ABSTRACTS

Abstracts for Oral Communications:

OC1.

CRITICAL ROLE OF PI3K/AKT IN THE RESTORATION OF ENDOTHELIAL FUNCTION BY HEME OXYGENASE-1 IN DIABETIC *DB/DB* MICE

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Objectives: Heme oxygenase-1 (HO-1), an inducible isoform of heme oxygenase, can exert vaso-protective effects. However, such vascular benefit in diabetic vasculopathy remains largely unclear. The present study aims at investigating the impact of HO-1 induction on endothelial function in diabetic *db/db* mice.
Methods: Diabetic *db/db* and non-diabetic *db/m+* mice were treated intraperitoneally with hemin (a potent inducer of HO-1) or vehicle for two weeks. The aortae were isolated and suspended in myograph for isometric force measurement; levels of marker proteins were determined by the Western blotting method; nitric oxide (NO) production in cultured human umbilical vein endothelial cells (HUVEC) was estimated using NO-sensitive DAF-DA fluorescence dye.
Results: Two-week hemin administration augmented endothelium-dependent relaxations in response to acetylcholine in *db/db* mouse aortae; this beneficial effect was antagonized by the co-treatment with the HO-1 inhibitor Sn(IV) mesoporphyrin IX dichloride (SnMP) and largely inhibited by the PI3K inhibitor wortmannin. Overnight treatment with hemin and bilirubin (a metabolic product of HO-1 activity) in organ culture reversed the impaired endothelial function in *db/db* mouse aortae and SnMP inhibited the effect of hemin but not that of bilirubin. Furthermore, hemin treatment increased the phosphorylation level of eNOS and Akt in diabetic mouse aortae. Hemin and bilirubin can restore the lost NO production in HUVECs exposed to high glucose and this effect was inhibited by the Akt inhibitor V and dominant-negative Akt transfection which suppresses the Akt expression. In addition, hemin treatment also improved the acetylcholine-induced relaxation in renal arteries from diabetic patients.
Conclusion: The present study demonstrates that the PI3K/Akt signaling pathway is likely to mediate the vascular benefit of HO-1 induction in restoring the impaired endothelial function in diabetic mice. The novel findings suggest that HO-1 induction could be a useful strategy for alleviation of diabetes-associated vascular dysfunction.

OC2.

EPAC-SELECTIVE CYCLIC AMP ANALOG, 8-PCPT-2'-O-ME-CAMP, INDUCED RELAXATION IN THE PORCINE CORONARY ARTERY IN A RAP1-INDEPENDENT MECHANISM

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Cyclic AMP (cAMP) is an important secondary messenger that regulates variety of cellular responses. The mechanism of action by cAMP through its classical target, protein kinase A (PKA), has been well established. Recently a novel cAMP target, cAMP-guanosine exchange factor (Epac) was reported to participate in cAMP-mediated cellular pathway. Epac1, an Epac isoform, is ubiquitously expressed in all tissue. It has been documented to involve in cardiac contraction, insulin secretion, and vascular relaxation response.

Our study aimed to study the role of Epac1 in the relaxation of porcine coronary artery. The vascular response was measured as isometric tension with organ bath technique. The existence of Epac1 in the porcine coronary artery was confirmed by immunoblotting. The Epac-selective cAMP analog, 8-pCPT-2'-O-Me-cAMP, was taken as an Epac1 activator. 8-pCPT-2'-O-Me-cAMP (≥30 μM) induced direct relaxation in both endothelium-intact and -denuded arterial rings that were contracted with thromboxane receptor agonists U46619. This effect was inhibited by the addition of PKA inhibitor, Rp-cAMPS (100 μM). Inhibition of Rap1, which is the downstream target of Epac1, by brefidin A (100 μM) did not downregualte the relaxation induced by 8-pCPT-2'-O-Me-cAMP. Although 8-pCPT-2'-O-Me-cAMP (1 μM) did not alter relaxation induced by the nitric oxide (NO) donor, sodium nitroprusside, and the ATP-sensitive potassium channel (K_{ATP} channel) activator, levcromakalim, it significantly inhibited adenylyl cyclase activator, forskolin-, induced relaxation in the presence of protein kinase G inhibitor.

Herein 8-pCPT-2'-O-Me-cAMP is able to modulate vascular tone in the porcine coronary artery and the effect was endothelium-independent. The vascular effect of 8-pCPT-2'-O-Me-cAMP does not involve either activation of Rap-1, NO signalling pathway, or K_{ATP} channels. In addition, PKA inhibitor depressed 8-pCPT-2'-O-Me-cAMP-induced relaxation whereas 8-pCPT-2'-O-Me-cAMP reduced the relaxation mediated by PKA activator. Meanwhile whether this indicates a synergy between Epac and PKA in vasorelaxation, or a competition between 8-pCPT-2'-O-Me-cAMP and cAMP or Rp-cAMPS for the cAMP binding site on the two proteins still requires further investigation.

ABSTRACTS

Abstracts for Oral Communications:

OC3.

ANTI-OBESITY DRUGS – ARE THEY UNDERUSED IN THE UNITED STATES?

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Introduction: Obesity is a major risk factor for coronary heart disease. We analysed the use of anti-obesity drugs in the United States in recent years.
Methods: We included 5332 (2630 men and 2702 women) participants of the National Health and Nutrition Examination Survey 2007-2008. We studied their demographic data, body mass index (BMI), and their weight and drug histories. Candidates were eligible for anti-obesity drugs if their BMI > 30.0 kg/m^2, or BMI > 27.0 kg/m^2 in the presence of one or more risk factors (hypertension, diabetes or dyslipidemia).
Results: 45.9% men and 45.0% women were eligible to take anti-obesity drugs. Although 85.1% knew they were overweight and 90.1% desired to lose weight, only 0.6% were on drugs during the previous month. 0.5% were on phentermine and 0.1% on orlistat. None were on sibutramine. During the preceding year, 2.2% took prescription drugs and 3.7% took non-prescription drugs to control weight. Furthermore, among eligible participants, only 61.9% changed their diet and 36.5% exercised to control weight.
Conclusions: Although obesity is highly prevalent, anti-obesity drug use was very low in the United States. This indicates that the withdrawal of sibutramine in 2010 would not have a large impact. More widespread changes in lifestyle are needed. Anti-obesity drugs should be considered for those who do not respond to lifestyle changes alone.

OC4.

EFFECTS OF ION CHANNELS ON PROLIFERATION AND DIFFERENTIATION IN HUMAN MESENCHYMAL STEM CELLS

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Background: Bone marrow-derived mesenchymal stem cells (MSCs) are a promising cell source for regenerative medicine; however, cellular physiology is not fully understood in human MSCs. The present study was to determine the potential role of the dominant functional ion channels, large-conductance Ca^{2+}-activated potassium (BKCa) channel, ether-à-go-go potassium (hEAG1) channel in regulating cell functions, including proliferation and differentiation, in human MSCs.
Methods: Ionic currents were recorded using a whole cell patch-clamp technique. Cell proliferation assay was made with MTT and ^{3}H-thymidine incorporation approaches. Cell cycle distribution was determined by flowcytometry. Lentivirus-based shRNA was used to knock down ion channels specificly. RT-PCR and Western blot analysis were applied. Adipogenic differentiation was visualized by Oil red O staining. Osteogenic differentiation was determined by alizarin red S staining.
Results: We found that paxilline and astemizole respectively reduced BKCa and hEAG1 current in human MSCs. The cell proliferation assay with MTT and ^{3}H-thymidine incorporation methods revealed that the inhibition of BKCa with paxilline and hEAG1 with astemizole decreased cell proliferation and reduced DNA synthesis rate in a concentration-dependent manner. Flowcytometry analysis displayed that paxilline and astemizole accumulated human MSCs at G0/G1 phase, and decreased cell population of S phase. Moreover, lentivirus-based shRNAs targeted to BKCa or hEAG1 channel remarkably reduced both mRNA and protein expression of BKCa or hEAG1 channel; and proliferation of human MSCs was reduced by BKCa-shRNAs or hEAG1-shRNAs. We also found these effects were accompanied by a decreased expression of cyclin D1 and cyclin E. In addition, we found that knock-down of BKCa or hEAG1 channels reduced differentiation ability of hMSCs. The expression level of PPARr or osteocalcin was decreased after the knock-down of KCNH1 or KCNMA1respectively when hMSCs were induced to adipogenic or osteogenic differentiation.
Conclusion: Our results demonstrate that BKCa and hEAG1 channels participate in the regulation of cell proliferation by promoting G0/G1 cells into cell cycling progression, and are also closely involved in cell differentiation in human MSCs.

ABSTRACTS

Abstracts for Oral Communications:

OC5.

EFFECT OF LUTEOLIN ON ENDOTHELIUM-DEPENDENT RELAXATION IS DIFFERENT BETWEEN MALE AND FEMALE RAT MESENTERIC ARTERIES: RELATIONSHIP WITH ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR AND CYCLOOXYGENASE PATHWAYS

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Background: Luteolin is a non-steroidal polyphenolic plant metabolite which has similar structure with estrogen. The present study focused on whether or not luteolin produced gender-specific effects on relaxations that were mediated by different endothelium-derived factors.
Methods: Mesenteric arteries were isolated from 45-50 weeks old male and female Sprague Dawley rats, and incubated in the organ chambers filled with Kreb's solution and bubbled with 95% O2 and 5% CO2. Endothelium-dependent relaxations mediated by nitric oxide (NO), endothelium-derived hyperpolarizing factors (EDHF) and cyclooxygenase (COX) products were studied.
Results: Our data showed that luteolin (10^{-5} M) enhanced endothelium-dependent relaxation in general and NO-mediated relaxations in both male and female rat mesenteric arteries, and the degree of these enhancements did not differ between the two genders. In the presence of indomethacin (10^{-6} M, COX inhibitor) and L-NAME (10^{-5} M, NO synthase inhibitor), EDHF-mediated relaxation were enhanced by luteolin in a greater manner in male than that in female. This phenomenon was also observed in relaxation mediated by intermediate conductance calcium-activated potassium channel (IK_{Ca}) and small calcium-activated potassium channel (SK_{Ca}). However, in the presence of L-NAME, UCL 1684 (10^{-6} M, SK_{Ca} antagonist) and TRAM-34 (10^{-6} M, IK_{Ca} antagonist), luteolin produced a smaller enhancement of COX-mediated relaxation in male than that in female.
Conclusions: In conclusion, luteolin selectively enhanced EHDF signaling pathways and this enhancement was greater in male than in female. On the other hand, luteolin caused greater activation of COX pathway in female than in male. Further study will focus on the expression of COX and potassium channels in both male and female rats.

OC6.

CARDIOPROTECTION OF THE NATURAL FLAVONE ACACETIN AGAINST ISCHEMIA/REPERFUSION INJURY IN ISOLATED RAT HEARTS

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Background: The natural flavone acacetin from Tianshan Xuelian has been reported to be a promising atrial-selective anti-atrial fibrillation agent. The present study was designed to investigate whether this compound would be cardiac protective against ischemia/reperfusion insult in isolated rat hearts.
Methods and Results: Isolated rat hearts were retrogradely perfused with a Langendorff apparatus. Electrocardiogram (ECG) and left ventricular pressure (LVP) of the heart were recorded using a Powerlab system. After a 30 min equilibrium perfusion, left anterior descending coronary artery was ligated for 30 min, then reperfused by releasing the ligation for 180 min. We found that ventricular fibrillation (VF, 81%) was observed in vehicle treated hearts (n=11) during reperfusion, and the incidence of VF was reduced to 77% and 25% in hearts treated with 1 (n=9) and 3 (n=9) μM acacetin. LVP was reduced to 26±6% and 37±4% of control at 30 min ischemia and after 120 min reperfusion, respectively, in vehicle group. In hearts treated with 3 μM acacetin, LVP was reduced to 44±7% and 54±8% of control. Myocardial infarction size/area at risk was decreased from 0.64±0.04 in vehicle-treated hearts (n=11) to 0.40±0.05 and 0.20±0.01 in hearts treated with 1 (n=9) and 3 (n=9) μM acacetin (P<0.01 vs. control). Acacetin (1 and 3 μM) decreased the release of lactate dehydrogenase (LDH) induced by reperfusion (30 min) to 70% and 34% of vehicle group. Apoptosis assay with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) revealed that acacetin significantly reduced DNA fragmentation (40±1.95% in vehicle control; 26±1.2% and 8±0.6% in 1 and 3 μM acacetin, n=8, P<0.01 vs control).
Conclusion: These results have demonstrated the novel information that acacetin is cardiac protective against ischemia/reperfusion insult in isolated rat hearts by reducing myocardial apoptosis, suggesting a new therapeutic indication of this flavone compound.