 2.0% respectively, the difference between these two groups were statistical significance (&chi2=7.88, P=0.019). (2) There were no statistically significant difference in genotype within steroid resistant NS group and non- steroid resistant NS group, hypertention group and non-hypertention group (P>0.05). CONCLUSIONS: There is an adenine insertion in locus rs10305936. The endothelin-A receptor gene polymorphisms in locus rs10305936 are related to the morbidity of nephrotic syndrome in children.

Session 12: Metabolism, Diabetes and Obesity

Keynote Lecture 6

Role of Endothelin in Vascular Dysfunction in Human Obesity and Diabetes

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Obesity and related disorders, including hypertension and type 2 diabetes, are associated with heightened risk of cardiovascular disease. Endothelin (ET)-1, the most potent vasoconstrictor peptide, also possesses important properties to stimulate the development and progression of the atherosclerotic process. It is therefore conceivable that increased ET-1 activity might participate in the derangement of adiposity-related vascular homeostasis. This concept is supported by the results of studies using receptor antagonists to show that the activity of endogenous ET-1 is indeed enhanced in overweight and obesity, as well as in type 2 diabetes. Also, increased ET-1 contributes to endothelial dysfunction related to obesity and type 2 diabetes, whereas decreasing ET-1 vasoconstrictor tone corrects the defect of endothelium-dependent vasodilation in these patients. Furthermore, ET-1-dependent forearm vasoconstriction is increased in overweight and obese, but not in lean hypertensive patients. In addition, in patients with central adiposity and the metabolic syndrome, enhanced intravascular ET-1 activity coexists with decreased nitric oxide (NO)-dependent vasodilator capacity, suggesting a prevalence of vasoconstrictor mediators in obese vessels. One of the mechanisms evoked to explain the development of vascular abnormalities in obesity deals with the physiological endothelial effects of insulin and their derangement in insulin-resistant states. Thus, in addition to NO-dependent vasodilator properties, insulin also stimulates ET-1 production. This action has been demonstrated by use of antagonists of ET-1 receptors in the forearm circulation of healthy subjects, where ET-1-dependent vasoconstriction is increased following local infusion of insulin and accompanies a concurrent rise in NO-mediated vasodilation. In the healthy state, therefore, there is a balance between insulin-stimulated release of ET-1 and NO, with a resulting neutral hemodynamic response to the hormone. In conditions of caloric excess and adiposity, by contrast, insulin resistance implies defective insulin-mediated vasodilation, leading in turn to impaired ability of the hormone to enhance its delivery and that of substrates to peripheral tissues. An important role of ET-1 in this abnormality is supported by studies showing that upregulation of the ET-1 system impairs NO-mediated vasodilation in the vessels of insulin-resistant patients with obesity or type 2 diabetes, whereas NO bioactivity is restored following blockade of ET-1 receptors. This notion is further strengthened by the observation that ET-1 receptor antagonism improves insulin sensitivity in obese patients with insulin resistance.
In conclusion, considerable evidence supports a mechanistic role of ET-1 in the pathophysiology of adiposity-related vascular dysfunction. Given the link between higher ET-1 activity and obesity, targeting the ET-1 system has hence the potential for effective cardiovascular prevention in this condition.

O-26

Molecular Mechanism for Suppression of Insulin Signaling by Endothelin-1 in Skeletal Muscle Cells

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Background: Endothelin-1 (ET-1) attenuates insulin-stimulated glucose uptake in human skeletal muscle, leading to the development of insulin resistance. However, the molecular mechanism underlying negative regulation of insulin receptor signaling by ET-1 remains unclear. The purpose of this study was to determine the inhibitory effects of ET-1 on insulin-induced Akt phosphorylation in rat skeletal muscle (L6) cells.
Methods: Western blot experiments were used to analyze changes in the phosphorylation levels of Akt at threonine 308 (Thr^{308}) and serine 473 (Ser^{473}). mRNA expression for two ET receptors (ET_AR and ET_BR) and the C-terminus region of G protein-coupled receptor kinase 2 (GRK2-ct) was detected by reverse transcription polymerase chain reaction. GRK2-ct was overexpressed in L6 cells using adenovirus-mediated gene transfer.
Results: mRNAs for ET_AR and ET_BR were detected on L6 cells as well as on human skeletal muscle. Insulin induced sustained Akt phosphorylation at Thr^{308} and Ser^{473}, which was completely abolished by a phosphatidylinositol 3-kinase (PI3K) inhibitor, LY294002. The insulin-induced phosphorylation of Akt was suppressed by addition of ET-1 after insulin stimulation. The inhibitory effect of ET-1 was counteracted by a $G_{\alpha q/11}$ protein inhibitor, YM-254890, and by a selective ET_AR antagonist, BQ-123. Overexpression of GRK2-ct to interfere with the function of endogenously expressed GRK2 cancelled the ET-1-induced suppression of insulin-stimulated Akt phosphorylation.
Conclusions: ET_AR activation with ET-1 suppresses insulin-induced, PI3K-medaited phosphorylation of Akt at Thr^{308} and Ser^{473} in a GRK2-dependent manner in skeletal muscle. These results indicate that both ET_AR and GRK2 are therapeutic targets for insulin resistance and type 2 diabetes.

O-27

Plasma Endothelin-1 Level is Associated with Cardiac Fibrosis and Diastolic Dysfunction in Diabetes

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Diabetes may affect cardiac structure and function independent to atherosclerosis and hypertension. Considering the increased risk of heart failure and cardiovascular event in diabetic cardiomyopathy, investigation of etiology and mechanism of this unique entity is important for developing potential therapy. Endothelin-1 (ET-1) has been associated with development of diabetic cardiomyopathy in pre-clinical study. This study aims to investigate correlation of plasma ET-1 with development of myocardial fibrosis and diastolic dysfunction in diabetes patient.Fourty-one diabetes and non diabetes patient with no history of myocardial infarction were recruited. Plasma ET-1 level were measured with radioimmunoassay, diastolic function were evaluated by Doppler echocardiography, and diffuse myocardial fibrosis were evaluated by post-contrast myocardial T1-relaxation time using cardiac MRI.Plasma ET-1 level is higher in diabetes group as compare to non diabetes (1.48±0.50 vs. 1.08±0.22 pg/ml, p< 0.05). All diabetes subjects developed diastolic dysfunction, with 17 (85%) had grade 2 and 3 diastolic dysfunction, compare to 13 (61,9%) non diabetes patient which showed normal diastolic function. Patient with grade 3 (severe) diastolic dysfunction showed higher plasma ET-1 level as compare to patient with normal diastolic function (1.78±0.50 vs. 1.09±0.19 pg/ml, p<0.05). Diabetes subject had shorter post-contrast T1-relaxation time-reflecting diffuse myocardial fibrosis (440.97±16.97 vs. 489.41±6.73 ms, p<.005), and correlates inversely to plasma ET-1 level (Spearman Coeff R = -0.394, p<0.05).In conclusion, higher plasma endothelin-1 level is associated with diffuse myocardial fibrosis and diastolic dysfunction in diabetes patient. This may provide additional evidence for the potential clinical use of endothelin-receptor blockade in preventing diabetic cardiomyopathy

O-28

Activation of ET-1-Mediated PKC-Epsilon/ERK1/2 Pathway Contributes to the Augmented Contractile Response in Aorta from Young Obese Rats

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Childhood obesity is an independent risk factor for cardiovascular diseases; however, the mechanisms of vascular alterations early in obesity remain unknown. Endothelin-1 plays a major role on vascular dysfunction in adiposity-related cardiovascular pathologies. Endothelin-1 induces vasoconstriction by RhoA/Rho-kinase and/or PKC pathways activation. Endothelin-1 also activates vascular ERK1/2. We hypothesized that early in obesity; endothelin-1-mediated activation of PKC/ERK1/2 augments vascular contraction in obese Zucker rats (OZRs). Aortic rings from young (6-7 week-old) OZRs and their lean counterparts were used. The isometric contraction to phenylephrine, U46619 (TXA2 mimetic), endothelin-1 and phorbol 12,13-dibutyrate (PKC activator) was increased in aorta from OZRs. The increased phenylephrine- and endothelin-1-induced vasoconstriction was corrected by inhibitors of PKC (GF-109203X) or MEK1/2 (U0126 and PD98059). The Rho-kinase inhibitor (Y-27632) did not change the contractile responses in OZRs. Vasorelaxation to Y-27632 was also similar in both groups. The ETA antagonist (BQ123) decreased endothelin-1-induced response and corrected the increased phenylephrine-induced contraction in OZRs. Western immunoblot indicated decreased ETA receptors expression in OZRs. Phosphorylation of PKC-alpha was decreased while PKC-beta did not change in OZRs. In contrast, OZRs displayed increased phosphorylation of MEK1/2, ERK1/2, PKC-epsilon and CPI-17, accompanied by decreased phosphorylation of the MAPK phosphatase (MKP-1). The increased phosphorylation of ERK1/2 in OZRs was corrected by either GF109203X or BQ123. Total calponin was decreased while MARCKs phosphorylation was not changed in OZRs. These results indicate that activation of endothelin-1-mediated PKC-epsilon/ERK1/2 pathway contributes to augment the contractile responses in aorta from young OZRs, providing evidence for a role of the endothelin-1 system on the impairment of the vascular function early in obesity. Funding: CNPq, FAPESP

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●効能・効果、用法・用量、警告・禁忌を含む使用上の注意、
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肺動脈性肺高血圧症治療薬

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【禁忌（次の患者には投与しないこと）】
（1）出血している患者（血友病、毛細血管脆弱症、上部消化管出血、尿路出血、喀血、眼底出血等）［出血を増大するおそれがある。］
（2）妊婦又は妊娠している可能性のある婦人（「妊婦、産婦、授乳婦等への投与」の項参照）

【効能・効果】
肺動脈性肺高血圧症

〈効能・効果に関連する使用上の注意〉
（1）原発性肺高血圧症及び膠原病に伴う肺高血圧症以外の肺動脈性肺高血圧症における有効性・安全性は確立していない。
（2）肺高血圧症のWHO機能分類クラスⅣ※の患者における有効性・安全性は確立していない。また、重症度の高い患者等では効果が得られにくい場合がある。循環動態あるいは臨床症状の改善がみられない場合は、注射剤や他の治療に切り替えるなど適切な処置を行うこと。

※WHO機能分類はNYHA（New York Heart Association）心機能分類を肺高血圧症に準用したものである。

【用法・用量】
通常、成人には、ベラプロストナトリウムとして1日120μgを2回に分けて朝夕食後に経口投与することから開始し、症状（副作用）を十分観察しながら漸次増量する。
なお、用量は患者の症状、忍容性などに応じ適宜増減するが、最大1日360μgまでとし、2回に分けて朝夕食後に経口投与する。

〈用法・用量に関連する使用上の注意〉
肺動脈性肺高血圧症は薬物療法に対する忍容性が患者によって異なることが知られており、本剤の投与にあたっては、投与を少量より開始し、増量する場合は患者の状態を十分に観察しながら行うこと。

【使用上の注意】（抜粋）
1.慎重投与（次の患者には慎重に投与すること）
（1）抗凝血剤、抗血小板剤、血栓溶解剤を投与中の患者（「相互作用」の項参照）
（2）月経期間中の患者［出血傾向を助長するおそれがある。］
（3）出血傾向並びにその素因のある患者［出血傾向を助長するおそれがある。］
2.重要な基本的注意
（1）本剤の有効成分は「ドルナー錠20μg」、「プロサイリン錠20」と同一であるが、用法・用量が異なることに注意すること。
（2）本剤から「ドルナー錠20μg」、「プロサイリン錠20」へ切り替える場合には、本剤最終投与時から12時間以上が経過した後に、「ドルナー錠20μg」、「プロサイリン錠20」をベラプロストナトリウムとして原則1日60μgを3回に分けて食後に経口投与することから開始すること。また、本剤と同用量の「ドルナー錠20μg」、「プロサイリン錠20」に切り替えると、過量投与になるおそれがあるため注意すること。
3.相互作用　併用注意（併用に注意すること）
抗凝血剤（ワルファリン等）、抗血小板剤（アスピリン、チクロピジン等）、血栓溶解剤（ウロキナーゼ等）、プロスタグランジンI_2製剤（エポプロステノール、ベラプロスト[注1)]）、エンドセリン受容体拮抗剤（ボセンタン）
注1）同一有効成分を含有する「ドルナー錠20μg」、「プロサイリン錠20」等との併用に注意すること。
4.副作用
原発性肺高血圧症及び膠原病に伴う肺高血圧症患者を対象とした臨床試験において総症例46例中、45例（97.8%）に副作用（臨床検査値異常を含む）が認められ、その主なものは頭痛34例（73.9%）、顔面潮紅31例（67.4%）、ほてり26例（56.5%）、嘔気13例（28.3%）、倦怠感13例（28.3%）、下痢10例（21.7%）、動悸8例（17.4%）、腹痛8例（17.4%）等であった。（承認時）
（1）**重大な副作用**
1）**出血傾向**［**脳出血**（頻度不明[注2)]）、**消化管出血**（頻度不明[注2)]）、**肺出血**（頻度不明[注2)]）、**眼底出血**（頻度不明[注3)]）］：観察を十分に行い、異常が認められた場合には投与を中止し、適切な処置を行うこと。
2）**ショック**（頻度不明[注2)]）、**失神**（10%未満）、**意識消失**（10%未満）：ショック、失神、意識消失を起こすことがあるので、観察を十分に行い、血圧低下、頻脈、顔面蒼白、嘔気等が認められた場合には投与を中止し、適切な処置を行うこと。
3）**間質性肺炎**（頻度不明[注2)]）：間質性肺炎があらわれることがあるので、観察を十分に行い、異常が認められた場合には投与を中止し、適切な処置を行うこと。
4）**肝機能障害**（頻度不明[注3)]）：黄疸や著しいAST（GOT）、ALT（GPT）の上昇を伴う肝機能障害があらわれることがあるので、観察を十分に行い、このような場合には投与を中止し、適切な処置を行うこと。
5）**狭心症**（頻度不明[注3)]）：狭心症があらわれることがあるので、異常が認められた場合には投与を中止し、適切な処置を行うこと。
6）**心筋梗塞**（頻度不明[注3)]）：心筋梗塞があらわれるとの報告があるので、異常が認められた場合には投与を中止し、適切な処置を行うこと。
注2）自発報告によるものについては頻度不明。
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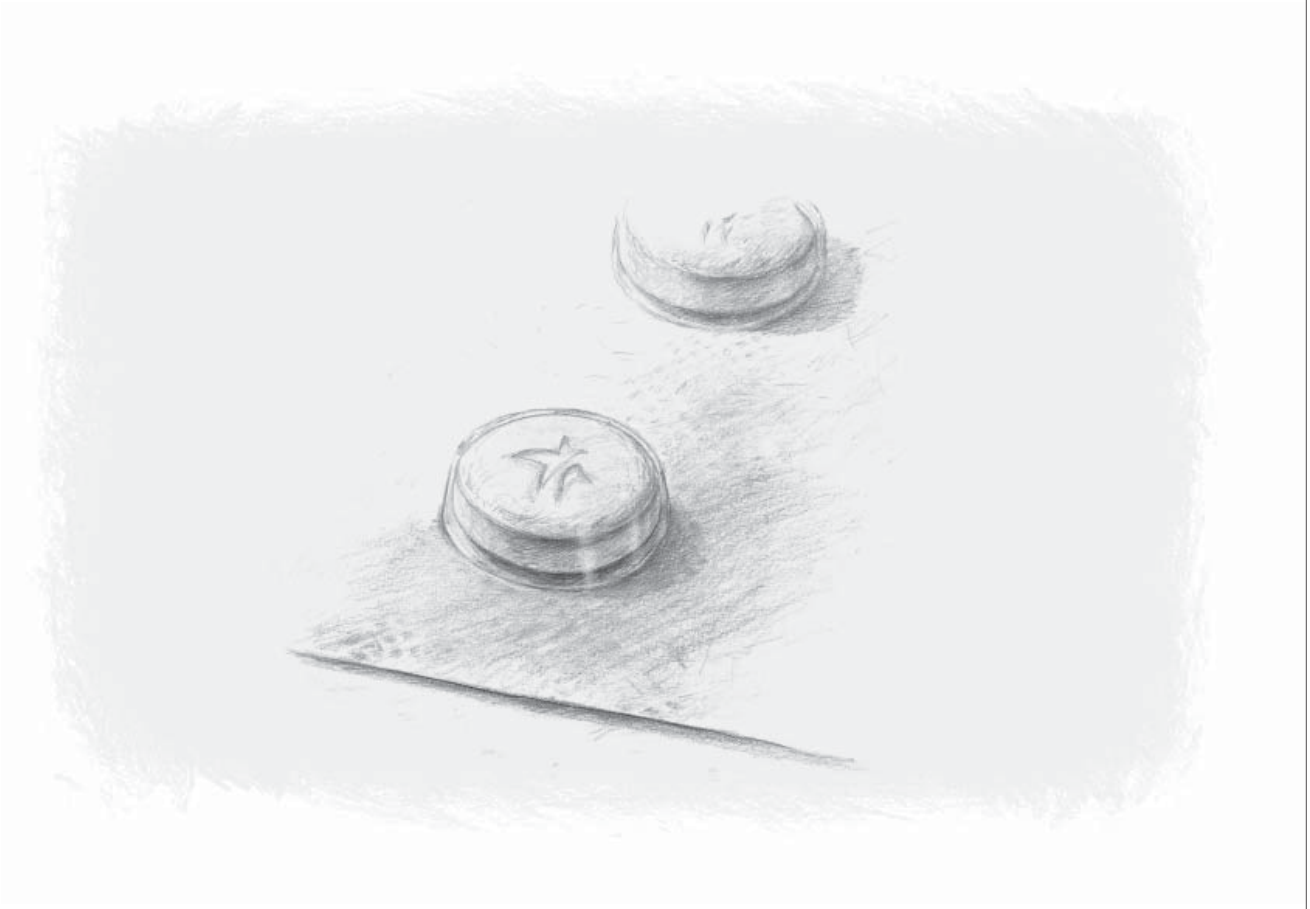

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製造元：オーバスネイチメディカル社

OrbusNeich

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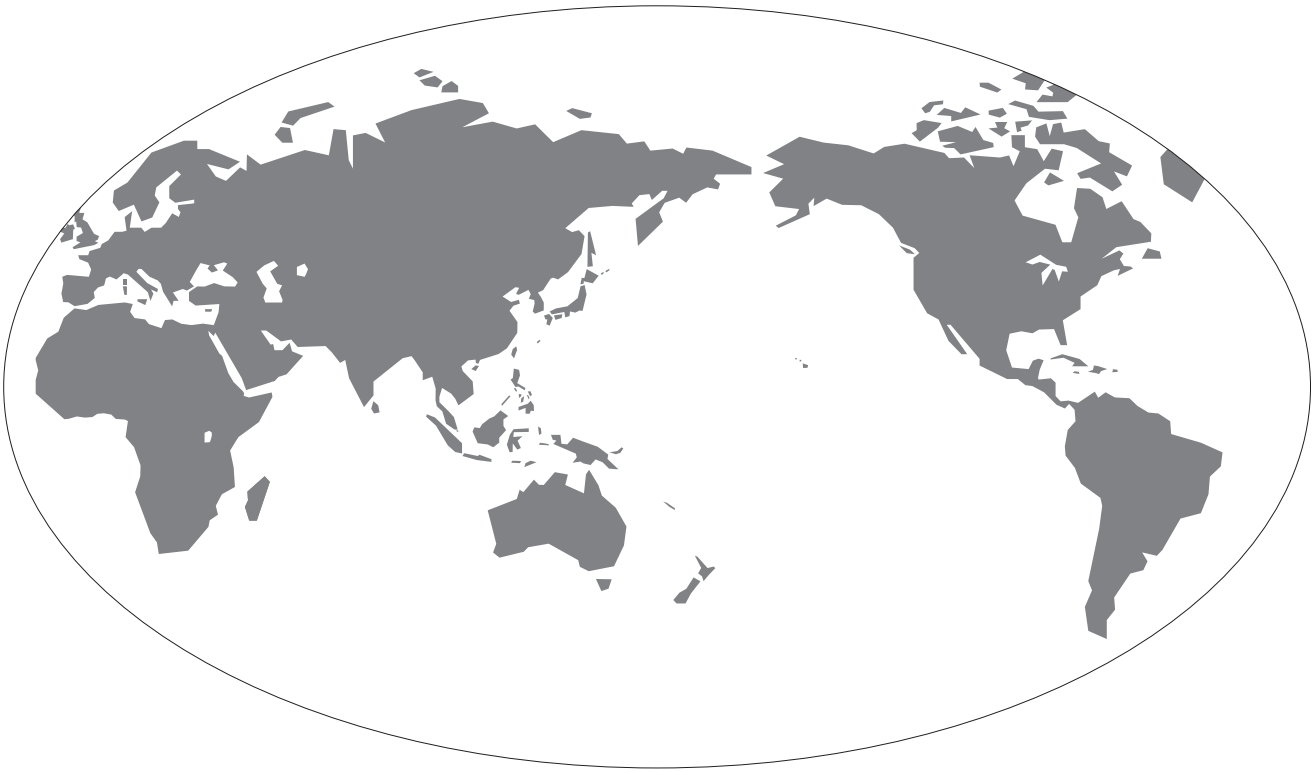

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アンチ・メタボな高血圧治療に。

Metabo sartan

【禁忌（次の患者には投与しないこと）】
1. 本剤の成分に対し過敏症の既往歴のある患者
2. 妊婦又は妊娠している可能性のある婦人
［「妊婦，産婦，授乳婦等への投与」の項参照］
3. アリスキレンを投与中の糖尿病患者（ただし，他の降圧治療を行ってもなお血圧のコントロールが著しく不良の患者を除く）［非致死性脳卒中，腎機能障害，高カリウム血症及び低血圧のリスク増加が報告されている。（「重要な基本的注意」の項参照）］

効能・効果

高血圧症

用法・用量

通常，成人にはイルベサルタンとして50～100mgを1日1回経口投与する。
なお，年齢，症状により適宜増減するが，1日最大投与量は200mgまでとする。

使用上の注意（一部抜粋）

1. 慎重投与（次の患者には慎重に投与すること）
（1）両側性腎動脈狭窄のある患者又は片腎で腎動脈狭窄のある患者（2）高カリウム血症の患者（3）重篤な腎機能障害のある患者（4）肝障害のある患者，特に胆汁性肝硬変及び胆汁うっ滞のある患者（5）脳血管障害のある患者（6）高齢者

2. 重要な基本的注意
（1）両側性腎動脈狭窄のある患者又は片腎で腎動脈狭窄のある患者においては，腎血流量の減少や糸球体ろ過圧の低下により急速に腎機能を悪化させるおそれがあるので，治療上やむを得ないと判断される場合を除き，使用は避けること。（2）高カリウム血症の患者においては，高カリウム血症を増悪させるおそれがあるので，治療上やむを得ないと判断される場合を除き，使用は避けること。また，腎機能障害，コントロール不良の糖尿病等により血清カリウム値が高くなりやすい患者では，高カリウム血症が発現するおそれがあるので，血清カリウム値に注意すること。（3）アリスキレンを併用する場合，腎機能障害，高カリウム血症及び低血圧を起こすおそれがあるため，患者の状態を観察しながら慎重に投与すること。なお，eGFRが60mL/min/1.73m^{2}未満の腎機能障害のある患者へのアリスキレンとの併用については，治療上やむを得ないと判断される場合を除き避けること。（4）本剤の投与によって，**一過性の急激な血圧低下**を起こすおそれがあるので，そのような場合には投与を中止し，適切な処置を行うこと。また，特に次の患者では低用量から投与を開始し，増量する場合は患者の状態を十分に観察しながら徐々に行うこと。1）血液透析中の患者　2）利尿降圧剤投与中の患者　3）厳重な減塩療法中の患者（5）本剤を含むアンジオテンシンII受容体拮抗剤投与中に重篤な肝機能障害があらわれたとの報告がある。肝機能検査を実施するなど観察を十分に行い，異常が認められた場合には投与を中止するなど適切な処置を行うこと。（6）降圧作用に基づくめまい，ふらつきがあらわれることがあるので，高所作業，自動車の運転等危険を伴う機械を操作する際には注意させること。（7）手術前24時間は投与しないことが望ましい。

3. 相互作用
併用注意（併用に注意すること）カリウム保持性利尿剤（スピロノラクトン，トリアムテレン等），カリウム補給剤（塩化カリウム），アリスキレン，非ステロイド性抗炎症薬（NSAIDs），COX-2選択的阻害剤

4. 副作用
承認時における副作用（自他覚症状）は，安全性評価対象例898例中117例（13.0%）に認められた。主なものは，めまい24例（2.7%），咳嗽14例（1.6%），頭痛10例（1.1%）であった。また，臨床検査値の異常変動は，臨床検査値が評価された安全性評価対象例896例中140例（15.6%）に認められた。主なものは，CK（CPK）上昇32例（3.6%），ALT（GPT）上昇21例（2.3%），AST（GOT）上昇18例（2.0%）であった。
（1）重大な副作用 1）**血管浮腫（頻度不明）**：顔面，口唇，咽頭，舌等の腫脹を症状とする血管浮腫があらわれることがあるので，観察を十分に行い，異常が認められた場合には投与を中止し，適切な処置を行うこと。2）**高カリウム血症（頻度不明）**：重篤な高カリウム血症があらわれることがあるので，観察を十分に行い，異常が認められた場合には，直ちに適切な処置を行うこと。3）**ショック，失神，意識消失（頻度不明）**：ショック，血圧低下に伴う失神，意識消失があらわれることがあるので，観察を十分に行い，冷感，嘔吐，意識消失等があらわれた場合には，直ちに適切な処置を行うこと。特に血液透析中，厳重な減塩療法中，利尿降圧剤投与中の患者では低用量から投与を開始し，増量する場合は患者の状態を十分に観察しながら徐々に行うこと。4）**腎不全（頻度不明）**：腎不全があらわれることがあるので，観察を十分に行い，異常が認められた場合には投与を中止し，適切な処置を行うこと。5）**肝機能障害，黄疸（0.1～1%未満）**：AST（GOT），ALT（GPT），Al-P，γ-GTPの上昇等の肝機能障害，黄疸があらわれることがあるので，観察を十分に行い，異常が認められた場合には投与を中止し，適切な処置を行うこと。6）**低血糖（頻度不明）**：低血糖があらわれることがある（糖尿病治療中の患者であらわれやすい）ので，観察を十分に行い，脱力感，空腹感，冷汗，手の震え，集中力低下，痙攣，意識障害等があらわれた場合には投与を中止し，適切な処置を行うこと。7）**横紋筋融解症（頻度不明）**：筋肉痛，脱力感，CK（CPK）上昇，血中及び尿中ミオグロビン上昇を特徴とする横紋筋融解症があらわれることがあるので，観察を十分に行い，このような場合には直ちに投与を中止し，適切な処置を行うこと。

■ その他の「使用上の注意」については添付文書等をご参照下さい。

長時間作用型ARB

薬価基準収載

Metabo sartan **イルベタン®錠** 50mg 100mg 200mg

イルベサルタン錠
IRBETAN®

処方せん医薬品注1)

注1）注意一医師等の処方せんにより使用すること

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IRB-KO-101A(C1) 審L9536　2013年6月作成　A41

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ベッセル内のガスは、高電圧をかけることで励起状態になり、308㎚の紫外線領域の波長の光子を発生させます。

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エキシマレーザ心内リード抜去システム

エキシマレーザ光をレーザシース（SLS II）内のファイバーを通じて、抜去する心内リードの癒着組織に照射し、剥離します。
リード抜去術には、LLD（リードロッキングデバイス）との併用を推奨します。

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医療機器承認番号：22000BZX00968000
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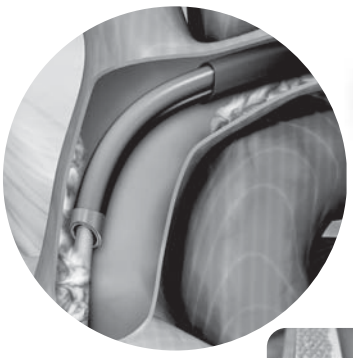

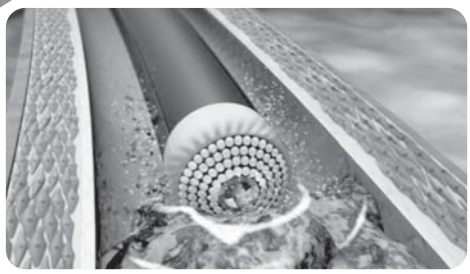

エキシマレーザ血管形成用カテーテル

冠動脈の動脈硬化病変を蒸散させるレーザカテーテルには次の２タイプがあります。

・Concentric Type
　–先端径：1.4㎜/1.7㎜/2.0㎜

販売名：エキシマレーザ血管形成用OSカテーテル
医療機器承認番号：21900BZY00070000

・Eccentric Type
　–先端径：1.7㎜/2.0㎜
　–側面にガイドワイヤールーメンを有し、光ファイバーが偏心状に配置されています。

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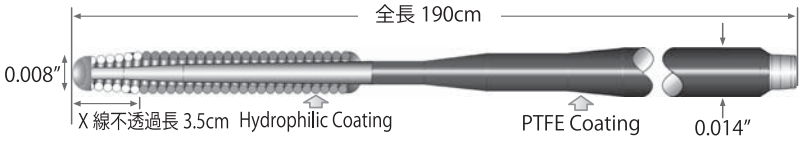
3gの先端荷重とテーパー構造（0.008”-0.014”）によりマイクロチャンネルを探る手技に適したガイドワイヤー



14 Universal 190 IJ14U1900A
14 Universal 300 IJ14U3000A

0.8gの先端荷重と190cm/300cm長をラインナップに持ち、選択性とサポート性を追及したガイドワイヤー

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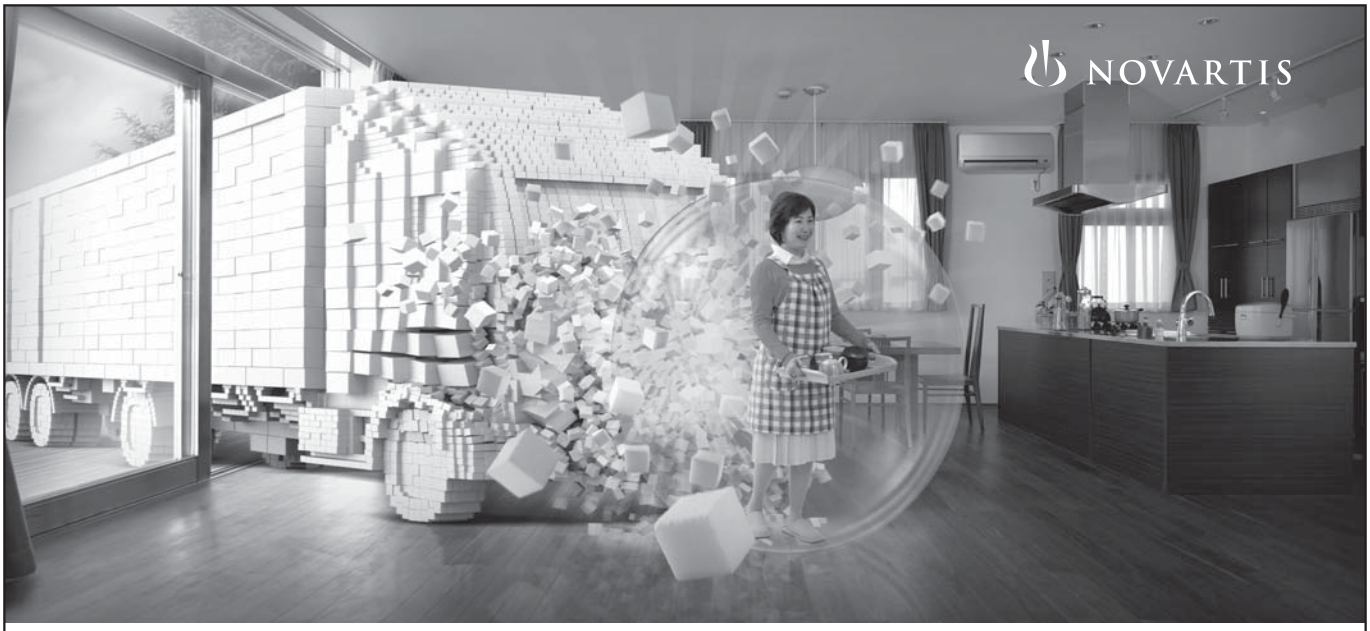

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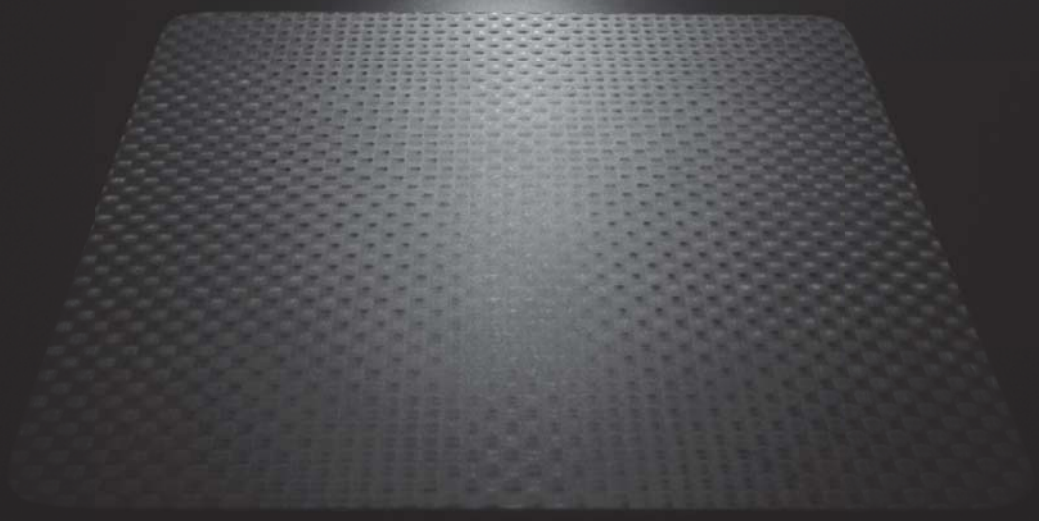

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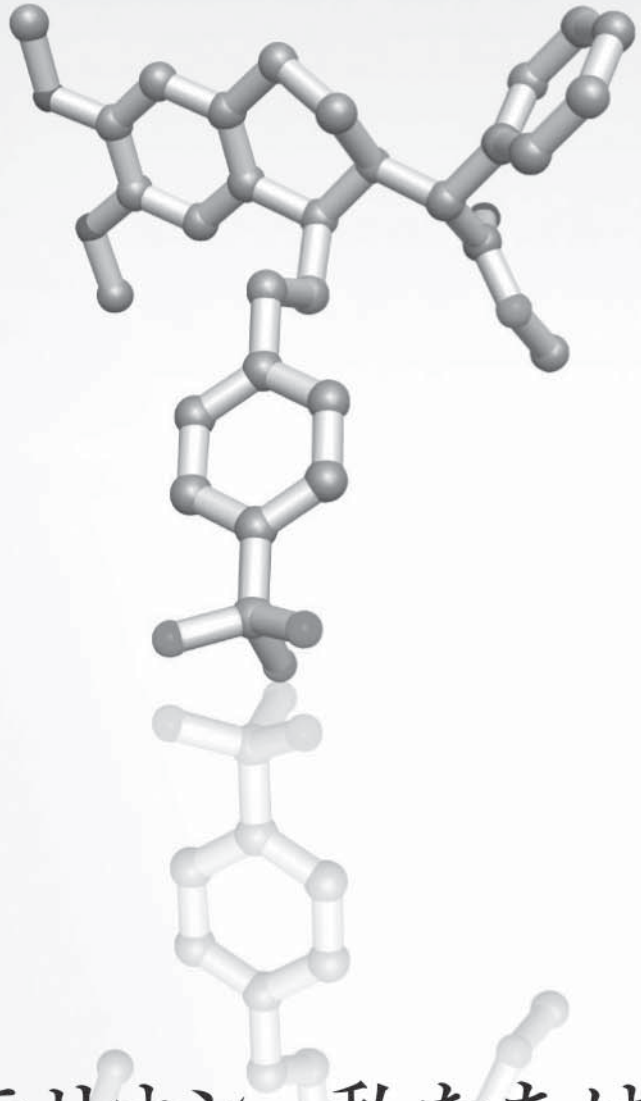

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