ABSTRACTS

Abstracts for Oral Communications:

OC1.

NOX4 IS A NOVEL SOURCE OF INTRACELLULAR ROS REQUIRED FOR OXIDIZED LDL-INDUCED MACROPHAGE DEATH

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An elevated plasma level of oxidized low density lipoproteins (OxLDL) is a biomarker for cardiovascular diseases including atherosclerosis. OxLDL promotes macrophage death, a hallmark of atherosclerotic lesions. In human monocyte-derived macrophages (HMDM), OxLDL increases intracellular ROS formation, which is absolutely required for OxLDL cytotoxicity (Asmis et al, Circ. Res. 2003; Wang et al., FRBM, 2006). However, the source of these ROS was not known. We now identified a new member of the Nox family, Nox4, in HMDM, and hypothesized that Nox4 mediates OxLDL-induced ROS formation and macrophage death. We found that in HMDM OxLDL up-regulated Nox4 mRNA expression but not Nox1, 2, 3 or 5 mRNA. OxLDL concomitantly up-regulated Nox4 and $p22^{phox}$ protein levels. Confocal microscopy studies showed that Nox4 appears to co-localize with $p22^{phox}$. Co-immunoprecipitation confirmed the association between $p22^{phox}$ and Nox4, suggesting that an active Nox4/$p22^{phox}$ complex is present in HMDM. Inhibition of MEK but not p38-MAPK or JNK prevented the up-regulation of Nox4 induced by OxLDL. Inhibition of MEK also prevented the OxLDL-induced increase in ROS formation and protected HMDM from OxLDL-mediated cell death, suggesting that Nox4 mediates both ROS formation and cell death induced by OxLDL. In contrast, inhibitors of p38-MAPK or JNK did not block OxLDL-induced ROS formation, and showed no protection against OxLDL. To confirm a mechanistic link between OxLDL-induced Nox4/$p22^{phox}$ induction, ROS production and macrophage death, we used gene silencing and over-expression approaches. Adenovirus-delivered siRNA directed against Nox4 suppressed OxLDL-induced ROS formation and macrophage death while ectopic Nox4 expression enhanced ROS formation and accelerated macrophage death induced by OxLDL. In summary, we demonstrate that the Nox4/$p22^{phox}$ complex is induced in HMDM in response to OxLDL stimulation via the MEK/ERK pathway. In conclusion, Nox4 mediates OxLDL-induced ROS formation and macrophage death, implicating monocytic Nox4 in the development and progression of atherosclerotic lesions.

OC2.

MATRIX METALLOPROTEINASE-9 (MMP-9) DELETION SLOWS CARDIAC AGING

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Cardiac aging is associated with decreased function of the left ventricle (LV) and higher prevalence of cardiovascular disease, but the mechanisms of cardiac aging are not fully understood. We have shown that matrix metalloproteinase-9 (MMP-9) regulates cardiac remodeling after myocardial infarction (MI), and LV MMP-9 levels increase from middle-aged to old mice. Accordingly, we tested the hypothesis that the age-related increase in MMP-9 regulates cardiac aging. We compared young (4-8 month old) and old (18-23 month old) wild type (WT) and MMP-9 null mice. For both WT and MMP-9 null mice, no increases in blood pressure were observed with age. By Doppler echocardiography, old WT mice showed lower early diastolic filling to atrial filling (E/A) ratios compared with young WT mice, indicating diastolic dysfunction with age. In contrast, old MMP-9 null mice showed similar E/A ratios as young WT and young null mice, suggesting that MMP-9 deletion preserves diastolic function with age. In addition, picro-sirius red (PSR) staining showed increased perivascular collagen in WT LV with age, while old MMP-9 null mice appeared to have less perivascular collagen deposition. Together, these data suggest that blocking MMP-9 function may slow cardiac aging in mice.

ABSTRACTS

Abstracts for Oral Communications:

OC3.

TOLL-LIKE RECEPTOR 4 DEFICIENCY ATTENUATES INSULIN RESISTANCE AND ENDOTHELIAL DYSFUNCTION ASSOCIATED WITH OBESITY AND DIABETES IN MICE

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Background: Aging and obesity are major risk factors for endothelial dysfunction and metabolic syndrome. Endothelial dysfunction is characterized by an impaired release of endothelium-derived relaxing (EDRF) and hyperpolarizing (EDHF) factors and enhanced production of contracting factors (EDCF). The Toll-like receptor 4 (TLR4) is a major target for lipopolysaccharide and saturated fatty acids, both of which are potent inducers of inflammation and insulin resistance. The present study was designed to evaluate the role of TLR4 in modulating metabolism and endothelial function in mice with loss-of-function mutation of TLR4.
Methods: $TLR4^{-/-}$ (C3H/HeJ) and wild type (C3H/HeOuJ) mice were subjected to standard or high fat diet. A type-2 diabetes animal model, double knockout in leptin receptor (Lepr) and TLR4 (DKO), was obtained by crossing $Lepr^{db/+}$ and $TLR4^{-/-}$ mice. Glucose and insulin tolerance tests (GTT and ITT) were carried out. Systolic blood pressure was measured by tail-cuff method. The animals were sacrificed at the age of 12 weeks. Rings of aorta, carotid artery and mesenteric artery (with or without endothelium) were suspended in a wire-myograph for measuring changes in isometric tension. Endothelial function was assessed by recording the responses to endothelium-dependent vasodilator and vasoconstrictor agonists.
Results: $TLR4^{-/-}$ mice under high fat diet feeding showed a better insulin sensitivity and lower blood pressure than wild type mice. DKO mice also demonstrated a significantly lower fasting blood glucose, serum cholesterol, lower blood pressureand better insulin sensitivity than $Lepr^{db/db}$ control mice. Acetylcholine-induced EDCF-mediated responses in carotid arteries were enhanced by aging, high fat diet, and genetic obesity, but were significantly attenuated by TLR4 deficiency. The contractions were inhibited by indomethacin, SC560, and S18886, but not NS398, suggesting the involvement of COX-1. Apocynin, MnTMPyP, catalase but not deferoxamine also inhibited the contractions, suggesting that reactive oxygen species may play a role. Acetylcholine-evoked hyperpolarizations were estimated in mesenteric arteries as endothelium-dependent relaxations blocked by Tram-34 and UCL-1684. The acetylcholine-induced EDHF responses were potentiated in $TLR4^{-/-}$ mice fed with control and high fat diet. The acetylcholine and sodium nitroprusside-induced relaxations in the aorta were not different in wild type and $TLR4^{-/-}$ mice.
Conclusion: Toll-like receptor 4 deficiency can prevent aging and obesity-induced insulin resistance and endothelial dysfunction possibly by decreasing oxidative stress.

OC4.

ENHANCEMENT OF ENDOTHELIAL NITRIC OXIDE SYNTHASE PREVENTS ENDOTHELIAL DYSFUNCTION INDUCED BY ASYMMETRICAL DIMETHYLARGININE

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Background & Objectives: Asymmetrical dimethylarginine (ADMA) is an endogenous L-arginine analogue that may competitively inhibit nitric oxide synthase (NOS). A growing body of evidence suggests the association between an elevated plasma level of ADMA and endothelial dysfunction. ADMA is now recognized as a risk factor for several cardiovascular diseases, such as hypertension and coronary artery disease. In the present study, we investigated the efficacy and mechanisms of AVE3085, a newly developed transcription enhancer of endothelial NOS (eNOS), with regard to its protection against ADMA-induced coronary endothelial dysfunction.
Methods: Porcine coronary small arteries (diameter 600 to 800 μm) were studied in a myograph for bradykinin (-10~-6.5 Log M)-induced, endothelium-dependent relaxation as well as endothelium-independent relaxation to sodium nitroprusside (-11~-4.5 Log M). Western blot experiments were performed to determine the protein expression of eNOS and the phosphorylation of eNOS at serine 1177 ($p\text{-eNOS}^{Ser1177}$) and at threonine 495 ($p\text{-eNOS}^{Thr495}$). The expression of nitrotyrosine was examined as well.
Results: Pre-incubation with ADMA (50 μM) for 1-hr significantly decreased the vasorelaxation of coronary arteries to bradykinin but not the relaxation to sodium nitroprusside. The maximal response to bradykinin decreased from 92.2±2.6 to 61.3±4.7 ($p<0.05$) and EC50 left shifted from 7.80±0.14 to 7.04±0.16 ($p<0.01$). Co-incubation with AVE3085 preserved bradykinin-induced vasorelaxation (94.6±1.6 *vs.* 86.7±3.2, $p>0.05$). ADMA markedly reduced the protein expressions of eNOS and $p\text{-eNOS}^{Ser1177}$ whereas increased the expressions of $p\text{-eNOS}^{Thr495}$ and nitrotyrosine ($p<0.05$). The decreased expression of eNOS and eNOS phosphorylation at serine 1177 was reversed by AVE3085. The increased expression of $p\text{-eNOS}^{Thr495}$ and nitrotyrosine was however, lowered by AVE3085 treatment ($p<0.05$).
Conclusions: AVE3085 protects against ADMA-induced endothelial dysfunction in coronary arteries. Enhancement of eNOS expression and activation as well as reduction of oxidative stress may account for the protective effect of AVE3085.
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ABSTRACTS

Abstracts for Oral Communications:

OC5.

POLYOL PATHWAY CONTRIBUTES TO THE ACUTE HYPERGLYCEMIA-INDUCED CONTRACTILE DYSFUNCTION IN PERFUSED HEART FROM RAT

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The polyol pathway, consisting of aldose reductase (AR) and sorbitol dehydrogenase (SDH), is an alternate metabolic route that converts excess glucose to fructose. It has been implicated in the development of various diabetic complications including nephropathy, retinopathy and cardiovascular disease. Previously, inhibition of AR was found to protect diabetic mice hearts from contractile dysfunction, suggesting that the polyol pathway contributes to hyperglycemia-induced contractile dysfunction. In this report we show that this glucose metabolic shunt also contributes to acute hyperglycemia-induced cardiac contractile dysfunction. Rat hearts were isolated and retrogradely perfused with either Krebs' buffer containing 10 μM AR inhibitor, Fidarestat, or 1 μM SDH inhibitor, CP-170,711, and subjected to acute hyperglycemia by perfusing with high glucose medium (33.3 mM) for 2 hours. The polyol pathway activity was measured by high performance liquid chromatography. Changes in the oxidative stress were determined by biochemical assays. We find that acute hyperglycemia-induced contractile dysfunction of the isolated perfused hearts is improved by pharmacological inhibition of the polyol pathway. The acute hyperglycemia-induced contractile dysfunction is most likely contributed by the changes in the activities of sacro/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and sodium calcium exchanger (NCX), two key players in Ca^{2+} regulation. Under acute hyperglycemia, SERCA is inactivated by the tyrosine nitration, which is contributed by high level of peroxynitrite. However, the activity of NCX is significantly increased, and the activation is probably contributed by reactive oxygen species. All these abnormalities were significantly attenuated by treatment with ARI or SDI. Thus, during acute hyperglycemia, polyol pathway-induced depletion of glutathione and increased level of superoxide probably inactivate SERCA, and stimulate NCX, leading to the abnormalities in contractile function.

OC6.

PROTECTIVE EFFECTS OF GINSENOSIDES AGAINST ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETIC MICE

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Diabetes is associated with endothelial dysfunction which contributes to the increased cardiovascular risks. The present study investigated whether or not ginseng extracts (PPD- or PPT-type ginsenosides) could restore endothelial function in the $lepr^{-/-}$ db/db mouse and the possible underlying cellular mechanisms. Db/db mice of 12-week-old were treated with PPD- or PPT-type ginsenosides (20 mg/kg/day) and vehicle for 14 days. Oral glucose tolerance test was performed after chronic administration of ginsenosides. Vascular function was assessed in aortas and femoral arteries. The expression of total and phosphorylation form of AMP-activated protein kinases (AMPK) and endothelial nitric oxide synthase (eNOS) were detected by Western blotting method. The present results show that PPD-type but not PPT-type ginsenosides improved oral glucose tolerance in db/db mice. Endothelium-dependent relaxations induced by acetylcholine were markedly impaired in diabetic mice and rescued by chronic treatment with PPD- or PPT-type ginsenosides. Acute inhibition of AMPK by compound C prevented the improvement of acetylcholine-induced relaxations in aortas and femoral arteries of PPD-treated but not PPT-treated db/db mice. Western blot analysis revealed that PPD increased the phosphorylation of AMPK and eNOS in db/db mouse aortas without affecting the total amount of AMPK or eNOS. PPT did not change the protein expression of AMPK phosphorylation. The present findings demonstrate that consumption of PPD- and PPT-type ginsenosides reverses endothelial dysfunction in diabetic mice. More importantly, PPD-type ginsenosides increase nitric oxide bioavailability through the increased phosphorylation of AMPK. These vasoprotective effects may account for a significant reduction of cardiovascular events in type 2 diabetic patients who receive ginsenoside therapy. (Supported by GRF/465308 and HKBU1/06C).

ABSTRACTS

Abstracts for Oral Communications:

OC7.

BINDING OF GENISTEIN WITH MEMBRANE ESTROGEN RECEPTOR AND THE POTENTIATING EFFECT OF GENISTEIN IN RAPID, NON-GENOMIC VASCULAR ACTION

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Background: Genistein is a phytoestrogen which enhances endothelial functions in a receptor-mediated manner. The present study was designed to characterize the mechanism involved in the rapid vascular actions of genistein and to determine whether genistein share the same receptor with estrogen in its non-genomic action.
Methods: Using tissue bath studies, isometric tension was measured in aortic rings isolated from 32-week-old male spontaneously hypertensive rats. The nuclear and membranous isoforms of estrogen receptor (ER)-α, ER-α66 and ER-α46, were cloned and expressed using a cell-free expression system. Binding study was performed subsequently.
Results: Genistein acutely potentiated acetylcholine-induced relaxation. This effect was insensitive to the transcription and translation inhibitors, actinomycin D and cycloheximide, respectively. The potentiation of acetylcholine and A23187-induced relaxation by genistein was inhibited by NF023 and GP antagonist-2A, the selective G_i and G_q α-subunit antagonists, respectively, but not by NF449, a selective G_s α-subunit antagonist. ER-α66 and ER-α46 were successfully cloned and expressed *in vitro*, with molecular sizes confirmed by Western blotting. 17β-estradiol bound to the ER-α66 and ER-α46 with similar affinity and genistein competed with 17β-estradiol for binding to both receptors.
Conclusion: The tissue bath studies demonstrate that rapid potentiating effect of genistein in acetylcholine-induced relaxation is non-genomic and G protein-coupled. In addition, our data also suggests that genistein may bind to nuclear and membranous estrogen receptors. Further studies are required to reveal whether the non-genomic vascular effect of genistein is mediated through the membranous estrogen receptors.

OC8.

CHRONIC TREATMENT OF VITAMIN D DERIVATIVES REDUCE ENDOTHELIUM-DEPENDENT CONTRACTIONS IN THE AORTA OF THE SPONTANEOUSLY HYPERTENSIVE RAT

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The available evidences suggest that vitamin D has cardiovascular effects besides regulating calcium homeostasis. Previous studies demonstrated that 1,25-dihydroxyvitamin D_3, the major metabolite of vitamin D, acutely reduce endothelium-dependent contractions induced by acetylcholine. To examine the chronic effect of 1,25-dihydroxyvitamin D_3, rats were treated with the vitamin D derivative for 6 weeks. The serum 1,25-dihydroxyvitamin D_3 level was significantly higher than the control while the mean arterial blood pressure was significantly lower. Aortic rings with or without endothelium were used for organ bath experiments. The release of prostacyclin and thromboxane A_2 after acetylcholine or A23187 stimulation were measured. The cytosolic-free calcium concentration was measured by confocal microscopy with the fluorescent dyes Fluo-4. Real time PCR was used to compare the mRNA level of COX-1, prostacyclin synthase, thromboxane synthase and eNOS between the control and treated groups. Both acetylcholine- and A23187-induced endothelium-dependent contractions were reduced significantly in the treated group. The acetylcholine-induced release of prostacyclin and the A23187-induced thromboxane A_2 was reduced in the treated group. There was no significant difference in cytosolic free calcium concentration caused by acetylcholine or A23187 between control and treated groups. COX-1 mRNA level was significantly inhibited in the treated SHR. These results demonstrate that chronic treatment of 1,25-dihydroxyvitamin D_3 modulates vascular tone by inhibiting the expression level of COX-1 mRNA which is a completely different mechanism as in the acute treatment. This chronic effect to EDCF may account for one of the factors that reducing the mean arterial blood pressure in the SHR rats.