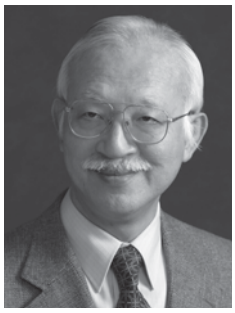
AS3-1

Ca_V CHANNELS AND THEIR MODIFIERS: MOLECULAR IDENTIFICATION OF THE BINDING SITES



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1976.03	*Graduate, Tohoku University School of Medicine (MD licensee)*
1980.03	*Graduate, Tohoku University Graduate School of Medicine, PhD (K+ channel opening by SG-75 (nicoradil))*
1980.07-1982.08	*University of Pennsylvania School of Medicine (PostDoc fellow in Department of Physiology)*
1982.08	*Assistant Professor, Department of Pharmacology, Tohoku University School of Medicine*
1993.10	*Associate Professor, Tohoku University School of Medicine*
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Ca^{2+} entry through voltage-gated Ca^{2+} channels (Ca_v channels) initiates a variety of cellular processes, including neurotransmitter release, muscle contraction, and gene expression even in non-excitable cells, *eg*. immune system. Ca_v channels are heteromultimers consisting of α_1, β, γ, and α_2/δ subunits. The α1 subunit is the pore-forming subunit and contains the key structural determinants required for gating, drug or toxin binding, and ion permeation. The α_1 subunit, therefore, is important targets of drug development for therapeutic agents of cardiovascular, neuronal, and other organ diseases. The binding sites in α_1 subunit of Ca_v1 channels for classical three types of Ca antagonists, phenylalkylamines (PAAs), dihydropyridines (DHPs), and benzothiazepine (BTZ), have been elucidated by the combined application of molecular and cellular biology, electrophysiology, and computer-assisted procedures. The models to show the blocking activity of Ca_v1 channels open the way of development new Ca_v channel modifiers to influence the activity of Ca_v2 or 3 channels. The progress in the studies of Ca_v channels and their modifiers including Ca antagonists is not ceased to pursue the development of novel agents for treatment of various disorders.

AS3-2

ACE-INHIBITORS AND ENDOTHELIAL FUNCTION

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Dr. Paul M. Vanhoutte obtained his M.D. degree at the University of Gent. He has been Professor of Pharmacology at the University of Antwerp, the Mayo Clinic (Rochester MN USA) and Baylor College of Medicine (Houston TX, USA). From 1992 to 2002, he was Director of Discovery Research at Servier. He currently is Head of the Department of Pharmacology and Pharmacy at the Li Ka Shing Faculty of Medicine of the University of Hong Kong. Dr. Vanhoutte has received the Doctor Honoris Causa degree of the Universities of Gent, Zürich, Antwerp, Montréal, of the Royal Melbourne Institute of Technology University in Melbourne, and of the Louis Pasteur University in Strasbourg.

The endothelium can mediate dilatations of arteries from animals and humans. Such endothelium-dependent relaxations of vascular smooth muscle are due to the release by the endothelial cells of potent nonprostanoid vasodilator substances. The best characterized endothelium-derived relaxing factor (EDRF) is nitric oxide (NO). In addition, the endothelial cells can evoke endothelium-dependent hyperpolarizations (EDHF-mediated relaxation) of the underlying smooth muscle. The release of relaxing factors can be initiated by shear stress, circulating hormones, thrombin, platelet products and locally produced autacoids (in particular bradykinin) . The release of EDRFs from the endothelium can be mediated by both pertussis toxin-sensitive (e.g. serotonin) and insensitive (e.g.bradykinin) G-proteins. In blood vessels from animals with regenerated and reperfused endothelium, and/or atherosclerosis, there is a selective loss of the pertussis-toxin sensitive mechanisms of EDRF-release which favors the occurence of vasospasm, thrombosis and cellular growth. Angiotensin Converting Enzyme (ACE) inhibitors such as perindopril protect kinins from breakdown and thus augment the release of both NO and EDHF caused by increases in shear stress and locally produced bradykinin. They also directly interact with bradykinin-receptors to augment their sentivities. These endothelial actions of ACE-inhibitors help to explain why they lower arterial blood pressure and exert protective effects against cardiovascular disease.

SS1-1

THERAPEUTIC STRATEGY FOR PERIPHERAL ARTERIAL DISEASE: PHARMACOLOGICAL THERAPY AND ANGIOGENISIS –ROLES OF SEROTONIN AND NITRIC OXIDE–

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1996PhD, Hiroshima University1988MD, Hiroshima University School of Medicine

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Endothelial dysfunction is an initial step in the development of atherosclerosis, resulting in cardiovascular complications. Peripheral artery disease (PAD) is one of the major manifestations of atherosclerosis. PAD is associated with increased cardiovascular morbidity and mortality. Patients with PAD have severe endothelial dysfunction. Recently, several investigators have shown that endothelial dysfunction in patients with PAD is reversible. Serotonin (5-hydroxytryptamine, 5-HT), released from activated platelets, has various subtypes of receptors and mediates both vasoconstriction and vasodilation as well as promoting platelet aggregation. Vasoconstricting effects of serotonin are mediated by 5-HT_{2A} receptors on vascular smooth muscle cells and platelets. PAD patients have higher plasma 5-HT concentrations than those in healthy subjects. Sarpogrelate hydrochloride, a selective 5-HT_{2A} antagonist, has been widely used as an anti-platelet agent for the treatment of PAD. By the blockade of 5-HT_{2A} receptors, sarpogrelate inhibits thrombus formation, suppresses platelet aggregation and inhibits vascular smooth muscle cell proliferation. Recently, we reported that sarpogrelate improves endothelial function in patients with PAD (*J Cardiovasc Pharm.* 2007; 49: 221). In addition, we have shown that autologous bone-marrow mononuclear cell (BM-MNC) implantation augments endothelium-dependent vasodilation in PAD patients who had rest pain and non-healing ulcers and who were not candidates for angioplasty or surgical revasularisation (*Circulation.* 2004;109:1215). Interestingly, a combination of BM-MNC implantation and sarpogrelate improves much more endothelial function in patients with critical limb ischemia.

SS1-2

EVIDENCE FOR A UNIQUE ROLE OF SEROTONIN IN THE SICK VESSEL SYNDROME

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Education

Year	*Degree*	*Institution*
1963	*M.D.*	*University of Chicago, Chicago, Illinois*

Post-Graduate Education

Year	*Position*	*Institution*
1964-66	*Resident, Internal Medicine*	*University of Chicago, Chicago, Illinois*
1967-70	*Research Internist*	*U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts*

Academic Appointments

Year	*Position*	*Institution*
1970-2008	*Cardiology Staff Physician*	*VA Medical Center, Iowa City, IA*
1999-present	*Professor of Cardiology*	*University of Iowa Hospitals & Clinics Iowa City, IA*

We have described the "sick vessel syndrome," characterized by exaggerated vasoconstriction and impaired vasodilatation (1). The net effect of these vasomotor abnormalities may be to predispose arteries to vasospasm and ischemia.

Serotonin plays a key role in the sick vessel syndrome. It is remarkable that a very wide variety of vascular diseases predispose to greatly exaggerated vasoconstrictor responses to serotonin. In our experience, serotonin is the stimulus that most consistently produces abnormal vasomotor responses in disease states. Atherosclerosis predisposes to vasospasm in response to serotonin in experimental animals (2), and serotonin can even produce complete coronary occlusion in patients with angina (3).

What is the mechanism for exaggerated vasoconstrictor responses to serotonin? Impairment of endothelium-dependent relaxation in atherosclerosis contributes to exaggerated vasoconstrictor responses to serotonin. Although endothelial dysfunction accounts for part of the augmented vasoconstrictor responses to serotonin in disease states, the major abnormality is altered signal transduction in smooth muscle, beyond the $5HT_{2A}$ receptor. Rho kinase plays a key role in augmented responses to serotonin in several disease states (4).

Rho kinase and ERK also appear to mediate migration of human aortic smooth muscle cells in response to stimulation of $5HT_2$ receptors (5). After vascular injury and proliferation of neointima in rabbits, smooth muscle cells in neointima and in arterial media are selectively hypercontractile to serotonin; the hyperresponsiveness is mediated by $5HT_{2A}$ receptors and rho kinase (6). In a human artery, both $5HT_{2A}$ and $5HT_{1B}$ receptors mediate vasoconstrictor responses to serotonin (7). It is of interest that inhibition of $5HT_{2A}$ receptors by sarpogrelate also attenuates intimal hyperplasia in vein grafts of rabbits (8).

There are important clinical implications of these findings. The overall concept is that, when platelets aggregate and release serotonin, inhibition of serotonergic receptors (especially $5HT_{2a}$ receptors) may have unique beneficial effects on blood vessels.

1. Heistad and Armstrong. Circulation, 89:2447-2450, 1994.
2. Shimokawa et al. Science, 221: 560-562, 1983
3. McFadden et al. N Engl J. Med., 324:648-654, 1991.
4. Kandabashi, Shimokawa,…and Takeshita. ATVB, 22:243-248, 2002.
5. Matsusaka and Wakabayashi. Biochem. Biophys. Res. Comm., 337:916-921, 2005.
6. Nishihira, Yamashita,…and Asada. J. Thromb. Haemost., 6:1207-1214, 2008.
7. Tanaka, Nakamura….Yamamoto. Clin. Exp. Pharmacol. Physiol., 35:836-840, 2008.
8. Kodama, Komori,…and Itoh. J. Vasc. Surg., 49:1272-1281, 2009.

SS2-1

VASCULAR FAILURE AND VASCULAR ENDOTHELIAL FUNCTION

Koichi Node

Cardiovascular and Renal Medicine, Saga University

He has expertise in vascular endothelial, arteriosclerosis, and vascular biology. His research and analysis focuses on the new treatments of ischemic heart disease and heart failure.
Since 2002 to present, he is a professor and chairman of cardiovascular and renal medicine at Saga University.

Atherosclerosis is characterized by the response of the vessel wall to chronic multifactorial injury leading to the formation of atheromatous or fibrous plaques. Endothelial dysfunction represents an initial stage of atherosclerosis. In addition to endothelial dysfunction, smooth muscle dysfunction, metabolic abnormalities of the vessel wall including inflammation, oxidative stress and breakdown of neurohormonal balance occur in various stages of atherosclerotic process. Now we propose a new clinical entity 'Vascular Failure' characterized as the integration of these vascular abnormalities. 'Vascular Failure' is not an anatomical disease entity but a comprehensive syndrome of failed vascular function that extends from risk factors to established atherosclerotic disease with arterial stenosis, and further to calcification of the vessel wall or serious vascular events that may be caused by plaque rupture and thromboembolic occlusion. We propose aggressive intervention to modify various risk factors, applying this integrated new entity 'Vascular Failure'.

SS2-2

LEARNING FROM THE LARGEST ARB MEGA-TRIALS: RESULTS FORM ONTARGET AND TRANSCEND

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Department of Nephrology and Hypertention, University of Erlangen-Nuremberg, Germany

Professor Roland Schmieder graduated in medicine at the University of Tübingen, Germany. His postgraduate medical training in nephrology, cardiology and hypertension was first at the Department of Medicine, University of Bonn, Germany, and later at the Department of Medicine, Section on Hypertensive Disease, New Orleans, USA. He is currently Professor of Medicine at the Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Germany. His research interests include hypertension, non-invasive cardiology and nephrology, and he focused his attention on the evaluation of early target-organ damage in primary and secondary hypertension. Professor Schmieder has participated in various clinical studies that have assessed the effect of antihypertensive therapy on left ventricular hypertrophy and renal haemodynamics. He is currently Council Member of the European Society of Hypertension.

There is substantial evidence that the presence of proteinuria or albuminuria is an important predictor of cardiovascular outcomes because it is an indicator of vascular disease, and hence provides a means to identify patients at greatest risk. Drugs that interrupt the renin-angiotensin system (RAS), such as angiotensin II receptor blockers (ARBs) or angiotensin-converting enzyme (ACE) inhibitors, not only lower blood pressure but also reduce inflammation oxidative stress and fibrotic processes and hence disease progression. Furthermore, reducing proteinuria using ARBs or ACE inhibitors reduces cardiovascular risk, as has been demonstrated in the IDNT, the IRMA-2 and the RENAAL studies in patients with diabetic nephropathy. In these studies, a reduction in albuminuria was associated with a proportional effect on renal protection; the greater the reduction, the greater the renal protection and cardiovascular complications. Comparisons between different agents that block the RAS are of particular interest. Similar to ACE inhibitors, telmisartan improves renal endothelial function and reduces the decline in glomerular filtration rate. Telmisartan was superior to losartan in the reduction of proteinuria in AMADEO™, despite a similar reduction in blood pressure. In ONTARGET®, conducted in broad range of patients at high risk of vascular events, telmisartan was as effective as ramipril in reducing the number of patients developing renal dysfunction but better tolerated. Based on these results, telmisartan offers a new option to reduce the development of renal dysfunction in patients with and without diabetes. In addition, ONTARGET® provides a unique opportunity to investigate the relationship between blood pressure, proteinuria and renal/cardiovascular outcomes. New data on these will be presented.

SS3-1

NEW MECHANISM OF CORONARY ENDOTHELIAL DYSFUNCTION IN HUMANS

Amir Lerman

The Division of Cardiovascular Diseases, Mayo Clinic, USA

Amir Lerman, MD, is Professor of Medicine, Division of Cardiovascular Diseases and Internal Medicine at the Mayo Clinic, Rochester, Minnesota. He serves as the Director of research of the Cardiac Catheterization Laboratory and the Director of the Chest Pain and Coronary Physiology Center and Clinic. Dr. Lerman has a special interests in the role of the endothelium in vascular tone with emphasis on the coronary circulation in atherosclerosis, acute coronary syndrome, and plaque vulnerability.

Coronary endothelial dysfunction is considered the early stage of atherosclerosis in association with future cardiovascular event. Thus, it is essential for us in order to understand the disease process as well as to attenuate the progression of disease to understand the mechanism of coronary endothelial dysfunction in humans. Previous studies focused on the role of endothelial derived vasodilators and vasoconstrictors in the regulation of endothelial function. In this current lecture, we will focus on the concept that coronary endothelial dysfunction represents imbalance between vascular injury and repair and we explore the role of inflammatory markers and oxidative stress as well as endothelial progenitor cells and the mechanism for coronary endothelial dysfunction in humans. The ability to investigate the role of these endogenous factors in the coronary circulation combined with novel imaging modalities may enhance our understanding of early atherosclerosis and coronary endothelial function in humans.

SS3-2

25 YEARS OF ENDOTHELIAL RESEARCH IN HUMANS: WHERE WE HAVE BEEN AND WHERE WE ARE HEADING

Peter Ganz

University of California, San Francisco, USA

Dr. Ganz has been a pioneer and a leader in translational vascular research. His interests have focused on understanding key aspects of human atherosclerosis including endothelial function, the biology of nitric oxide, vascular inflammation and plaque vulnerability. Dr. Ganz has authored over 200 peer reviewed journal articles, many published in the most prestigious journals. He has served on steering committees of numerous clinical trials. Many fellows who worked in Dr. Ganz's laboratory have gone onto key leadership positions in cardiology nationally and internationally.
Dr. Ganz received his M.D. from Harvard Medical School, completed his residency at the Massachusetts General Hospital and cardiovascular fellowship at the Brigham and Women's Hospital. He spent nearly 25 years directing research in the Cardiac Catheterization Laboratories at the Brigham and Women's Hospital and Harvard Medical School. In 2008, he assumed the role of the Chief of Cardiology and the Director of the Center of Excellence in Vascular Research at the San Francisco General Hospital and the University of California, San Francisco. Dr. Ganz is the Maurice Eliaser Distinguished Professor of Medicine at the University of California, San Francisco.

Much progress has occurred in the understanding of the biology of the endothelium since the discovery of endothelium-dependent vasorelaxation by Furchgott in the rabbit aorta in 1980 and our first published description of endothelial function in humans in 1986. Nitric oxide (NO) mediates many of the protective functions of the endothelium. In humans, endothelial dysfunction has been linked to all known risk factors for atherosclerosis. It has been detected in conduit arteries and in resistance arterioles and in coronary as well as peripheral arteries. This recognition of endothelial dysfunction as a systemic disorder has facilitated non-invasive testing of endothelial function in accessible peripheral arteries. Clinical investigations strongly support an anti-atherogenic role for NO. Coronary endothelial dysfunction in cardiac transplant recipients is associated with rapidly progressive vasculopathy. Endothelial dysfunction in the brachial artery is associated with rapid progression of carotid intima-media thickness. Numerous studies demonstrate a strong association between endothelial dysfunction and cardiovascular events. Commonly used therapies in the treatment of atherosclerosis reverse endothelial dysfunction. Improvement in endothelial function also partly accounts for the lipid-independent benefits of statins. The observation in clinical studies that therapeutic interventions restores normal endothelial function in some subjects but fails to do so in others has been used to differentiate clinical responders from non-responders. As endothelial function is a central component cardiovascular disorders, it is used by the scientific community as well as by pharmaceutical industry to gain pathophysiologic insights and develop novel therapies. Human endothelial studies are an extraordinary example of successful translational research.

O1-1

ENHANCED ANGIOTENSIN II FORMING ACTIVITY IN MONONUCLEAR CELLS BY ELEVATED FFA: IMPLICATION IN ENDOTHELIAL DYSFUNCTION AS MOBILE RENIN-ANGIOTENSIN SYSTEM

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Objectives: Release of free fatty acids (FFA) from adipose tissue was implicated in insulin resistance and endothelial function in subjects with visceral fat obesity. We previously demonstrated that elevated FFA caused endothelial dysfunction that was prevented by inhibition of renin-angiotensin system in humans. However, little was known as to mechanisms for FFA-mediated activation of renin-angiotensin system (RAS) and resultant endothelial dysfunction.

Methods and Results: We measured activity of the circulating RAS, RAS in the vasculature and mononuclear cells after the elevation of FFA by the lipid/heparin infusion in healthy male subjects. Effect of FFA on leukocyte activation was also assessed by the count of adhesive leukocytes ex vivo. Although parameters of the circulating RAS and vascular responses to ANG I and II were not affected by elevated FFA, total, chymase-dependent, cathepsin G-dependent ANG II forming activity was significantly enhanced by elevated FFA. Elevated FFA also significantly increased the number of adhesive leukocytes and this reaction was attenuated by RAS inhibition.

Conclusion: Elevated FFA by the lipid/heparin infusion in human, mimicking lipid profile in subjects with visceral fat obesity and insulin resistance, significantly enhanced ANG II forming activity in human mononuclear cells, which was implicated in FFA-induced endothelial dysfunction as mobile renin-angiotensin system presumably through RAS-dependent leukocyte activation.

O1-2

VASCULOPROTECTIVE ROLE OF NITRIC OXIDE SYNTHASE SYSTEM AGAINST VASCULAR LESION FORMATION IN MICE IN VIVO

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Background: Nitric oxide synthase (NOS) system consists of three isoforms: nNOS, iNOS, and eNOS. Due to substantial compensatory interactions among the three NOSs, the role of the entire NOS system in vascular lesion formation remains to be fully elucidated. In this study, we addressed this point in mice lacking the complete NOS system (triply n/i/eNOS-/- mice) that we have recently developed.

Methods and Results: Permanent ligation of a unilateral carotid artery was performed in 2-month-old wild-type (WT), singly nNOS-/-, iNOS-/-, and eNOS-/-, and triply n/i/eNOS-/- mice (n=12-16). At 2 weeks after the carotid artery ligation, neointimal formation, constrictive vascular remodeling, and adventitial infiltration of inflammatory leukocytes were noted in the ligated carotid arteries of all genotypes. While neointimal formation (intima/media ratio, 0.19±0.02 in WT) was noted in nNOS-/- (0.51±0.03) and eNOS-/- genotypes (0.58±0.03), it was most accelerated in n/i/eNOS-/- genotype (1.01±0.08, $P<0.01$ vs. other genotypes). While constrictive vascular remodeling (vascular circumference ratio, 0.95±0.05 in WT) was noted in nNOS-/- (0.80±0.02) and iNOS-/- genotypes (0.79±0.02), it was most accelerated in n/i/eNOS-/- genotype (0.70±0.03, $P<0.05$ vs. other genotypes). Furthermore, while adventitial infiltration of inflammatory leukocytes (cell number, 52±9 in WT) was noted in nNOS-/- (100±6), iNOS-/- (84±5) and eNOS-/- genotypes (94±6), it was again most accelerated in n/i/eNOS-/- genotype (272±23, $P<0.01$ vs. other genotypes).

Conclusions: These results provide the first direct evidence that the NOS system in its entirety plays an important protective role, in a cooperative manner, against inflammatory vascular lesion formation caused by blood flow disruption in mice in vivo.

O1-3

CYCLOPHILIN A PROMOTES VASCULAR OXIDATIVE STRESS AND ACCELERATES DEVELOPMENT OF ANGIOTENSIN II-INDUCED AORTIC ANEURYSMS

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Introduction: Cyclophilin A (CyPA) is a chaperone protein secreted from vascular smooth muscle cells (VSMC) in response to reactive oxygen species (ROS), that stimulates VSMC migration and inflammatory cell migration in vitro. We hypothesized that VSMC-derived CyPA contributes to AAA pathogenesis due to its proinflammatory properties.

Methods and Results: To determine the role of CyPA in abdominal aortic aneurysm (AAA) formation, ApoE-/- and ApoE-/-CyPA-/- mice were infused with angiotensin II (Ang II) for 4 weeks. There were no differences in blood pressure and cholesterol between ApoE-/- and ApoE-/-CyPA-/- mice before and after Ang II treatment. Ang II-induced AAA formation and aortic rupture was frequently observed in ApoE-/- mice (89% and 40%). In contrast, ApoE-/-CyPA-/- mice were completely protected from Ang II-induced AAA formation and aortic rupture (0% and 0%). The incidence of AAA was 63% in CyPA+/+ marrow-transplanted ApoE-/- mice, while the incidence of AAA in ApoE-/-CyPA-/- mice remained 0% after transplantation of CyPA+/+ bone marrow cells. In situ and gelatin zymography demonstrated that CyPA was required for matrix metalloproteinase (MMP) activation in aortic wall. Finally, VSMC-specific CyPA overexpressing mice revealed augmented Ang II-induced MMP activity in the vascular wall.

Conclusion: Vascular-derived CyPA contributes to AAA pathogenesis in mice by increasing proinflammatory cytokine expression, inflammatory cell migration, and MMP activation.

O1-4

RAMP2 IS THE KEY DETERMINANT OF THE VASCULAR FUNCTIONS OF ADRENOMEDULLIN

Takayuki Shindo, Takayuki Sakurai, Akiko Kamiyoshi, Yuka Shindo, Hisaka Kawate, Nobuyoshi Iinuma, Takuma Arai, Takahiro Yoshizawa, Teruhide Koyama, Natsumi Shimoyama, Ryuuichi Uetake and Akihiro Yamauchi

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Adrenomedullin (AM) is a potent vasodilating peptide involved in both the pathogenesis of cardiovascular diseases and circulatory homeostasis. In the present study, we generated knockout mice of receptor activity-modifying protein 2 (RAMP2), a small membrane protein and a modulator of G-protein coupled receptor, and showed that it is the key determinant of the vascular functions of AM.

Like AM-/- embryos, RAMP2-/- embryos died in utero at mid-gestation due to vascular fragility that led to severe systemic edema and hemorrhage. They also showed accumulation of pericardial effusion suggestive of cardiac failure. The expression of AM was upregulated by 5-fold in RAMP2-/-, showing a compensatory response. Vascular endothelial cells (ECs) in RAMP2-/- were severely deformed and detached from the basement membrane. Expression of tight junction, adherence junction and basement membrane molecules by ECs was diminished in RAMP2-/-, leading to paracellular leakage, which likely explains the severe edema and hemorrhage.

Unlike their homozygous knockout littermates, RAMP2+/- mice survived until adulthood. In RAMP2+/- mice, the aortic expression of RAMP2 was reduced to half that seen in wild-type mice. RAMP2+/- mice had higher blood pressures than wild-type mice. With acute infusion of AM, RAMP2+/- mice showed a smaller blood pressure response than wild-type mice. In RAMP2+/- mice, reduced RAMP2 expression impaired neovascularization and caused vascular hyperpermeability. Conversely, ECs overexpressing RAMP2 showed enhanced capillary formation, firmer tight junctions and reduced vascular permeability.

Our findings demonstrate that RAMP2 is the key determinant of AM's vascular functions and RAMP2 is essential for angiogenesis and vascular integrity.

O1-5

PATHOPHYSIOLOGICAL RELEVANCE OF UNCOUPLED ENDOTHELIAL NITRIC OXIDE SYNTHASE IN CARDIOMYOCYTE INJURY TRIGGERED BY PHENYLEPHRINE-INDUCED HYPERTROPHY

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Pathophysiological relevance of uncoupling state of endothelial nitric oxide synthase (eNOS) in phenylephrine (PE)-induced cardiomyocyte remodeling remains unclear. We here defined pathophysiological role and mechanism of generation of reactive oxygen species (ROS) such as superoxide through uncoupled eNOS in PE-induced hypertrophic cardiomyocytes. In cultured rat cardiomyocytes, eNOS protein markedly increased with concomitant hypertrophy at 48 h after prolonged PE treatment (10 μM). When intracellular nitric oxide (NO) and superoxide levels were monitored, both basal levels of NO and superoxide markedly elevated at 96 h after the prolonged PE treatment. The superoxide generation was associated with increased uncoupling state of eNOS and mitochondrial NADPH oxidase activation. Thus, uncoupling of eNOS likely in part accounts for superoxide generation by the prolonged PE exposure, thereby inducing apoptotic cell death. Furthermore, cardioprotective effects of a novel NOS inhibitor, DY-9836 well correlated with the inhibition of aberrant superoxide generation. DY-9836 treatment also protected cardiomyocytes from breakdown of caveolin-3/dystrophin, which are major components to scaffold eNOS in cardiomyocyte caveolae. Taken together, generation of superoxide through uncoupled eNOS mediates PE-induced injury in hypertrophied cardiomyocytes.

Lu YM, Fukunaga K et al. Biochem. Pharmacol. 2007;74:1727-1737

Lu YM, Fukunaga K et al. Mol Pharmacol. 2009;75:101-112

O2-1

DIFFERENT ROLES OF BONE MARROW-DERIVED PROGENITOR CELLS IN THE PATHOGENESIS OF VASCULAR DISEASE

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Background: Prognosis of patients with severe cardiovascular diseases still remains poor, while recent reports have shown that angiogenesis with bone marrow (BM)-derived progenitor cells is a promising new therapeutic strategy. Accumulated evidence suggests that circulating endothelial progenitor cells (EPCs) contribute to the formation of new blood vessels. In contrast, several recent reports showed the existence of BM-derived progenitor cells that differentiate into α-smooth muscle actin $(SMA)^{+}$ cells, which may participate in the progression of atherosclerosis and lung fibrosis. However, it is controversial whether those BM-derived progenitor cells are beneficial or harmful in nature. So, in this study, we examined individual roles of smooth muscle progenitor cells (SMPCs) and EPCs.

Methods and Results: In patients with pulmonary arterial hypertension (PAH), the number of circulating VEGF-2^{+}/c-kit^{+} progenitors, which are considered as SMPCs, was increased compared with controls. In animal model, pravastatin ameliorated hypoxia-induced PAH associated with a decrease in the number of SMPCs accumulating to the pulmonary artery adventitia through suppression of SDF-1/CXCR4 pathways. Mice lacking EpoR in non-erythrocyte lineage $(EpoR)^{-/-}$-rescued mice) demonstrated impaired angiogenesis in hindlimb ischemia associated with an impaired EPC mobilization.

Conclusions: These results indicate that progenitor cells play differential roles in the pathogenesis of cardiovascular diseases; EPCs may enhance therapeutic angiogenesis, whereas SMPCs may be harmful as they accelerate arteriopathy. Although the promotion of BM-derived progenitor cells could be a new therapeutic strategy for the treatment of cardiovascular diseases, we should consider the types and characteristics of BM-derived progenitor cells when treating patients with ischemic cardiovascular diseases.

O2-2

ROLE OF BONE MARROW STEM CELLS IN THE PATHOGENESIS OF PULMONARY HYPERTENSION AND PHARMACOLOGICAL MODULATION

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Background: Bone marrow (BM) stem cells differentiate into various vascular cells under pathological conditions. We investigated whether different BM stem cells, hematopoietic (HSC) and nonhematopoietic stem cells, differentiate into discrete cell lineages in pulmonary vascular disease (PVD) in mice with pulmonary hypertension (PH) and whether stem cell kinetics may be modulated by a pulmonary vasodilator, an endothelin receptor antagonist.

Methods: Experiment1. Wild-type mice lethally irradiated and transplanted with whole BM cells (n=25) or cells from c-kit$^+$Sca-1$^+$Lin$^-$ HSC-rich fraction (n=12) from littermates expressing enhanced green fluorescent protein (eGFP) were exposed to hypobaric hypoxia (380mmHg) for 21 days, or kept in the ambient air. Experiment2. BM chimeric mice (n=54) transplanted similarly with whole eGFP-positive BM cells were exposed to hypobaric hypoxia (380mmHg) or kept in the ambient air, injected with saline or bosentan sodium salt (30mg/kg/d, ip) for 21 days. After the treatment period, right ventricular systolic pressure (RVSP) were evaluated. Tissue sections were immunostained and analyzed by using laser scanning confocal microscopy, quantitatively.

Results: BM-derived macrophages and endothelial cells, which were increased in lungs in mice associated with PH, PVD, and right ventricular hypertrophy induced by chronic hypoxia, mainly originate from HSC-rich and non-HSC-rich fractions, respectively. Bosentan promoted BM-derived endothelial cell incorporation but inhibited macrophage infiltration into lungs in inhibiting PH in BM-chimeric mice exposed to chronic hypoxia.

Conclusions: BM-derived endothelial cells and macrophages of different stem cell origins contribute to PVD in mice. Modulation of BM-derived vascular cells could be a new mechanism of bosentan in inhibiting PH.

O2-3

ADIPONECTIN MEDIATES THE BENEFICIAL EFFECT OF PPARγ AGONIST ROSIGLITAZONE ON ENDOTHELIAL FUNCTION IN TYPE II DIABETIC MICE

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Background: Therapeutic benefit of peroxisome-proliferator-activated receptor (PPAR) agonist has been demonstrated in vascular disorders such as hypertension and atherosclerosis. PPARγ activation is also known to improve insulin resistance and lipid metabolism in type II diabetes. The present study investigated whether chronic PPARγ activation by rosiglitazone could improve endothelial function in an animal model of type II diabetes, db/db mice.

Methods: db/db mice received oral treatment of rosiglitazone or vehicle for 6 weeks. Plasma glucose was monitored during the treatment, and plasma insulin and adiponectin were measured. Vascular reactivity of isolated blood vessels was studied in myograph. Protein expressions were detected by western blotting.

Results: Rosiglitazone improved glucose tolerance, and reduced plasma insulin, total cholesterol, and triglyceride level. Rosiglitazone treatment restored the impaired endothelium-dependent relaxations in aortas, renal arteries, and resistant mesenteric arteries of db/db mice. Western blotting analysis showed an increased phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser^{1177} and phosphorylation of 5'AMP-activated protein kinase (AMPK) at Thr^{172} without affecting the total amount of eNOS or AMPK in rosiglitazone-treated mouse aortas. Moreover, rosiglitazone increased the plasma adiponectin level. Adiponectin treatment improved endothelial function in db/db mouse aortas, and increased phosphorylation of eNOS and AMPK. Improved endothelial function by PPARγ activation was inhibited in adiponectin knockout db/db mice.

Conclusions: The present study provides novel findings showing that the vasoprotective effect of rosiglitazone in type II diabetes by increasing NO bioavailability through adiponectin-AMPK signaling pathway. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences, and Focused Investment Scheme)

O2-4
BLACK TEA POLYPHENOLS PROTECT ENDOTHELIAL CELL FUNCTION

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Tea, the most popular beverage worldwide, is consumed in three basic forms; green tea, black tea and oolong tea. Tea contains over 4,000 chemicals some of which are bioactive. In recent years there has been a mounting interest in understanding the cardiovascular and metabolic benefits of polyphenolic flavonoids in tea, which can be used as a supplement in patients. Diverse cardioprotective effects of consuming tea or tea polyphenols have been described on pathological conditions, e. g. hypertension and atherosclerosis. Theaflavins are another class of polyphenol pigments found in black tea, however, little is known about their bioactivity in the vascular system. We have recently demonstrated that black tea and its theaflavins cause dilatations of rat aortas via endothelial nitric oxide-dependent mechanisms and the tea polyphenols are very effective in protecting endothelial function agonist oxidative stress. Chronic administration of black tea extracts in ovariectomized female rats restored endothelial function that was impaired during estrogen deficiency. The present results support the vascular benefit of consumption of black tea, which is equal to that of drinking green tea in terms of their endothelial cell protection and antioxidant capacity. (Supported by Hong Kong GRF, CUHK Focused Investment Scheme and CUHK Li Ka Shing Institute of Health Sciences).

O2-5

INDUCTION OF CARDIAC HYPERTROPHY BY RHO-ASSOCIATED KINASE 2 IS MEDIATED BY DOWNREGULATION OF FOUR-AND-A-HALF LIM-ONLY PROTEIN 2

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Previous studies have shown that Rho-associated kinase (ROCK) inhibitors could prevent the development of cardiac hypertrophy. However, mutant mice with targeted deletion of ROCK1 develop cardiac hypertrophy to the same extent as wild-type (WT) mice suggesting that ROCK2 may be involved in the hypertrophic process. To determine whether ROCK2 mediates cardiac hypertrophy, cardiomyocyte-specific ROCK2 null mice (c-ROCK2$^{-/-}$ mice) were generated using conditional ROCK2flox/flox mice and alpha-myosin heavy chain promoter-driven Cre recombinase transgenic mice. The c-ROCK2$^{-/-}$ mice are viable, and under basal condition, hemodynamic parameters, cardiac anatomy, and heart function were similar compared to wild-type (WT) mice. However, following angiotensin II (Ang II) infusion (400 ng/kg/min, 28 days), c-ROCK2$^{-/-}$ mice exhibited substantially less increase in heart to body weight ratio, left ventricular mass, myocyte cross-sectional area, hypertrophy-related fetal gene expression, intraventricular fibrosis, and cardiac apoptosis compared to WT mice. Yeast 2-hybrid screening of a human cDNA library identified the four-and-a-half LIM-only protein-2 (FHL2) as a binding partner of ROCK2 but not ROCK1. The FHL2 expression was elevated in c-ROCK2$^{-/-}$ hearts and ROCK2 activation leads decreased FHL2-mediated inhibition of serum response factor (SRF) and extracellular signal-regulated mitogen-activated protein kinase (ERK). Indeed, SRF and ERK activities were both decreased in the hearts of Ang II-stimulated c-ROCK2$^{-/-}$ mice. These results indicate that ROCK2 mediates cardiac hypertrophy through its inhibitory interaction with FHL2 and suggest that selective ROCK2 inhibitors or factors, which could upregulate FHL2 may be beneficial in the treatment of cardiac hypertrophy.

O3-1

THE CATECHIN-INDUCED REDOX-SENSITIVE ACTIVATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IS CRITICALLY DEPENDENT ON HYDROXYL MOIETIES

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Objectives: Several rich sources of polyphenols strongly increase the endothelial formation of nitric oxide (NO), a potent vasoprotecting factor, via the redox-sensitive activation of the PI3-kinase/Akt pathway leading to the phosphorylation of endothelial NO synthase (eNOS). The present study examined the molecular mechanism underlying the stimulatory effect of catechins on the endothelial formation of NO.

Methods: Vascular reactivity was assessed using porcine coronary artery rings in the presence of indomethacin and charybdotoxin plus apamin. The phosphorylation level of Akt and eNOS was assessed in cultured coronary artery endothelial cells by Western blot analysis. Both natural and synthetic catechins were evaluated.

Results: (-)-Epigallocatechin-3-O-gallate (EGCg) caused endothelium-dependent relaxations in coronary artery rings and the phosphorylation of Akt and eNOS in cultured endothelial cells. Both of these responses were inhibited by the membrane permeant analogue of SOD, MnTMPyP, whereas native SOD was without effect. They were also minimally affected by inhibitors of major cellular enzymatic sources of reactive oxygen species including NADPH oxidase, xanthine oxidase, cytochrome P450 and the mitochondrial respiration chain. Following replacement of all hydroxyl groups by O-methyl groups, the EGCg derivative induced neither endothelium-dependent relaxations nor phosphorylation of Akt and eNOS.

Conclusions: EGCg causes endothelium-dependent relaxations of coronary arteries via the redox-sensitive Akt-dependent activation of eNOS in endothelial cells. The stimulatory effect is critically dependent on the presence of hydroxyl groups possibly leading to auto-oxidation of this catechin.

O3-2
BIPHASIC EFFECTS OF INSULIN ON ENDOTHELIAL SENESCENCE

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Insulin is a main treatment tool and plays an important role in aging. Little is known about the effects of insulin on cellular senescence. The effects of insulin were investigated on senescence of human umbilical venous endothelial cells (HUVECs). Senescence-associated-β-galactosidase (SA-β-gal), human telomerase activity and telomere length were evaluated. High glucose increased SA-β-gal activity and decreased telomerase activity in 3 days. All concentrations of insulin also promoted endothelial senescence under normal glucose, but physiological concentrations of insulin decreased SA-β-gal, increased telomerase activity, and prevented telomere length shortening under high glucose. This was associated with reduced reactive oxygen species generation as well as increased NO production and endothelial NO synthase (eNOS) expression. Interestingly, high concentrations (10^{-7}-10^{-6} M) of insulin potentiated high-glucose-induced SA-β-gal activity, decreased telomerase activity, and shortened telomere length. Transfection of small-interfering RNA targeting eNOS reduced the anti-senescence effect of physiological concentrations of insulin but was without effect on insulin-associated endothelial senescence. Thus, high-glucose-induced endothelial senescence has the factors of both stress-induced and replicative senescence. Physiological concentrations of insulin delay cellular senescence by NO-dependent and telomere-related mechanism and may retard atherosclerosis formation under high glucose, while all concentrations of insulin under normal glucose or high concentrations of insulin under high glucose promote cellular senescence in an eNOS-independent way. These unique dual effects of insulin offer important clues for the regulation and pathophysiology of endothelial cell senescence in diabetes.

O3-3

POSITIVE FEEDBACK MECHANISM OF NO BY DIMETHYLARGININE DIMETHYLAMINOHYDROLASE-2 EXPRESSION VIA CYCLIC GMP INDUCTION IN ENDOTHELIAL CELLS

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Background: Dimethylarginine dimethylaminohydrolase (DDAH) is an enzyme that metabolizes asymmetric N^G,N^G-dimethyl-L-arginine (ADMA) and N^G-monomethyl-L-arginine (MMA), competitive endogenous inhibitors of nitric oxide synthase (NOS). However, it remains unknown whether NO itself influences DDAH activity to regulate NO generation in a biofeedback mechanism. We studied the effects of NO itself on the intracellular ADMA/MMA and DDAH expression and enzymatic activities in primary rat aortic endothelial cells (RAECs).

Methods: RAECs were isolated from Sprague-Dawley rats. DDAH mRNA levels were quantified by real-time RT-PCR, DDAH protein determined by Western blot and immunohistochemistry, intracellular arginine and methylarginines contents by HPLC, DDAH enzyme activities using [^{3}H]-MMA as substrates, and NOx in cultured medium by Greiss method.

Results: The NO donors SNAP and NOR3 did not influence the DDAH-1 mRNAs, but induced steady-state DDAH-2 mRNA and protein levels. SNAP upregulated DDAH enzymatic activity and reduced the MMA and ADMA contents. Suppression of DDAH-2 mRNA using small interfering RNA technology abrogated NO-induced DDAH-2 expression. The cGMP agonists 8-bromo cGMP and C-type natriuretic peptide also stimulated DDAH-2 gene and protein levels and increased nitrite/nitrate released into the culture supernatant. SNAP-induced DDAH-2 expression was inhibited by the protein kinase G inhibitor KT5823 and a soluble guanylate cyclase inhibitor ODQ, suggesting a mediatory role for cGMP in NO-induced DDAH2 expression.

Conclusions: Thus, NO acts on endothelial cells to induce DDAH-2 expression via a cGMP-mediated process to reduce ADMA/MMA. Thus, the DDAH-2-ADMA/MMA-eNOS regulatory pathway and NO-induced cGMP constitute a positive-feedback loop that ultimately serves to maintain NO levels in the endothelial environment.

O3-4

MODULATION OF RENIN-ANGIOTENSIN SYSTEM BY RENIN INHIBITOR ALISKIREN IMPROVES ENDOTHELIAL FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Aliskiren is the first orally renin inhibitor approved for the treatment of hypertension. Clinical studies suggest that aliskiren is effective in hypertension and diabetic nephropathy. The present study aimed to investigate whether direct renin inhibition by aliskiren can improve endothelial function and nitric oxide (NO) bioavailability in spontaneously hypertensive rats (SHRs).

Methods: SHRs and Wistar Kyoto rats (WKYs) were treated with vehicle and aliskiren for 8 weeks. Blood pressure was monitored biweekly. Changes in vascular reactivity in isolated aortas and intralobar renal arteries were studied in organ bath and myograph. Protein expression of endothelial NO synthase (eNOS), superoxide dismutases (SODs), angiotensin II type 1 receptor (AT1R) and nitrotyrosine were detected by Western blotting.

Results: Blood pressure lowering effect of aliskiren was prominent in SHRs but absent in WKYs. Endothelium-dependent relaxations to acetylcholine in SHRs were improved by aliskiren and endothelium-dependent contractions to acetylcholine in the presence of L-NAME was diminished in aliskiren-treated SHRs. Western blotting showed an increased phosphorylation of eNOS at ser1177 without affecting the total eNOS level, upregulation of SOD, as well as downregulation of AT1R and nitrotyrosine in aortas of aliskiren-treated SHRs. By contrast, aliskiren had minimal effects on above-mentioned markers in WKYs.

Conclusions: The present study provides novel evidence demonstrating that direct renin inhibition can effectively protect endothelial function in hypertension by augmenting NO bioavailability, thus in support of the therapeutic benefit of aliskiren in patients with hypertension. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme)

O4-1

LECTIN-LIKE OXIDIZED LDL RECEPTOR-1 (LOX-1) AS A NOVEL RECEPTOR FOR REMNANT-LIKE LIPOPROTEIN PARTICLES (RLP) IN VASCULAR SMOOTH MUSCLE CELLS

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Remnant-like lipoprotein particles (RLPs) have been implicated in vascular dysfunction and atherogenesis. In this study, we examined whether lectin-like oxidized LDL receptor-1 (LOX-1) acts as a receptor for RLPs in vascular smooth muscle cells (VSMCs). RLPs were isolated from human plasma by immunoaffinity gel mixture of anti-apolipoprotein A-1 and anti-apolipoprotein B-100 monoclonal antibodies. DiI-labeled RLPs were taken up by CHO-K1 cells stably expressing LOX-1 but not by wild-type CHO-K1 cells. Uptake of DiI-labeled RLPs was competitively suppressed by excess amounts of unlabeled RLPs or oxidized LDL (Ox-LDL), indicating that binding sites for RLPs and Ox-LDL on the LOX-1 molecule are identical or overlapped. RLPs induced LOX-1 expression and cell migration in VSMCs, which were significantly suppressed by LOX-1-specific siRNAs. AG1478, an inhibitor of epidermal growth factor (EGF) receptor phosphorylation, as well as by CRM197 which neutralizes heparin-binding EGF-like growth factor (HB-EGF), also suppressed RLP-induced LOX-1 upregulation and migration of VSMCs, indicating the involvement of HB-EGF shedding. Furthermore, PD98059, SB203580 and wortmannin, inhibitors of extracellular regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K), respectively, significantly blocked RLP-induced LOX-1 upregulation, and Y27632, a selective inhibitor of Rho kinase (ROCK) significantly suppressed RLP-induced cell migration. In conclusion, LOX-1 is a novel receptor for RLPs, which mediates LOX-1 expression and cell migration, involving shedding of HB-EGF and subsequent EGF receptor phosphorylation, and its down-stream signals including ERK, p38 MAPK, PI3K and Rho. LOX-1-mediated uptake of RLPs thus may play important roles in vascular dysfunction and atherogenesis.

O4-2

ANGIOPOIETIN-LIKE 2 IS A POTENT VASODILATOR AND HYPOTENSIVE PROTEIN IN MICE

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Angiopoietin-like 2 (Angptl2) is a 57 kDa protein released in the blood whose function and receptor remain unknown. Our objective was to study its effect on vasomotor tone and blood pressure regulation in mice. We purified an Angptl2-GST fusion protein from the media of stably transfected HEK 293 cells, by affinity chromatography on glutathione Sepharose. In vitro, exogenous addition of purified Angptl2 (1 nM) induced 64±7% relaxation (n=10) of preconstricted (U46619, 30 nM; thromboxane A2 analog) isolated mesenteric arteries from normal C57BL6 mice. This response was endothelium-independent (62±13%; n=4), while acetylcholine-induced relaxation was abolished in denuded arteries($P<0.05$). In intact arteries, while the nitric oxide synthase inhibitor N-nitro-L-arginine (L-NNA, 100 uM) magnified to 82±5% (n=9, $P<0.05$) Angptl2-induced relaxation, this response was fully prevented (n=4, $P<0.05$) by ODQ (1 uM), an inhibitor of the soluble guanylate cyclase. In vivo intra-carotid injection of Angptl2 (6 ug/kg, 200 uL bolus) induced a rapid drop in systolic blood pressure from 89±3 (n=9) to 70±3 mm Hg ($P<0.05$). The time to recover 50% of the pre-injection blood pressure (t50%) value was 250±63 sec. In the presence of L-NNA (5 mg/kg) or ODQ (1 mg/kg), baseline blood pressure increased ($P<0.05$) from 86±1 to 111±3 mm Hg and to 87±7 mm Hg, respectively; the hypotensive effect of Angptl2 (-19±2 mm Hg) was potentiated by L-NNA (-33±3 mm Hg), or prevented by ODQ (-2±2 mm Hg; $P<0.05$). These data point to a new physiological vasodilatory and hypotensive pathway activated in the nanomolar range byAngptl2 in mice.

O4-3

NBCn1 IS THE ONLY Na^+,HCO_3^- COTRANSPORTER IN VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS IN SITU: IMPORTANCE FOR VASCULAR TONE REGULATION

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We investigated the regulation of intracellular pH (pH_i) in vascular smooth muscle (VSMCs) and endothelial cells (ECs) of mouse mesenteric arteries using fluorescence microscopy. The importance of Na^+,HCO_3^- cotransport for arterial tone regulation was studied.

In arteries from NBCn1-/- mice, intracellular acidification of ECs after extracellular Na^+ removal in the presence of CO_2/HCO_3^- and amiloride was abolished. In VSMCs from NBCn1-/- mice, the Na^+- and HCO_3^--dependent pHi recovery after an NH_4^+-prepulse was abolished. In ECs and VSMCs, NBCn1 knockout reduced steady-state pH_i (0.1-0.3 units).

The response of isolated arteries to norepinephrine (NE) was unaffected by NBCn1 knockout but the decrease in EC50 for NE upon treatment with L-NAME was reduced in arteries from NBCn1-/- mice. Relaxation to acetylcholine (ACh) was smaller in arteries from NBCn1-/- mice. The difference in ACh-response disappeared after treatment with L-NAME. The response to the NO-donor SNAP, the expression of endothelial NO-synthase (eNOS) and the EC Ca^{2+}-response to ACh were all unaffected by NBCn1 knockout.

We conclude that NBCn1 is the only Na^+,HCO_3^- cotransporter in ECs and VSMCs in situ. We propose that NBCn1 is important for normal NO-mediated vasorelaxation through Ca^{2+}-independent modulation of eNOS.

O4-4

ROLE OF RHO KINASE IN THE INHIBITION OF ENDOTHELIUM-DEPENDENT VASORELAXATION TO ISOPRENALINE BY TP RECEPTOR ACTIVATION

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Background: Activation of Thromboxane A_2 (TP) receptors causes potent vasoconstriction, which contributes to increased vascular tone < blood pressure. The present study was designed to examine the hypothesis that stimulation of TP receptor impairs endothelium-dependent vasorelaxation to isoprenaline via Rho kinase mechanisms.

Methods: SD rat carotid arteries were isolated < suspended in myograph for the measurement of changes in isometric tension. The production of nitric oxide < intracellular calcium levels in primary cultured aortic endothelial cells was assayed with imaging technique. Phosphorylated eNOS protein was determined with western blot.

Results: U46619 concentration-dependently inhibited relaxation to isoprenaline in rings with or without endothelium. Treatment with Rho kinase inhibitors, Y27632 (2 μM) or HA 1077 (10 μM) improved agonist-evoked relaxation only in rings with endothelium < neither Rho kinase inhibitors affected isoprenaline-induced relaxation in L-NAME or ODQ-treated rings with endothelium. Isoprenaline stimulated a rise in nitric oxide production < intracellular calcium in cultured rat endothelial cells. Increased nitric oxide (NO) production was inhibited by U46619 (100 nM) < this effect was prevented by treatment with Y27632, which were verified by phosphorylated-eNOS level with western blot results.

Conculsions: The present results demonstrate that activation of Rho kinase is the primary mechanism that underlies TP-receptor-mediated inhibition of endothelial NO production < endogenous NO-dependent relaxation in response to isoprenaline.

O5-1

NITRIC OXIDE SYNTHASE AND SOLUBLE GUANYLYL CYCLASE ACTIVATION ARE REQUIRED FOR HYPOXIC ENDOTHELIUM-DEPENDENT CONTRACTIONS OF THE PORCINE CORONARY ARTERY

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The contractile response to hypoxia has been studied in the lung, and is termed hypoxic pulmonary vasoconstriction. However this response is also observed in coronary arteries. The present study investigated the mechanism underlying this contractile response in isolated porcine coronary arteries. Isometric tension was measured in rings with or without endothelium. In quiescent preparations, the contractile response to hypoxia was only observed in rings with endothelium and was abolished by indomethacin and S18886, demonstrating the involvement of cyclooxygenase products and TP receptor activation, respectively, in the phenomenon. In contracted preparations, the hypoxic response was also endothelium-dependent and was reduced by indomethacin and S18886. The endothelium-dependent hypoxic contractions were abolished by L-NAME, ODQ and NS2028 in both quiescent and contracted preparations. The addition of DetaNONOate in the presence of L-NAME restored the hypoxic response, suggesting the involvement of the nitric oxide pathway. Assay of the cyclic GMP content showed no change upon exposure to hypoxia in preparations with and without endothelium. These experiments suggest that hypoxic endothelium-dependent contractions of the porcine coronary artery depend on more than one signaling pathway.

O5-2

TESTOSTERONE ACTIVATES ENDOTHELIAL NITRIC OXIDE SYNTHASE THROUGH NON-GENOMIC SIGNALING OF ANDROGEN RECEPTOR

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Background: Epidemiological studies have shown that androgen deficiency in elderly men is associated with the higher incidence of cardiovascular disease. Although vasodilator action of testosterone has been reported, its action on endothelial nitric oxide synthase (eNOS) is not known. The purpose of this study was to investigate the effects of testosterone on eNOS activity in human aortic endothelial cells.

Methods and Results: Physiological concentrations of testosterone (1-100 nmol/L) augmented nitric oxide (NO) production, as measured by DAF-2 fluorescence, at 10-30 min. NOS activity as measured using the L-citrulline assay was twice increased by testosterone compared to vehicle. In parallel, testosterone rapidly induced the increase in phosphorylation levels of eNOS (Ser1177) in a concentration-dependent manner, peaking at 30 min after stimulation. eNOS activation by testosterone was associated with the increase in phosphorylation levels of Akt and was blocked by an Akt inhibitor, SH-5 and a PI3K inhibitor, wortmannin, suggesting that PI3K/Akt pathway is involved. The effects of testosterone on eNOS/NO were abolished by androgen receptor antagonists, nilutamide and flutamide, and by siRNA for androgen receptor, but were not inhibited by estrogen receptor antagonist ICI 182780 and transcriptional inhibitor actinomycin D, suggesting the non-genomic activation of eNOS via androgen receptor.

Conclusions: These results indicate that the non-genomic signaling of androgen receptor triggered by testosterone induces a rapid activation of eNOS and subsequent NO production via PI3K/Akt and pathway in endothelial cells. These provide a novel insight into vascular protective effects of testosterone.

O5-3

ANGIOTENSIN II IMPAIRS ENDOTHELIUM-DEPENDENT VASODILATATION VIA TYROSINE PHOSPHORYLATION OF THE ENDOTHELIAL NO SYNTHASE

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The proline-rich tyrosine kinase Pyk2 phosphorylates eNOS on tyrosine 657, thus inhibiting its activity and limiting NO production. Since Pyk2 can be activated by angiotensin (Ang) II and reactive oxygen species, we speculated that the Pyk2-mediated inhibition of eNOS contributes to the loss of NO bioavailability in pathologies associated with increased Ang II and H_2O_2 levels.

Ang II and H_2O_2 both enhanced the tyrosine phosphorylation of Pyk2 and eNOS and H_2O_2 (1 - 100 μmol/L) stimulated the phosphorylation of eNOS Tyr657 without affecting that of Ser1177. At these concentrations, H_2O_2 also limited basal NO production and markedly impaired NO production in response to bradykinin and ionomycin. H_2O_2 (30 μmol/L) also induced phosphorylation of eNOS on Tyr657 in murine aortae and impaired acetylcholine-induced vasodilatation. Endothelial overexpression of a dominant negative Pyk2 mutant protected against H_2O_2-induced endothelial dysfunction. Correspondingly, carotid arteries from eNOS-/- mice in which the endothelium was transduced with an adenovirus expressing the non-phosphorylatable eNOS Y657F mutant were protected against H_2O_2. Chronic (3 week) treatment of wild-type mice with Ang II considerably increased levels of Tyr657 phosphorylated eNOS in aortae and this was again associated with a clear impairment in endothelium-dependent vasodilatation.

Taken together, Pyk2 activation under pathological conditions, i.e. increased Ang II and H_2O_2 concentrations, causes the phosphorylation of eNOS on Tyr657 attenuating NO production and endothelium-dependent vasodilatation. This mechanism, in addition to eNOS uncoupling, may underlie the endothelial dysfunction observed in cardiovascular diseases associated with increased activity of the renin-angiotensin system and elevated redox stress.

O5-4

AGING INDUCES ENDOTHELIAL DYSFUNCTION WHILE SPARING ARTERIAL THROMBOSIS

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Background: Aging is the major cardiovascular risk factor. Cardiovascular events occur due to arterial thrombosis; however, it is not known whether aging itself promotes arterial thrombosis in the absence of any other risk factor and atherosclerotic lesions. Unlike humans, wild type mice on a regular diet do neither develop cardiovascular risk factors other than aging nor spontaneous atherosclerosis; thus, they represent an ideal model to investigate age as an independent factor.

Methods and Results: 105 week old male C57BL/6 mice were compared to 11 week old controls. Organ chamber experiments revealed that aortas of old mice exhibit endothelial dysfunction in response to acetylcholine with reduced bioavailability of nitric oxide ($p<0.01$ for maximal relaxation to acetylcholine). In contrast, arterial thrombosis induced by photochemical injury did not differ in old and young animals (-0.1±6.9 minutes, $p=0.99$, $n=5$). Arterial tissue factor expression and activity, prothrombin time and partial thromboplastin time, and shear stress dependent platelet adhesion were similar in the two groups (p=n.s. for all parameters). Histological analysis of aortic root, carotid artery, and descending aorta did not reveal any differences except for sporadic single cells staining positive for Oil-red-O in the old mice. Fasting blood glucose and total plasma cholesterol were similar in the two groups, while plasma triglycerides were lower in the old mice.

Conclusions: Aging per se, despite inducing endothelial dysfunction, does not promote arterial thrombosis. This study underscores the importance of controlling modifiable risk factors in old patients.

O5-5

MODULATION OF CALCIUM-ACTIVATED K^+ CHANNELS BY CHRONIC SHEAR STRESS ALTERATIONS IN THE RAT MESENTERIC ARTERIAL BED

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Aim: We tested the hypothesis that chronic alterations in wall shear stress (WSS) modulate expression and contribution of endothelial small- and intermediate Ca^{2+}-activated K^+ channels ($K_{Ca}2.3$ and $K_{Ca}3.1$, respectively) to ACh-induced EDHF-mediated relaxations (EDHF response).

Methods and Results: We measured the expression and participation of the $K_{Ca}2.3$ and $K_{Ca}3.1$ channels in the EDHF response in mesenteric resistance arteries (MA) of 12-weeks old WKY rats exposed for 1 day (1d) or 7 days (7d) to either a low (-90%; LF), high (+90%; HF) or normal blood flow (NF) in vivo.

In LF-1d and LF-7d a larger $K_{Ca}2.3$-mediated signalling was observed compared to NF as evidenced by (i) a larger inhibition of the EDHF response by the selective $K_{Ca}2.3$ blocker apamin (0.5 μmol/L), (ii) an enhanced relaxing response to the K_{Ca} channel opener NS309 in the presence of the selective $K_{Ca}3.1$ antagonist TRAM-34 (0.3 μmol/L), (iii) an increased $K_{Ca}2.3$ channel protein expression.

In HF-1d and HF-7d a larger $K_{Ca}3.1$-mediated signalling was observed compared to NF as evidenced by (i) a larger inhibition of the EDHF response by TRAM-34, (ii) an enhanced response to NS309 in the presence of apamin, (iii) an increased $K_{Ca}3.1$ channel protein expression.

Conclusion: $K_{Ca}2.3$ and $K_{Ca}3.1$ channels are differentially modulated by WSS in small MA.

O6-1

VASOHIBIN-1 PREVENTS CELLULAR SENESCENCE AND MAINTAINS VASCULAR ENDOTHELIAL CELLS

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Background/Purpose: We isolated vasohibin-1 that is specifically expressed in endothelial cells (ECs) in response to angiogenic factors (VEGF and FGF-2), and inhibits angiogenesis in vitro and in vivo. In general, angiogenesis stimulators promote EC survival and vascular growth, whereas angiogenesis inhibitors promote EC death and vascular regression. The aim our study is to characterize the role of vasohibin-1 in EC survival/death.

Methods/Results: We established vasohibin-1 overexpressing MS1 clones. MS1 is a mouse endothelial cell line. Vasohibin-1 inhibited proliferation and migration of MS1. To examine the role of vasohibn-1 in cell survival, we exposed those clones to cellular stress; H2O2 or serum starvation. The clones showed the resistance to cell death. Next, we used primary endothelial cells, human umbilical endothelial cells (HUVECs). Using vasohibin-1 adenoviral vector, we transiently overexpressed vasohibin-1 in HUVECs. Likewise, vasohibin-1 inhibited cell proliferation and stress-induced cell death of HUVECs. To further examine the role of vasohibin-1 against cellular senescence, we exposed HUVECs to H2O2, and stained the cells with senescence-associated β-galactosidase (SA-β-gal), which is a biomarker of cellular senescence. Cellular senescence was inhibited in vasohibin-1 overexpressing HUVECs. Conversely, when vasohibin-1 was knocked-down with siRNA in HUVECs, the cells turned enlarged and flattened shape that is typically cellular senescence-phenotype, and SA-β-gal positive cells also significantly increased.

Conclusion: An angiogenic inhibitor, vasohibin-1, increased the resistance of ECs to cellular stresses, and exhibited protective effects against cellular senescence. Therefore, we propose that vasohibin-1 is involved in the maintenance of ECs by increasing stress tolerance.

O6-2

SMALL G PROTEIN RhoA AND Rac COORDINATELY REGULATE STROMAL CELL DERIVED FACTOR-1α-MEDIATED ANGIOGENESIS IN A NITRIC OXIDE DEPENDENT FASHION

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Background: Stromal cell derived factor-1α(SDF1α), a chemokine that controls angiogenesis, has been demonstrated to activate eNOS and small G proteins. However, the molecular mechanism(s) underlying SDF1α-mediated angiogenesis and the role of small G proteins have yet to be elucidated.

Methods: Human umbilical venous endothelial cells were cultivated and subjected to in vitro angiogenesis assay using matrigel. Small G proteins (Rho, Rac, and Cdc42) acitivities were determined by pull-down assay using specific recombinant proteins. Rho kinase (RK) activity was assessed by changes in the MYPT1 phosphorylation level using immunoblot. Rho and RK activities were inhibited by overexpression of its dominant negative (dn) form by adenoviral vector. mDia activity was suppressed by siRNA.

Results: SDF1α activated endothelial Rac and Rho, not Cdc42. Akt/eNOS phophorylation, of which level is positively regulated by Rac/PI3K, was enhanced by SDF1α. SDF1α-induced Akt/eNOS phosphorylation was reversed by PI3K inhibitor wortmannin and by eNOS inhibitor L-NAME. In contrast, SDF1α had no effect on RK activity and inhibition of RK exhibited no effect on the Akt/eNOS phosphorylation. In vitro angiogenesis promoted endothelial Rac and Rho activation, which were enhanced by SDF1α and inhibited by wortmannin and L-NAME. Functional deletion of Rho inhibited in vitro angiogenesis, on which RK inhibition had no effect. mDia, another downstream target of Rho, remarkably disrupted in vitro angiogenesis.

Conclusion: SDF1α-mediated angiogenesis is simultaneously regulated by Rac and Rho. Rac promotes PI3K/Akt/eNOS activation. Rho activation links to its downstream target mDia, not to Rho-kinase, presumably leading to endothelial elongation.

O6-3

THE STEADY-STATE EXPRESSION LEVEL OF CONNEXIN43 IS MAINTAINED BY THE PI3K/AKT PATHWAY

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Gap junction in vascular walls plays an important role in vascular homeostasis. It has been already reported that endothelial cells express the gap junction proteins, connexin 37 (Cx37), Cx40 and Cx43, and the expression of Cxs is not uniform in the endothelium of the vessels, depending on the size of vessels. Among them, Cx43 are well-known to be related to be hypertension. The turnover of Cx43 protein is very fast and faster than that of Cx43 mRNA in the steady-state of the cells. This means that the maintaining of a high-level of Cx43 proteins in vascular cells under normal condition is contributed to the regulation of Cx43 mRNA expression. To investigate the mechanism to keep Cx43 expression level, the effects of protein kinase inhibitors on the basal expression of Cx43 were examined. It was found that the specific PI3K inhibitor LY294002 significantly decreased the steady-state expression levels of Cx43 mRNA and protein in the cells. Furthermore, dominant-negative Akt expression reduced both Cx43 expression and gap junction activity. These results suggest an important role of PI3K/Akt in the regulation of basal Cx43 expression. A promoter assay for Cx43 and an actinomycin D chase experiment revealed that PI3K/Akt modulated Cx43 expression post-transcriptionally via mRNA stability. The present findings could provide new insights into the molecular understanding of Cx43 expression.

O6-4

CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 IS INVOLVED IN ENDOTHELIUM-DEPENDENT CONTRACTIONS OF THE AORTA OF THE SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Phospholipase A2, a regulatory enzyme found in most mammalian cells, catalyzes membrane phospholipids to arachidonic acid. There are two major cytosolic types of the enzyme, calcium-dependent (cPLA2) and calcium-independent (iPLA2) phospholipase A2. It could be expected that cPLA2 is the major form of PLA2 in producing endothelium-dependent contracting factors (EDCF) since Ca^{2+} plays a crucial role in the process. The present study investigated whether or not iPLA2 plays a role in such responses of the aorta of the spontaneously hypertensive rat (SHR) as well as the control Wistar-Kyoto rat (WKY).

Methods and Results: Immunohistochemistry showed that iPLA2 was densely distributed in endothelial cells of both SHR and WKY aorta. At 10 μM, the selective iPLA2 inhibitor, bromoenol lactone (BEL), abrogated endothelium-dependent contractions induced by both acetylcholine and A23187. At 5 μM, only the contractions induced by acetylcholine were inhibited. Incubation with arachidonic acid methyl ester together with BEL restored the contractions. Similar results were obtained from the measurement of the release of prostacyclin and thromboxane A2. The involvement of store-operated calcium channels (SOC) was demonstrated by the inhibitory effect of the SOC inhibitor, SKF96365, on endothelium-dependent contractions.

Conclusions: iPLA2 plays a substantial role in generating endothelium-derived contracting factors and both calcium-dependent and -independent pathways are involved in the process.

O6-5

SIRT1 PROMOTES CELL PROLIFERATION AND PREVENTS CELLULAR SENESCENCE THROUGH TARGETING LKB1 IN PRIMARY CULTURES OF PORCINE AORTIC ENDOTHELIAL CELLS

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Background: Endothelial dysfunction is an early and persistent vascular abnormality during the development of cardiovascular diseases (CVD). The biological age of endothelial cells of patients with CVD is often older than their chronological age. In fact, endothelial cell aging is a common process preceding the development of vascular dysfunctions. Sirtuins are a family of proteins critically involved in caloric restriction-mediated anti-aging activities. The present study evaluated the roles of SIRT1 in the process of replicative senescence during prolonged culture of porcine aortic endothelial cells (PAECs).

Methods and Results: PAECs were harvested from the aorta of female pig hearts and cells passaged at regular intervals. After four passages, PAECs tended to de-differentiate and eventually reached senescence. The cumulative population doubling was 19.18 from passage one to four, at which the cells showed decreased telomerase activities, lost their proliferative capacities and original morphology, but acquired a “fried egg” appearance. The expression levels of SIRT1 were progressively decreased during this process. Over-expression of SIRT1 stimulated endothelial cell proliferation and eNOS-phosphorylation. By contrast, the protein levels of serine threonine kinase 11 (STK11, LKB1), was upregulated in senescent PAECs. Transient transfection of vector encoding LKB1 inhibited endothelial cell proliferation, decreased eNOS-phosphorylation and increased the senescence-associated β-galactosidase activities. On the other hand, replacement of SIRT1 reduced LKB1 levels and antagonized its effects. Further analysis suggested that SIRT1 could interact with and regulate the acetylation status of LKB1.

Conclusion: In endothelial cells, SIRT1 exhibits its protective functions and anti-aging activities at least partly by targeting LKB1.

P1-1

THE AKT-MEDIATED ACTIVATION OF ENDOTHELIAL NO SYNTHASE BY A RED WINE EXTRACT INVOLVES PROCYANIDIN DIMERS AND OLIGOMERS, AND CONJUGATED ANTHOCYANINS

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Objectives: Previous studies have shown that red wine phenolic extracts strongly enhance the endothelial formation of nitric oxide (NO), a vasoprotective factor, by causing the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO synthase (eNOS). Red wine phenolic extracts are complex mixtures of several hundreds of compounds. Therefore, the purpose of the present study was to identify active polyphenols using multi-step bioguided fractionation of the red wine extract.

Methods: The red wine phenolic extract was submitted to a bioguided fractionation using chromatographic methods. Cultures of porcine coronary artery endothelial cells were used to determine the phosphorylation level of Akt and eNOS by Western blot analysis. Phenolic compounds in active fractions were identified by MALDI-TOF and HPLC-MS techniques.

Results: The first step of fractionation yielded 9 fractions of which 4 of them significantly increased the phosphorylation level of Akt and eNOS in endothelial cells. The active fractions contained mainly procyanidins and some anthocyanin compounds. The fractionation of one of the active fractions yielded 11 sub-fractions; all of these sub-fractions significantly increased the phosphorylation level of Akt and eNOS. The analysis of the phenolic compounds indicated that these sub-fractions contained mainly mixtures of procyanidin dimers and conjugated anthocyanins.

Conclusion: The red wine extract contains several types of phenolic compounds, which are able to cause the Akt-mediated activation of eNOS by phosphorylation in endothelial cells. The active phenolic compounds include predominantly procyanidins dimers and oligomers, and several conjugated anthocyanins.

P1-2

CHRONIC INTAKE OF RED WINE POLYPHENOLS BY YOUNG RATS PREVENTS THE DEVELOPMENT OF AN ENDOTHELIAL DYSFUNCTION

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Introduction: Aging is associated with an endothelial dysfunction & vascular oxidative stress. This study examined whether chronic intake of red wine polyphenols (RWPs), which are known to stimulate the endothelial formation of NO & EDHF, & to have antioxidant properties, by young adult rats is able to prevent the subsequent development of an endothelial dysfunction with age.

Methods: Vascular reactivity of mesenteric artery rings from young (12 weeks) & old (40 weeks) male Wistar rats was assessed in organ chambers. Young adult rats received either solvent (3 % ethanol), RWPs extract (25 or 50 mg/kg/day) or the antioxidant apocynin (100 mg/kg/day) in the drinking water from week 16 until week 40. The expression level of eNOS, arginase 1, angiotensin II receptors, NADPH oxidase subunits & nitrotyrosines was assessed by immunohistochemistry & reactive oxygen species (ROS) by dihydroethidine. Exercise capacity was assessed by an endurance capacity test.

Results: Aging is associated with an endothelial dysfunction charaterized by blunted endothelium-dependent relaxations, oxidative stress, an upregulation of eNOS, arginase 1, NADPH oxidase subunits (nox-1, p22phox), AT1 and AT2 receptor expression. Intake of RWPs or apocynin starting at week 16 until week 40 prevented the induction of endothelial dysfunction & normalized oxidative stress & the expression of the different proteins. In addition, exercise capacity was significantly improved in 40-week old rats receiving RWPs or apocynin.

Conclusions: These findings indicate that intake of RWPs prevents endothelial dysfunction in aging by normalizing vascular oxidative stress & the expression of eNOS, NADPH oxidase & angiotensin receptors.

P1-3

ANTI-ATHEROGENIC EFFECT OF OLEANOLIC ACID APPEARS UNRELATED TO ENDOTHELIAL RELEASE OF NITRIC OXIDE

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The present study investigated the mechanisms by which oleanolic acid, a component of olive oil, increases release of nitric oxide (NO), and investigated the effect of oleanolic acid in an atherosclerotic animal model. Measurements of isometric tension, NO concentration, and endothelial cell calcium were performed in isolated rat mesenteric arteries. 8 weeks ApoE-/- mice were feed a Western-type diet in combination with oleanolic acid (ApoE-/--OA; 100 mg/kg/day), fluvastatin (ApoE-/--fluvastatin; 5 mg/kg/day), or vehicle (ApoE-/--Vehicle). Wild type mice were used as negative controls. Oleanolic acid, caused endothelium-dependent relaxation in large and small mesenteric arteries from rats. The release of NO was calcium-independent and due to phosphorylation of Akt and endothelial NO synthase at serine1177. In the animal study total plasma cholesterol levels were similar among all groups of ApoE-/- mice, while atherosclerotic plaque area was reduced in aorta from oleanolic acid-treated ApoE-/- and fluvastatin-treated ApoE-/- mice, compared to vehicle-treated ApoE-/-mice. Compared to vehicle and fluvastatin-treated ApoE-/- mice, aortic segments from wild type and oleanolic acid-treated ApoE-/- mice had a greater response to phenylephrine and less expression of inducible NO synthase. Acetylcholine relaxation was not improved in aortae from oleanolic acid or fluvastatin treated ApoE-/- mice. Oleanolic acid increases release of NO in isolated rat arteries and chronic administration of oleanolic acid has a pronounced anti-atherogenic effect in ApoE-/- mice, but the anti-atherogenic effect of oleanolic acid seems unrelated to endothelial release of NO.

P1-4

RED WINE POLYPHENOLS IMPROVE ENDOTHELIAL DYSFUNCTION IN THE MESENTERIC ARTERY OF OLD RATS: ROLE OF OXIDATIVE STRESS

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Introduction: Aging is associated with blunted endothelium-dependent relaxations & vascular oxidative stress. The aim of the present study is to determine whether intake of red wine polyphenols (RWPs), which are known to have antioxidant properties & to stimulate the endothelial formation of nitric oxide (NO) & endothelium-derived hyperpolarizing factor (EDHF), improves the endothelial function in old rats.

Methods: Vascular reactivity of mesenteric artery rings from young (12 weeks) & old (55 weeks) male Wistar rats, was assessed in organ chambers. Old rats received either solvent (3% ethanol), RWPs extract (100 mg/kg/day) or the antioxidant apocynin (100 mg/kg/day) in the drinking water for 4 weeks. The expression level of eNOS & arginase 1, angiotensin II receptors (AT1R and AT2R), NADPH oxidase subunits & nitrotyrosines was assessed by immunohistochemistry, & the formation of reactive oxygen species (ROS) by dihydroethidine.

Results: Aging is associated with blunted endothelium-dependent relaxations to acetylcholine & an excessive vascular formation of ROS & peroxynitrites. Both the NO- & the EDHF-mediated relaxations are reduced. Aging upregulated eNOS, arginase 1, NADPH oxidase subunits (nox-1, p22phox), AT1R & AT2R expression. RWPs & apocynin treatments improved endothelial dysfunction, normalized oxidative stress & the expression of the different proteins in old rats.

Conclusion: The present findings indicate that aging is associated with blunted endothelium-dependent relaxations involving an increased oxidative stress. Intake of RWPs for 4 weeks by old rats is able to improve endothelial dysfunction, & to normalize vascular oxidative stress & the expression of eNOS, arginase 1, NADPH oxidase & angiotensin receptors.

P1-5

EPIGALLOCATECHIN GALLATE CAUSES ENDOTHELIUM DEPENDENT CONTRACTIONS IN THE RAT AORTA

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Background: The regular consumption of green tea is associated with a reduced risk of cardiovascular disease. Among the catechins of green tea, epigallocatechin gallate (EGCG) is the most abundant and possesses anti-oxidant and vasodilator properties. The release and actions of endothelium-derived contracting factors (EDCF) involve oxygen-derived free radicals and contribute to endothelial dysfunction in hypertension. The present study was designed to evaluate the effects of EGCG on endothelium-dependent contractions.

Methods: Aortae, with and without endothelium, derived from 36 weeks old spontaneous hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats were studied in organ chambers for isometric tension recording.

Results: From 10^{-9} to 10^{-5} M, EGCG did not inhibit endothelium-dependent contractions evoked by acetylcholine or adenosine triphosphate in aortae of both SHR and WKY rats, partly indicating that EGCG did not improve vascular function by preventing generation of EDCF. On the other hand, 10^{-6} to 10^{-4} M of EGCG significantly induced concentration-dependent contractions, which were larger in preparations with than in those without endothelium. Moreover, L-NAME (inhibitor of endothelial NO synthase] potentiated and indomethacin (inhibitor of cyclooxygenases) abolished EGCG's effects on vasocontractions. The contractions to EGCG were larger in SHR than in WKY aortae.

Conclusions: EGCG does not inhibit EDCF-mediated responses in the rat aorta. However, higher concentrations of the catechin cause both endothelium-dependent and independent increases in tension which are inhibited by NO and involve the production of vasoconstrictor prostanoids both in the endothelial cells and in vascular smooth muscle.

P1-6

SIRTUIN-1 AND AMP-DEPENDENT PROTEIN KINASE MEDIATE ARTERIAL RELAXATIONS CAUSED BY THE POLYPHENOL, S17834

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Polyphenols, such as resveratrol, are known activators of sirtuin-1 (sirt1), an NAD-dependent class III deacetylase capable of promoting cardiovascular protection, metabolic health, and longevity. Our studies have shown that the synthetic polyphenol, S17834, activates sirt1 and AMP-dependent protein kinase in HepG2 cells, and has potent antioxidant, anti-inflammatory, and anti-atherosclerotic actions in mice. This study reports potent direct smooth muscle vasodilator properties of S17834 that were noted following studies in which the compound proved to be antihypertensive in type 1 or type 2 diabetic low density lipoprotein receptor-deficient mice. S17834 fully relaxed mouse and rat aortic rings denuded of endothelium and contracted with phenylephrine. The concentration causing 50% relaxation was 0.5 - 1 μM, which was 100-fold less than that of resveratrol. Rings with intact endothelium were less sensitive to S17834 than rings without endothelium. The sirt1 inhibitors, nicotinamide (5 mM) or sirtinol (20 μM) significantly inhibited relaxations to S17834 in endothelium-denuded aortic rings. The sirt1 activator, SRT1720, relaxed only 85% and was 10 times less potent than S17834. The AMP kinase activators, AICAR or A769662 induced incomplete relaxations at 1 - 2 mM and 25-50 μM, respectively. Relaxations to S17834 were significantly shifted to the right by the AMP kinase inhibitor, compound C (30 μM). These data indicate that S17834 has potent direct smooth muscle vasodilator actions mediated via sirt1 and AMP kinase. They also suggest that effective targeting of sirt1 and AMP kinase may be promising avenues for antihypertensive therapy.

P1-7

CHRONIC ANTIOXIDANT CATECHIN PRESERVES H2O2-DERIVED eNOS DILATIONS AND MAINTAINS CEREBRAL BLOOD FLOW IN AGING ATHEROSCLEROTIC MICE

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The decline in cerebrovascular endothelial function and cerebral blood flow (CBF) regulation observed with age and cardiovascular diseases is associated with increased free radical production. We hypothesized that chronic antioxidant treatment with catechin would slow atherosclerosis progression and maintain cerebrovascular functions. Atherosclerotic (ATX: LDLR-/-;ApoB+/+) 3 m/o mice were treated with catechin (30 mg/kg/day) for 9 months. C57Bl6 (WT) mice were used as controls. Endothelium-dependent dilations (EDD) to acetylcholine were recorded in pressurized cerebral arteries. Thoracic aorta atherosclerotic plaque area rose from 2±1 to 65±2% (3 to 12 m/o), but was limited to 42±3% by catechin. EDD decreased with age in WT (45±6 to 20±2%) and even further in ATX (26±2 to 7±2%). During EDD, eNOS-derived H2O2 release (quantify by DCFDA oxidation) decreased with age in WT (203±28 to 93±12 a.u.) but not in ATX (105±6 to 109±13 a.u.). Catechin improved EDD (24±4%) and H2O2 release (157±16 a.u.). NOS inhibition (L-NNA) reduced EDD in 3 and 12 m/o WT and 3 m/o ATX (23±2, 5±2 and 13±4 %, respectively), but not in 12 m/o ATX (10±3%); catechin however, restored the inhibitory effect of L-NNA (5±1 %). The increase in CBF induced by whisker stimulation (laser Doppler flowmetry) was similar in 3 and 12 m/o WT (35±2 and 33±1%), but lower at both ages in ATX mice (19±5 and 20±1%, respectively). Catechin restored CBF responses (30±1%). In conclusion, chronic catechin treatment limits endothelial dysfunction by protecting the eNOS pathway, and restores the coupling between CBF and increased neuronal activity in ATX mice.

P2-1

GENETIC DELETION OF POLY (ADP-RIBOSE) POLYMERASE PROMOTES OXIDATIVE STRESS INDUCED ENDOTHELIAL DYSFUNCTION

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Background: Increased production of reactive oxygen species (ROS) and loss of endothelial NO bioavailability are key features of vascular disease. Activation of the nuclear enzyme poly (adenosine diphosphate [ADP]-ribose) polymerase (Parp) is considered a downstream effector of oxidative stress, leading to NAD+ consumption and ATP depletion. We assessed the effect of genetic PARP-1 deletion on vascular function in paraquat-induced oxidative stress.

Methods and Results: Parp-1(-/-) and wild-type (WT) mice were injected with paraquat (10 mg/kg per kg of body weight) or sodium chloride (control) 24 hours prior to experiment. The aortae were harvested and examined in organ chambers for isometric tension recording. Paraquat treated (PQ) Parp-1(-/-) mice displayed markedly impaired endothelium-dependent relaxations, and N(omega)-nitro-L-arginine methyl ester (L-NAME)-induced contractions were significantly enhanced in PQ Parp-1 (-/-) mice. In contrast, untreated Parp-1 (-/-) mice did not develop such alterations; moreover endothelial function remained unaffected in WT mice with or without PQ. In the presence of superoxide dismutase (SOD) and catalase, endothelium-dependent relaxations were completely restored in PQ treated Parp-1 (-/-) mice, but were not affected in the other groups. Pretreatment with indomethacin completely reversed vascular dysfunction in the aortae of PQ treated Parp-1(-/-) mice, but did not affect vasodilatation in the other groups.

Conclusions: Mice lacking the Parp-1 gene exhibit a decreased resistance to oxidative stress. The endothelial dysfunction occurring under these conditions is mediated by an enhanced production of vasoconstrictor metabolites of arachidonic acid (endothelium-derived contracting factor).

P2-2

PHARMACOLOGICAL INHIBITION OF ADIPOCYTE-FATTY ACID BINDING PROTEIN (A-FABP) IMPROVES ENDOTHELIAL FUNCTION IN MALE APOLIPOPROTEIN E-KNOCKOUT MICE

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Adipocyte-fatty acid binding protein (A-FABP) modulates inflammatory responses in macrophages and may play a role in formation of foam cells and atherosclerotic plaques. A-FABP is markedly upregulated in regenerated porcine coronary arterial endothelial cells. The project were designed to investigate the presence (or not) of A-FABP as well as endothelial function at early stages of atherosclerosis in the aorta of 8, 12 and 18 weeks old male C57 apolipoprotein E-knockout ($ApoE^{-/-}$) mice. The effect was determined by treatment with a selective A-FABP inhibitor, BMS 309403, in 12-week old $ApoE^{-/-}$ mice. A-FABP was detected by immunoflorescent staining in the endothelium of the aorta at 12, but not 8 weeks. In myograph experiments, the endothelium-dependent relaxations to acetylcholine and UK14304 (a selective α2-adrenoceptor agonist) were reduced significantly in the $ApoE^{-/-}$ mice at 8 and 12 weeks on, respectively, compared to those obtained in wild type mice. Relaxations to the calcium ionophore A23187 were diminished significantly only from 18 weeks. Treatment with the A-FABP inhibitor significantly improved the relaxation to acetylcholine and UK14304 but not that to A23187 without affecting the plasma lipid profile. In conclusion, A-FABP was detected in male atherosclerotic-prone $ApoE^{-/-}$ mice since the age of 12 weeks. Endothelial dysfunction was observed as early as at 8 weeks of age and deteriorated until 18 weeks, as judged from the reduced relaxations to acetylcholine, UK14304 and A23187. Endothelial dysfunction can be alleviated by treatment with an A-FABP inhibitor, suggesting that A-FABP may be a novel target for the treatment of endothelial dysfunction.

P2-3

EFFECT OF NADPH OXIDASE INHIBITOR AND SUPEROXIDE DISMUTASE MIMETIC ON THE EXPRESSION OF NITRIC OXIDE SYNTHASE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Both oxidative stress and the expression of nitric oxide synthase (NOS) are higher in spontaneously hypertensive rats (SHR) than in Wistar-Kyoto rats. To clarify the relationship between oxidative stress and the NOS expression, we compared the effect of chronic treatment with a NADPH oxidase inhibitor, apocynin and a superoxide dismutase mimetic, tempol in SHR.

Method: 5 week-old, male SHR were randomly divided into three groups; a control group, an apocynin group or a tempol group, and treated with vehicle, apocynin or tempol in drinking water for 8 weeks. The NADPH oxidase activity in the kidney and plasma and urine H2O2 were measured. The expression of endothelial and neuronal NOS (eNOS and nNOS) proteins in the kidney and the thoracic aorta was analyzed using Western blots.

Results: Systolic blood pressure was lower in the drug-treatment groups than in the control group. Plasma creatinine was significantly lower in the tempol group than in the control group. NADPH oxidase activity was lower in the drug-treatment groups than in the control group, Plasma and urine H2O2 were lower in the apocynin group and higher in the tempol group than in the control group, The expression of eNOS and nNOS proteins in the kidney and the thoracic aorta was lower in the apocynin group and higher in the tempol group than in the control group.

Conclusions: The expression of eNOS and nNOS proteins in SHR was changed conversely by apocynin and tempol. Oxidative stress, especially H2O2 may upregulate the expression of NOS in SHR.

P2-4

QUINONE-BASED SYSTEM FOR THE CONTROLLED INDUCTION OF OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN ISOLATED ARTERIES

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We designed an original system (patent subject) for controlled generation of oxygen free radicals and tested it for applicability in evaluating the effects of oxidative stress upon endothelium-dependent relaxation. We used a quinone, that turns into semi-quinone radical when irradiated in UV. The latter is able to transform molecular oxygen into singlet oxygen, which then forms reactive oxygen species, predominantly superoxide. The intensity and duration of UV irradiation of the samples were precisely controlled. We used lucigenin amplified chemiluminescence to determine optimal quinone concentration (0.5 mM) and optimal irradiation duration. Experiments were done in triplicate, either without irradiation of the quinone solution (control) or with irradiation for three different durations. Chemiluminescence revealed a linear relation between free radical generation and irradiation duration. In separate experiments we applied the same irradiation protocol to 5 ml batches of Krebs-Henseleit solution (containing 0.5 mM quinone), which were immediately used to incubate rings of rat mesenteric arcade and first order branches, mounted and equilibrated in the chamber of a wire myograph. Using this procedure of controlled oxidative stress we induced an endothelial dysfunction, without affecting the contractile responses, elicited by phenylephrine and high extracellular potassium, or the endothelium-independent relaxation by nifedipine. Using inhibition of nitric oxide synthase and cyclooxygenase we showed that this reduction of endothelium-dependent relaxation is due to reduced NO bioavailability, which is partially compesated by an amplification of the EDHF component, as also shown in other studies regarding the effects of oxidative stress upon endothelium-dependent relaxation. Supported by Romanian Grant CNCSIS-A1222/2007-2008.

P2-5

TOLL-LIKE RECEPTOR 4 INCREASES OXIDATIVE STRESS BY INHIBITING EXTRACELLULAR SUPEROXIDE DISMUTASE IN ANGIOTENSIN II-INDUCED HYPERTENSION

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Objective: Toll-like receptor 4 (TLR4) and angiotensin II (AngII) induce vascular remodeling through the production of reactive oxygen species (ROS) in the vascular wall. AngII has been also shown to increase anti-oxidant enzyme extracellular superoxide dismutase (ecSOD). However, the relationship between TLR4 and ecSOD in regulating ROS remains unknown. We investigated the effects of TLR4 on ROS and ecSOD in AngII-induced hypertension *in vivo* .

Methods and Results: $TLR4^{-/-}$ and wild-type (WT) mice were randomly subjected to administration of AngII or norepinephrine (NE) for 2 weeks. The control WT and $TLR4^{-/-}$ mice showed little differences in superoxide ($\cdot O_2$-) content, wall-to-lumen ratio, perivascular fibrosis, and the expression of ecSOD and Cu/ZnSOD in the abdominal aorta, and blood pressure (BP). Both drugs equally increased BP compared with the control WT and $TLR4^{-/-}$ mice. In WT mice, AngII induced vascular remodeling and a 10-fold increase in both $\cdot O_2$- content and ecSOD compared with the control WT mice ($p<0.05$). In contrast, in $TLR4^{-/-}$ mice, AngII did not affect $\cdot O_2$- content and vascular remodeling, whereas AngII increased ecSOD by 2-fold compared with the WT mice ($p<0.05$). AngII did not affect Cu/ZnSOD, and NE showed little effects on these indices in WT and $TLR4^{-/-}$ mice.

Conclusion: TLR4 may increase ROS by inhibiting the upregulation of ecSOD in Ang II-induced hypertension *in vivo* .

P2-6

LOW LUMINAL PH AGGRAVATES FATTY ACID BOUND ALBUMIN INDUCED O2•- PRODUCTION IN RENAL PROXIMAL TUBULAR CELL

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It is known that fatty acid bound albumin induces oxidative stress in proximal tubular cells. Although luminal pH of proximal tubule decreases according to the reabsorption of bicarbonate, it is not examined well how luminal pH affects the production of superoxide, O2•-. Therefore, we examined the role of luminal pH in the O2•- production.

We applied oleic acid bound albumin (OA-alb, 15g/L) to the HK-2 cells, human proximal tubule cell line, and measured the production with dihydroethidium. The O2•- production was evaluated as the increasing rate of ethidium intensity under real time fluorescent microscopy. When buffer pH is above 6.6, the production was not significant (O2•- production; 0.15 ± 0.06 unit/sec at pH 6.9 and 0.34 ± 0.15 unit/sec at pH6.6, p=0.297). However, by decreasing buffer pH to 6.4, OA-alb increased the production to 1.45 ± 0.28 unit/sec (p =0.026 versus pH 6.6). A NADPH oxidase activity inhibitor, apocynin blunted the production by 56%.

In summary, the luminal acidic pH aggravates OA-alb induced O2•- production via NADPH oxidase in proximal tubular cells. This superoxide might contribute to vasa-recta constriction and stimulate tubular reabsorption of Na.

P2-7

GENETIC DELETION OF OX40 LIGAND SUPPRESSES THE DEVELOPMENT OF ATHEROSCLEROSIS IN APOLIPOPROTEIN E-DEFICIENT MICE

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Background: Atherosclerotic lesions are characterized by infiltration of inflammatory cells, for which immunological mechanism may be involved. It has been demonstrated that atherosclerosis is associated with enhanced vasa vasorum formation. OX40, a membrane-bound molecule of the tumor-necrosis-factor-receptor superfamily, is expressed in activated T-cells, while OX40 ligand (OX40L) is expressed in activated macrophages and endothelial cells. In this study, we examined whether OX40/OX40L system is involved in the pathogenesis of atherosclerosis.

Method and Results: We first demonstrated that endothelial cells in human coronary atheroma were immunopositive for OX40L (n=5). We then examined whether or not the OX40/OX40L system influences the development of atherosclerosis in apolipoprotein E-deficient ($ApoE^{-/-}$) mice. $ApoE^{-/-}$ mice and $ApoE^{-/-}$/OX40L-deficient ($ApoE^{-/-}/OX40L^{-/-}$) mice were fed on a high-fat diet starting at 4 weeks of age. After 8 weeks, aortic en face analysis with oil-red O staining demonstrated that the extent of aortic atheroma was significantly less in $ApoE^{-/-}/OX40L^{-/-}$ mice as compared with $ApoE^{-/-}$ mice (9.6±2.2% vs. 13.6±3.5%, $P<0.01$). To elucidate the role of OX40L in vasa vasorum formation for atherogenesis, we examined adventitial vascularity by immunostaining and confirmed that the number of blood vessels in adventitia was significantly less in $ApoE^{-/-}/OX40L^{-/-}$ mice as compared with $ApoE^{-/-}$ mice (16.0±3.2 vs. 33.5±16.1 vessels/HPF, $P<0.01$, n=8). Furthermore, capillary formation in implanted matrigel was significantly less in OX40L-/- mice as compared with WT mice.

Conclusions: These results indicate that the OX40/OX40L system plays an important role in neovascularization of atheroma. Thus, the OX40/OX40L system could be a new therapeutic target for atherosclerosis.

P3-1

CANDESARTAN IMPROVES IMPAIRED ENDOTHELIAL FUNCTION IN HUMAN CORONARY ARTERY

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Background: Endothelial dysfunction is associated with increased risk for cardiovasclular events. Several studies have documented that angiotensin II receptor blockers (ARB) improve peripheral endothelial function. However, the effect of candesartan on coronary endothelial function remains unclear. The purpose of this study was to ascertain the beneficial effect of candesartan on the human coronary artery.

Methods and Results: 24 patients with right coronary artery (RCA) disease were enrolled. They were divided into two groups; control group (n=10) and candesartan (4-8 mg/day) group (n=14). After PCI to RCA, the coronary blood flow (CBF) was measured in the left anterior descending artery without stenosis by using intracoronary doppler-tipped guide-wire. We evaluated the coronary endothelial function as an increased CBF ratio by intracoronary acethylcholin infusion (30 μg / min). The ratio of increased CBF in both groups was below 300%, implying the endothelial function in these patients was impaired. After treatment with candesartan for six months, increased CBF ratio was compared with that before treatment. In the candesartan group, CBF significantly increased from 199 ± 20% to 337 ± 27% ($P < 0.005$), whereas CBF did not change in the control group (192 ± 11% vs 184 ± 15%, $P = 0.84$). Relative change of CBF ratio on the candesartan group was significantly greater than the control group (1.90 ± 0.8 vs0.99 ± 0.3, $P < 0.001$).

Conclusions: Inhibition of the coronary vascular Angiotensin receptor with ARB increased the acethylcholin induced CBF. Our results suggest that candesartan improved coronary endothelial dysfunction in human coronary artery.