ABSTRACTS

Abstracts for Posters:

P1.

Effects of Activator of Peroxisome Proliferator-activator Receptor-γ on ET-1 Induced Cardiomyocyte Hypertrophy and Regulation of Calcineurin/NFAT Signal Pathway

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Objectives: It was suggested that calcineurin/NFAT activation was not only sufficient to cause hypertrophy but also necessary for hypertrophy development. Peroxisome proliferator-activated receptor-γ(PPAR-γ) has been manifested as Negative regulators of cardiac hypertrophy. Although crosstalk between PPAR-γ and NFAT was reported in Human T Lymphocytes, adipocytes and tumor cells, it's still unknown whether such a link exists in cadiocytes.
Methods: [3H] leucine incorporation assay was performed to measure protein synthesis. Reverse transcription-polymerase chain reaction (RT-PCR) was applied to analyze the mRNA level of atrial natriuretic factor (ANF) and PPAR-γ. Western blot analysis was performed to investigate the calcineurin/NFAT protein level on the modulatory effects of ET-1 and rosiglitazone. Immunofluorescence analysis was used to examine the cellular localization of calcineurin/NFAT.
Results: In the present study, we found that calcineurin enzymatic activity, mRNA, and protein levels are increased in cultured neonatal rat cardiomyocytes by endothelin-1 (ET-1). While application of rosiglitazone, PPAR-γ activator, inhibited ET-1 induced increase of calcineurin enzymatic activity and prevented nuclear translocation of calcineurin/NFAT in cardiomyocytes. Moreover, co-immunoprecipitation studies showed that rosiglitazone strongly induced the association of NFAT with PPAR-γ.
Conclusion: These results suggest that activation of PPAR-γ inhibits ET-1 induced cardiac hypertrophy through regulating calcineurin/NFAT signaling pathway.

P2.

The Role of Polyol Pathway in the Pathogenesis of Diabetic Cardiomyopathy

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Diabetic cardiomyopathy (DC) is a cardiac failure due to hyperglycemia. The disease affects cardiac structure and function in the absent of coronary atherosclerosis. The cellular and molecular mechanisms leading to hyperglycemia-induced ventricular myocardial structural and functional alternations remain poorly understood. In diabetic animals increased flux of glucose through the polyol pathway has been implicated in the pathogenesis of cataract, retinopathy, neuropathy, and nephropathy. Aldose reductase (AR) is the first and rate-limiting enzyme of the polyol pathway. A recent study showed that AR activity is elevated in the diabetic mouse heart. We propose that increased AR activity in the hearts of diabetic animal might increase oxidative stress as it has been shown in other tissues, leading to cardiomyopathy. We found that elevated polyol pathway activity is responsible for the alteration of cardiac function and Ca^{2+} homeostasis in both acute and chronic hyperglycemia, leading to contractile abnormalities. We believe that the mechanism may due to increased oxidative stress and redox active molecules mediated by polyol pathway. Experiments to demonstrate this hypothesis is in progress.

P3.

High Glucose Induces Endoplasmic Reticulum Stress in COX-2 Dependent Manner in HUVECs

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Stress in the end POST http://www.icsm-hk.org/abstractsubmit.phing of C/EBP-homologous (CHOP) protein and Glucose-regulated protein 78 (GRP78/BiP), can result in apoptosis. The aim of this study was to explore the role of ER stress in high glucose (HG)-induced apoptosis in human umbilical vein endothelial cells (HUVECs).
Methods: HUVECs were exposed to low glucose (5.5 mmol/L) and high glucose (30 mmol/L) for 12~60 h, then cell proliferation and apoptosis were determined. The expression of cyclooxygenase-2 (COX-2), GRP78 and CHOP proteins were also evaluated by western blotting analysis.
Results: After incubated with high glucose for 48 h, the cell proliferation decreased and the number of apoptotic cells increased in HUVECs. High glucose induced a time-dependent increase in the levels of GRP78 and CHOP expression in HUVECs. Incubation of endothelial cells with high glucose also increased the expression of COX-2 protein. The number of apoptotic cells after prolonged high glucose exposure was significantly reduced in the presence of the COX inhibitor indomethacin. Indomethacin also abolished HG-induced GRP78 and CHOP expression.
Conclusions: High glucose can up-regulate GRP78 and CHOP expression in COX-2 dependent manner in HUVECs, which eventually lead to the cell apoptosis.
(This work was supported by the National Natural Science Foundation of China (No. 30400094))

ABSTRACTS

Abstracts for Posters:

P4.

Properties of Adenosine-Metabolising Enzymes Extracted from Vascular Endothelial Cells

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Adenosine is formed extracellularly by ecto-5'-nucleotidase during exercise vasodilation. It was also suggested that adenosine was released from vascular endothelial cells during hypoxia. However, it is not known whether adenosine originating from endothelial cells is formed intracellularly by cytosolic-5'-nucleotidase or extracellularly by ecto-5'-nucleotidase.

Objectives: In this study, we investigated whether the endothelial cells were capable of forming adenosine intracellularly in sufficient quantities for it to diffuse out into the extracellular space. The vascular endothelial cells were isolated from rat skeletal muscle, purified and homogenized. The cytosolic and ecto-enzymes for adenosine metabolism, including 5'-nucleotidases (5'N), adenosine deaminase (AD) and adenosine kinase (AK) were separated, and their properties were examined in these cell homogenates.

Methods: The vascular endothelial cells from hindlimb muscles of young male SD rats were separated and further purified by immunomagnetic precipitation using $CD31^+$ DynaBeads. Then, the fresh intact cells were homogenized, and further separated into membrane and cytosolic fractions, by ultra-centrifugation. The adenosine-metabolising enzymes were assayed using methods based on those previously reported by our lab. Enzyme kinetic parameters, such as Vmax and Km values, were calculated using Prism.

Results: The enzymes were assayed using different concentrations of substrates (adenosine or AMP). The maximal velocities (Vmax; nmol/min/mg protein) were 2.1±0.3 for AD and 15.4±2.3 for AK, and the Michaelis constants (Km, which are a measure of the affinity of the enzyme for its substrate; μM) were 41.9±34.6 for AD and 120.2±45.7 for AK (pH 7.0, n=11). The 5'N activities were examined at different pH values from 6.0 to 8.0. Both ecto- and cytosolic-5'N had their lowest Vmax at pH 7.0, whereas, the Vmax values remained similar at all other pH values tested. Furthermore, cytosolic-5'N had a Vmax which was almost 2.5-fold higher than that of membrane-bound 5'N at all pH values tested. The Km of 5'N was not altered by pH depression within the physiological range.

Conclusions: Cytosolic-5'N has a higher activity than membrane-bound 5'N, which is the opposite of the situation in muscle cells, and suggests that adenosine may be formed intracellularly in vascular endothelial cells. The activity of 5'N was lowest at pH 7.0, which suggests that any deviation of pH from its normal value may trigger the formation of adenosine in endothelial cells.

P5.

Role of Ca^{2+}/Calmodulin-dependent Protein Kinase II in Cardioprotective Effect of Estrogen

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Estrogen, the primary female sex hormone, has been shown to confer cardioprotection by down-regulating the β_1-adrenoceptor and suppressing the expression and activity of the protein kinase A (PKA), a messenger of the G protein S/Adenyly Cyclase/cAMP/PKA pathway, which mediates the action of β_1-adrenoceptor stimulation. We hypothesize that estrogen may also suppress the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), another signaling pathway mediating the action of β_1-adrenoceptor stimulation, which plays an important role in regulation of the apoptotic signaling pathway. We first used western blot to detect the expression of CaMKII in female rats undergoing sham operation or ovariectomized with (Ovx+E) and without (Ovx) estrogen replacement rats. Both the expression and phosphorylation of CaMKIIδc were up-regulated in the Ovx rats, which was restored to normal by estrogen replacement. Furthermore, in the isolated perfused heart the infarct size and the release of lactate dehydrogenase induced by ischemic insult were significantly greater in Ovx than the sham and Ovx+E rats. Upon blockade of CaMKII with a selective inhibitors, KN93 (2.5 μM), or blockade of PKA with its inhibitor, KT5720 (2 μM), the infarct size was reduced in Ovx rats. Together our data showed that estrogen suppresses the CaMKIIδc, thus protecting the heart against ischemic insult.

ABSTRACTS

Abstracts for Posters:

P6.

Junctional Adhesion Molecule-1 Gene Polymorphisms in Central Obesity and Raised Blood Pressure

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Objectives: Junctional adhesion molecule-1 (JAM-1) in the autonomic nervous system is implicated in the development of hypertension in spontaneous hypertensive rats. We investigated the association of single nucleotide polymorphisms (SNPs) in the JAM-1 gene (*F11R*) with hypertension and central obesity in Hong Kong Chinese.

Methods: Seven tagging SNPs were identified in the HapMap database. Genotyping was performed using Sequenom MassArray in 263 hypertensive subjects and 393 normotensive controls, of whom 263 matched the cases in age and sex.

Results: When subjects on anti-hypertensive medication were excluded, rs790056 and rs2774276 were associated with lower systolic blood pressure (TT: 124.8±18.3 mmHg versus TC+CC: 120.2±15.5 mmHg, *P*=0.004 and CC: 124.7±18.5 mmHg versus CG+GG: 120.5±15.1 mmHg, *P*=0.007, respectively). Comparing 213 subjects with central obesity with 213 controls matched for sex and age, rs2481084 and rs3737787 were associated with lower odds of central obesity (odds ratio=0.516, *P*=0.002 and odds ratio=0.540, *P*=0.005, respectively). All these associations remained significant after correction for multiple testing. Analysis of statistically similar SNPs suggested that the causative variants for systolic blood pressure were located in *F11R*, while those for central obesity could be due to causative variants in the transcription factor 1 gene immediately upstream.

Conclusions: JAM-1 plays a role in blood pressure regulation, not only in rats but also in man. The link between JAM-1 and central obesity merits further investigation.

P7.

Chronic Nicotine Administration Alters Vascular Reactivity in Mouse Cerebral Arteries

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Smoking is a major health hazard with detrimental effects on the cerebral circulation, which include a decrease in cerebral blood flow and a high risk for stroke. Nicotine, a vasoactive substance in tobacco, can enhance vasoconstriction. However, the mechanisms underlying how nicotine constricts cerebral arteries are incompletely understood. The present study aimed at investigating the effects of chronic nicotine administration on the reactivity of isolated mouse cerebral arteries (internal diameter less than 100 μm) by measuring isometric force in myograph. The results show that the contractions to different receptor-dependent agonists such as phenylnephrine, U46619, endothelin-1, serotonin and angiotensin II were significantly greater in cerebral arteries from nicotine-treated mice than in the control mice. By contrast, the contraction induced by 60 mM KCl was comparable between the two groups. In addition, the endothelium-dependent relaxations induced by acetylcholine were impaired in the nicotine-treated mice. The present study provides novel information on the effects of chronic nicotine administration on mouse cerebral vascular reactivity. Further experiments will be carried out on the genetically engineered mice to examine the possible mechanisms underlying endothelial dysfunction in nicotine-treated mice.

(Supported by CUHK Li Ka Shing Institute of Health Sciences and Focused Investment Scheme)

ABSTRACTS

Abstracts for Posters:

P8.

Prostaglandin F_{2A} Acts as the Major Endothelium-derived Contracting Factor in the Hamster Aorta

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Endothelial dysfunction is associated with the increased production and release of endothelium-derived contracting factors (EDCFs), whose identity is still poorly defined due to their heterogeneity in different vascular beds and species. We demonstrated previously the essential role of cyclooxygenase (COX)-2 in endothelium-dependent contractions in young healthy hamsters. The aim of the present study was to identify the possible EDCF candidate(s). Solutions that bathed the aortae in the presence of L-NAME (plus inhibitors) and acetylcholine were collected and assayed for the amount of arachidonic acid metabolites released. The levels of six prostanoids, or their derivatives were measured by enzyme immunoassay (EIA) kits and they included prostaglandin (PG) $F_{2\alpha}$, PGE_2, PGD_2, 6-keto $PGF_{1\alpha}$ (for PGI_2), thromboxane (TX) B_2 (for TXA_2) and 8-isoprotanes. Among the six assayed prostanoids, only the release of $PGF_{2\alpha}$ and PGI_2 evoked by acetylcholine was inhibited or abolished by treatment with celecoxib (a specific COX-2 inhibitor) or 2-APB (a non-selective cation channel blocker), but not by valeryl salicylate (a specific COX-1 inhibitor). To confirm the role of $PGF_{2\alpha}$ and PGI_2 as EDCFs, isometric force measurement was performed on aortic rings in the presence of L-NAME. Only did exogenous $PGF_{2\alpha}$ elicit vasoconstriction at a concentration that corresponded to the amount released endogenously. By contrast, PGI_2 failed to trigger any contraction *per se*, even at a concentration 50 times higher than the one detected in the EIA study. In summary, the present results show that $PGF_{2\alpha}$ a COX-2 derived prostanoid, acts as a physiological EDCF in the hamster aorta. Since the lipid profile of hamster resembles that of human, the present findings may suggest hamster as a useful model for studies in relation to COX-2-mediated vascular complications and dyslipidemia.

P9.

Up-regulated Angiotensin II Type 1 Receptors Mediate Endothelial Dysfunction in DB/DB Diabetic Mice

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Alterations in the vascular renin-angiotensin system (RAS) play a role in the pathophysiology of endothelial dysfunction in diabetes. In this study, we have investigated the mechanisms underlying the up-regulated angiotensin II type 1 receptors (AT1R)-associated effects on vascular function in the $lepr^{-/-}$ db/db mouse. Db/db mice of 12-week-old were treated with valsartan (AT1R blocker, 10 mg/kg/day), enalapril (angiotensin converting enzyme inhibitor, 10 mg/kg/day) or vehicle for 6 weeks. Vascular function was assessed in aortae in myograph. Expression of angiotensin II in aortae was evaluated by immunofluorescence, and the expression of angiotensin-converting enzyme and AT1R was investigated by Western blot. The present study shows a pathogenic role of AT1R in the development of oxidative stress-related endothelial dysfunction in the aortae from db/db diabetic mice, which is related to the activation of NAD(P)H oxidases and generation of superoxide anions. The increases in the level of angiotensin II in diabetic arteries were indicated by the enhanced expression of angiotensin converting enzyme. Chronic treatment with selective RAS blockers significantly improves the endothelial function in db/db mice. The novel findings of the present investigation provide useful insights into new therapeutic strategies against the development of endothelial dysfunction in diabetes.

(Supported by CUHK Li Ka Shing Institute of Health Sciences and Focused Investment Scheme)

ABSTRACTS

Abstracts for Posters:

P10.

Cardioprotective Effect of Melatonin on Impaired Calcium Homeostasis, Myocardial and Ischemia-reperfusion Injuries in Chronically Hypoxic Rats

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Chronic hypoxia (CH) deteriorates myocardial functions and impairs calcium homeostasis in sarcoplasmic reticulum (SR). We hypothesized that administration of melatonin protects against myocardial and ischemia-reperfusion (I/R) injuries by improving SR calcium handling in CH rats. Adult Sprague-Dawley rats were daily administered with melatonin (MCH, 10mg/Kg/day of body weight, i.p.) or vehicle (VCH, 2% ethanol in normal saline) and exposed to hypoxia (10% O_2 in air) for 4 weeks. The ratio of heart/body weight, left ventricular hypertrophy and levels of malondialdehyde were significantly increased in the VCH group compared with the normoxic control, but the increased levels were lowered in the MCH group. Levels of LDH leakage before ischemia, during I/R and infarct size of the isolated perfused hearts were also significantly attenuated in the MCH group compared with the vehicle group. Spectroflurometric studies showed that decreases in SR calcium content and I/R-induced calcium overload were remarkable in VCH rat cardiomyocytes. The I/R-induced impairments were restored in the MCH group. Levels of SR Ca^{2+}-ATPase protein expression were decreased in the VCH but not the MCH group. Results suggest that melatonin is cardioprotective against CH-induced myocardial injury with ameliorated calcium homeostasis in the SR of cardiomyocytes during I/R.

(Study was supported by research grants from Research Grants Council, HKSAR, and the University Research Council of the University of Hong Kong)

P11.

Higher Fruit Intakes Are Associated with Higher C-Reactive Protein in Patients with Type II Diabetes

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Background: Prior studies have shown that higher intakes of fruit are associated with a lower C-reactive protein (CRP) and risk of metabolic syndrome. However, limited data on the relation between fruit intakes and inflammatory marker concentration in patients (pts) with diabetes mellitus (DM) are available.

Methods & Results: We studied the relationships between the fruit intakes with high sensitive CRP (hsCRP) in 86 type II DM pts (60±9 yrs, 48F) and 96 age and sex matched healthy controls (59±8 yrs, 60F). Dietary intakes were assessed with the use of a validated food-frequency questionnaire for Chinese, and anthropometric and blood pressure measurements and fasting blood sample were taken from all pts. After adjusting for the calories intakes, there were no significant differences in fruit/kcal (0.03±0.03 vs. 0.03±0.02 g/kcal per day, P=0.82) intake between DM pts and controls. The plasma concentration of hsCRP was significantly inverse correlated with the fruit (r=-0.22, p=0.03) in controls but was positive correlated with the DM pts (r=0.41, p<0.001). Even after adjusting for potential confounders (age, anthropometric measurement, calories intake and serum cholesterol level), these negative and positive correlations between plasma concentration of hsCRP with fruit in controls (r=-2.91, p=0.005) and DM pts (r=0.37, p=0.001) remained significant.

Conclusion: In contrast to normal subjects, higher intakes of fruit are associated with a higher inflammatory marker as determined by hsCRP in pts with type II DM.

P12.

Expression and Role of Adrenomedullin and Its Receptor Proteins in LPS-Stimulated Rat Macrophage

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Background: Adrenomedullin (AM) is a vasorelaxant peptide that has important roles in cytokine regulation in the inflammatory response as well as the cardiovascular homeostasis. Patients with dysregulated level of plasma AM were correlated with a variety of diseases, including hypertension, heart failure, myocardial infraction and septic shock. We have shown that AM production was stimulated by lipopolysaccharide (LPS) and AM exerted significant effect on production of inflammatory cytokines, including interleukin (IL)-6, tumor necrosis factor-α (TNF-α) and IL-1β. Administration of AM and AM binding protein-1 (AMBP-1) increases the cardiovascular stability and decreases tissue injury in septic animals. However, little is known about the regulation of AMBP-1 and AM receptors proteins: calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein (RAMP-2). The present study aimed to study the effect of AM on the anti-inflammatory cytokine, IL-10, and investigate the regulatory role of cytokines on the expression of AMBP-1 and AM receptors.

Methods: A rat alveolar macrophage cell line, NR8383 was used. Cells were stimulated by LPS in the presence or absence of AM or other inflammatory mediators. Productions of IL-6 and IL-10 were measured by ELISA at 6 and 24 hr after stimulation. Basal and LPS-stimulated expressions of AMBP-1, CRLR and RAMP-2 were determined by TaqMan real-time PCR using β-actin as an internal control.

Results: LPS increased IL-10 production at 12 to 24 hr after stimulation. LPS-induced IL-10 production in NR8383 cells were significantly increased by AM and TNF-α, but markedly reduced by interferon-γ (IFN-γ). AMBP-1 was constitutively expressed by NR8383 cells and no significant effect of LPS or cytokines was found on AMBP-1 expression. For the AM receptor proteins, LPS significantly reduced RAMP-2 expression at 6 to 24 hr after stimulation but there was no significant effect of cytokines on basal or LPS-stimulated RAMP-2 and AMBP-1 expression. On the other hand, LPS markedly increased CRLR gene expression at 6 hr which was gradually reduced after 24 hr of stimulation. IL-10 and IFN-γ significantly reduced LPS-stimulated CRLR expression by 53% and 48.3% respectively while TNF-α and IL-6 showed no significant effect.

Conclusion: Our results suggest that AM might play a role in the regulation of LPS-stimulated IL-10 in macrophage. In addition, LPS and inflammatory mediators, including IL-10 and IFN-γ might be involved in the regulation of CRLR expression and RAMP-2 in septic conditions.

ABSTRACTS

Abstracts for Posters:

P13.

Participation of Heme Oxygenase and Nitric Oxide Synthase in the Regulation of Endothelium-dependent Vasodilatation After Different Levels of Exercise Volume

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Objective: To examine different levels of exercise volume on endothelium-dependent vasodilatation and role of vessel heme oxygenase (HO) and endothelial/inducible nitric oxide synthase (eNOS and iNOS).
Methods: Male Sprague-Dawley rats were assigned to sedentary control, acute (2 weeks) or chronic (6 weeks) treadmill running at moderate intensity (50% maximal aerobic velocity) for 2 hr/day (moderate volume), or 3 hr/day (high volume). Endothelium-dependent vessel function was examined in isolated thoracic aorta. Co-localization of aortic HO-1/HO-2 and eNOS/iNOS was observed with immunofluorescence study.
Results: As compared with sedentary control, acute and chronic endurance training enhanced endothelium-dependent relaxation ($P<0.01$). While acetylcholine-induced dilation was completely inhibited by NOS inhibitor in sedentary controls, the dilation in endurance training groups was only partly blocked by NOS inhibition (inhibition rate was 98±3%, 79±6% and 77±5% in sedentary control, acute and chronic endurance training, respectively, $P<0.01$). The remnant dilation in endurance training groups was further abrogated by HO inhibition, with concomitant elevation in aortic eNOS as well as HO-1 and HO-2. In contrast to endurance exercise, high volume intense training resulted in mild hypertension with significant impairment in endothelium-dependent vasodilatation and elicited profuse increases in both iNOS and eNOS (P<0.01).
Conclusion: Endothelium-dependent vasodilatation can be improved by endurance exercise of moderate volume but impaired by chronic intense training of high volume. Elevation of vessel eNOS and HO-1/HO-2 may contribute to the enhanced vasodilatation, which can be offset by intense training and elevation in vessel iNOS.

P14.

Depletion of Circulating Endothelial Progenitor Cells in Normal Subjects with Depression

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Introduction: Although the mechanisms remain unclear, depression is associated with endothelial dysfunction and increase risk of cardiovascular disease (CVD). Recent studies suggest that circulating endothelial progenitor cells (EPC) play an important role in endothelial repair and correlate with endothelial function. However, there is no data on the relationship between the level of circulating EPCs and depression status.
Methods and Results: We studied 132 healthy normal individuals (55±8 yrs, 59 men) without prior cardiovascular diseases or diabetes. All subjects has coronary artery calcium<10 as assessed by cardiac computed tomography. The numbers of circulating CD34+EPCs were determined by flow cytometry, and the depression status was estimated by Depression Anxiety Stress Scales. The median depression score of the study population is 4 (range 0 to 34). Forty-two pts (32%) had a high depression score as defined by ≥75% percentile depression score of all subjects (>=8). There were no significant differences in age (55±9 vs. 54±10 yrs), sex (45 vs. 40% male) and prevalence of history of hypertension (23 vs. 22%), hyperlipidaemia (27 vs. 41%) and smoking (30 vs. 29%) between the subjects with or without high depression score (all P>0.05). However, subjects with high depression scores had a significantly lower CD34+ EPC percentage (4.28±2.59 vs. 5.50±3.96%, P=0.037) than those without high depression score. CD34+ EPC percentage have a negative correlation with depression score (r=-0.307, P=0.040) and a positive correlation with hs-CRP value (r=0.330, P=0.027), central pulse wave velocity (PWV) (r=0.261, P=0.004), and heart-ankle PWV (ha PWV) of both lower limb (right ha PWV: r=0.189, P=0.039; left ha PWV: r=0.205, P=0.024).
Conclusions: Our result demonstrates that depression was associated with a lower level of circulating EPCs in the normal individuals without significant cardiovascular diseases. This finding suggests that depletion of EPC in normal subjects with depression may contribute to the development of endothelial dysfunction.

ABSTRACTS

Abstracts for Posters:

P15.

KMUP-1 Reduces Monocrotaline-induced Pulmonary Artery Hyperplasty and Cell Proliferation, Involving ENOS and Rock Expression

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eNOS and ROCK II play an important role in pulmonary hypertension (PH), which leads to hyperplasia of the pulmonary artery smooth muscle cells (PASMCs). ROCK II upregulation precedes the development of pulmonary vascular remodeling. MCT produces endothelial injury that develops severe PH. In this study, PH was induced by monocrotaline (MCT; 60 mg/kg, i.p.). Administration of KMUP-1 HCl (5 mg/kg/day PO or 1 mg/kg/day, i.p.) for 3 weeks reversed MCT-induced PH and exposed concomitant ROCK II inhibition, eNOS elevation in lung tissue and inhibition of hyperplasia in pulmonary artery. KMUP-1 further decreased the pulmonary artery wall thickness on MCT-induced PH rat from 59.8±2.7% to 30.6±8.6% (5 mg/kg/day, PO) and 40.8±3.4% (1 mg/kg/day, i.p.), indicating pulmonary artery anti-hyperplasty KMUP-1 HCl decreased the cell proliferation, analyzed by MTT test. It is concluded that KMUP-1 HCl is effective in reducing PH, which are associated with a decrease of ROCK II and increase of NOS expression. KMUP-1 HCl may represent a novel treatment against the progression of PH.

P16.

Effect of Hyperkalemia on Large Conductance Calcium-activated Potassium Channels in the Isolated Smooth Muscle Cell of Porcine Coronary Arteries: Relevance to Cardiologic Arrest

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Objectives: Hyperkalemic solutions are widely used for cardioplegia and organ preservation. Large conductance calcium-activated potassium channels (BK_{Ca}) play an important role in the regulation of vascular tone. However, little is known about the effect of hyperkalemia on the BK_{Ca} activity. This study was designed to investigate the effect of hyperkalemia on BK_{Ca} in the isolated smooth muscle cell (SMC) of coronary arteries.
Methods: SMCs were enzymatically isolated from porcine coronary arteries dissected from fresh pig hearts. Primary cultures of the SMCs (usually 5-7 days) were used for patch-clamp study. The effect of K^+ with different concentrations (5.4, 20, 60, or 120 mM) was investigated on whole-cell BK_{Ca} current with a holding potential of -60 mV (n=5 for each concentration).
Results: The rise of extracellular K^+ in bath solutions significantly resulted in increase of BK_{Ca} currents in a K^+-concentration dependent manner in comparison with the control solution (5.4 mM: 31.6±3.4pA/pF; 20 mM: 73.6±11.4pA/pF; 60mM: 108.6±20.6pA/pF; 120 mM: 135.2±20.5 pA/pF, $p<0.05$ one-way ANOVA).
Conclusions: Raise of extracellular K^+ concentration increases BK_{Ca} activity of the porcine coronary arterial SMCs in a dose-dependent manner. This may be an important part of the effect of hyperkalemic cardioplegia on the coronary circulation and therefore may have clinical implications in the heart preservation.

ABSTRACTS

Abstracts for Posters:

P17.

Melatonin Attenuates Aortic Inflammation, Endothelial Dysfunction and Hypertensive Response to Chronic Intermittent Hypoxia in Rats

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Chronic intermittent hypoxia (CIH) is one of the important factors contributing to the pathogenesis of hypertension in patients with obstructive sleep apnea. Melatonin was reported to alleviate hypertension and to attenuate oxidative injury and inflammation. The aim of this study was to investigate the protective mechanism of melatonin against inflammation and endothelial dysfunction induced by CIH in rat aorta. Male 28-day-old Sprague-Dawley rats were treated with IH for 8 hr/day diurnally after daily intraperitonal injection of melatonin (10 mg/kg) or vehicle (2% ethanol in saline) for 14 and 21 days. Mean, diastolic and systolic blood pressures were significantly elevated in the vehicle-treated but not in the melatonin-treated rats by 21-day hypoxic treatment when compared with the normoxic control. There were significant increases in levels of malondialdehyde and the mRNA expressions of NADPH oxidase, pro-inflammatory mediators (TNFα, iNOS, COX-2) and adhesion molecules (ICAM-1, VCAM-1 and E-selectin) of the aortic artery in the vehicle group treated with 14-day hypoxia preceding the hypertensive response. Also, levels of NO, endothelial-dependent relaxation and the mRNA expressions of eNOS, antioxidant enzymes (glutathione peroxidase, catalase and copper/zinc superoxide dismutase) were remarkably lowered in the hypoxic rats. Administration of melatonin significantly reduced expressions of NADPH oxidase, pro-inflammatory mediators and adhesion molecules. Moreover, levels of NO, endothelial function, expressions of eNOS and antioxidant enzymes were restored by melatonin. These results suggest that melatonin is protective against CIH-induced endothelial dysfunction via an antioxidant and anti-inflammatory mechanism.
(Study was supported by research grants from Research Grants Council, HKSAR, and the University Research Council of the University of Hong Kong)

P18.

Functional Upregulation of Proinflammatory Cytokines and Local Inflammation in the Rat Carotid Body During Chronic Hypoxia

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The structure and function of the carotid body (CB) are greatly altered during chronic hypoxia (CH). Recent studies showed the expression of interleukin (IL)-1 receptor and IL-6 receptor in the CB, suggesting a role of proinflammatory cytokines in the chemoreceptor function. The present study aimed to examine the hypothesis that the expression of proinflammatory cytokines, namely IL-1β, IL-6 and tumor necrosis factor (TNF)α, plays a role in the rat CB in CH. Immunohistochemistry showed that the expressions of cytokines and their receptors IL-1r1, gp130 and TNFr1 were localized in the lobules of chemosensitive glomus cells containing tyrosine hydroxylase. Levels of the protein and mRNA expression of which were significantly increased in the CB of CH rats when compared with the normoxic (Nx) controls. Application of exogenous cytokines (0.1-10 nM) concentration-dependently elevated intracellular calcium ($[Ca^{2+}]_i$) responses to acute hypoxia in the dissociated fura-2-loaded glomus cells and showed a significantly greater response in CH than Nx. Moreover, the gene transcripts of inflammatory mediator inducible nitric oxide synthase and chemokines (MCP-1, CCR2, MIP-1α, and ICAM-1) were increased in the CB of CH rats. These results collectively suggest that the increased expressions of proinflammatory cytokines play a functional role in the CB with local inflammation during CH.
(Study was supported by research grants from Research Grants Council, HKSAR, and the University Research Council of the University of Hong Kong)

ABSTRACTS

Abstracts for Posters:

P19.

Protective Effect of Black Tea Against Brain Damage After Transient Middle Cerebral Artery Occlusion in Rats – A Proteomics Approach

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Black tea possesses biological activities as being anti-oxidative, anti-viral, and anti-inflammatory. The purposes of the present study are to (1) verify whether or not black tea could reduce focal ischemia/reperfusion-induced brain injury in a rat model of Middle Cerebral Artery Occlusion (MCAO) and (2) to understand the underlying possible mechanisms by a proteomics approach. Male Sprague-Dawley rats were allowed to consume black tea for 3 months before subjected to a middle cerebral artery 2-hr occlusion followed by 46-hr reperfusion. Black tea consumption ameliorated the infarction volume, reduced the neurological deficit total score and TUNEL-positive staining. The results demonstrate that black tea exerts a neuroprotective action. Global protein analysis was performed on the lesion and sham-control cerebral cortex using two-dimensional gel electrophoresis. Protein spots with more than a 3-fold change in intensity were identified by mass spectrometry. Two-dimensional gel electrophoresis resolved about 1000 protein spots, of which only fifteen proteins were significantly up-regulated after ischemia/reperfusion and black tea could attenuate the increased levels of these proteins. Identified spots are prohibitin, actin, serum albumin precursor, tubulin α-1 chain, probable protein disulfide isomerase, glutamine synthetase, complement C4A anaphylatoxin, α enolase, aspartate aminotransferase, dihydropyrimidinase related protein-2 (DRP-2), pyruvate kinase, tropomyosin, fibroblast isoform, heat shock cognate 71 KD protein and transketolase. These proteins are involved in inhibiting DNA synthesis, cell motility, mediating local inflammatory processes, vascular permeability, glycolysis, axon elaboration, stabilizing cytoskeleton and protein folding. The present results on protein biomarkers indicate that black tea consumption can ameliorate cerebral ischemia/reperfusion-associated cell proliferation, inflammation, cytoskeletal network, and neuroregeneration.

(This study was supported by Research Grants Council of Hong Kong SAR and Li Ka Shing Institute of Health Sciences. FPL, LMY, and WTW were supported by CUHK Focused Investment Scheme)

P20.

Effect of Carvedilol on Pacemaker Current of Atrial Myocytes of Older Rats

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Objective: This article investigated the effect of Carvedilol (Car) on pacemaker current (I_f) of atrium myocytes of old rats (22-24 mo).

Methods: The single atrium myocytes of the rats were isolated by Langendorf perfusion device. I_f current was recorded with whole-cell patch clamp way.

Results: I_f densities decreased significantly by Car. At the test potential of -150 mV, the average densities of If were decreased from 3.2±0.4 pA/pF to 2.4±0.3 pA/pF by Car (1.0 μmol/L, P<0.01). I_f current was inhibited by Car in voltage-dependent way and Car significantly blocked I_f current at more negative test potential. Current of I_f was reduced by Car in concentration-dependent way ranged from 0.1 to 30.0 μmol/L, with IC_{50} of 1.37 μmol/L (95% confidence limit: 0.57~1.96 μmol/L). Furthermore, steady state activiation curves were shifted to negative by Car. The voltages half-maximal activation ($V_{1/2}$) were -96.2±2.3 mV (Car treatment) and -87.5±4.7mV (control). It demonstrated that I_f current activiated procedure was partly blocker by the effect of Car.

Conclusion: This study suggested that Carvedilol could decrease I_f density of atrium cells of older rats.

P21.

An 80-year-old Patient with Mitochondrial tRNA Gene Mutation Presenting Hypertension as the Main Symptom

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Mutations in mitochondrial DNA (mtDNA) have been implicated in the pathogenesis of different clinical syndromes. We speculated that mutations in mtDNA may also be associated with essential hypertension. Therefore, we investigated the role of mtDNA defects in hypertension by screening for the mitochondrial DNA mutation by sequencing of the entire mitochondrial genome. In this report we describe a patient who has been suffering from hypertension but who has survived to age 80. The patient had a long history of hypertension. Sequence analysis of mtDNA in this patient identified a A-to-G transition at position 4295 (A4295G) in the $tRNA^{Ile}$ gene and 14693 (C14693U) in the $tRNA^{Glu}$. We found that the protein expression in mitochondrial was severely impared. The tRNA expression was also impaired. Thus, mitochondrial dysfunctions, caused by the mutation of mitochondrial tRNA, may have a potential modifier role in increasing the occurrence of the hypertension.

ABSTRACTS

Abstracts for Posters:

P22.

A4435G Mutation in the Mitochondrial TRNA^{MET} Gene as a Modulator of Metabolic Syndromes

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Objective: Patients have a variable of clinic phenotypes as cardiac hypertrophy, cardiac dilatation and CHD risk equivalent-diabetes mellitus. To find the relationship between mitochondrial DNA (mtDNA) mutation and hypertension, we did mitochondrial DNA analysis in 2,000 patients with essential hypertension.
Methods: The mitochondrial genome mutation was detected by sequence analysis of mitochondrial DNA from enrolled patients with essential hypertension. Then we collected and did statistic analyses on the clinical data and genetic characteristics of the family.
Results: We found a sporadic mitochondrial tRNAMet A4435G mutation by mtDNA sequence analysis. The patient was diagnosed to have essential hypertension since the age of 44 years with the present of cardiac hypertrophy and left ventricular dilatation and complicated with hyperlipidemia, abdominal obesity and type 2 diabetes mellitus which were diagnosed as metabolic syndrome. The double time in lymphoblastoid cell line increased significantly compared with the normal control (P<0.05). The rate of oxygen consumption was significantly decreased in the patient's mutant cell line.
Conclusions: This suggests that mitochondrial mutation play an important role in pathogenesis of hypertension, abdominal obesity, hyperlipidemia and type 2 diabetes mellitus, which was diagnosed as metabolic syndrome.

P23.

Depletion of Circulating Endothelial Progenitor Cells is Associated with Impairment of Endothelial Independent Dilatation of Brachial Artery in Patients with Scleroderma

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Background: Although the mechanism remains unclear, vascular dysfunction is one of the hallmarks of scleroderma (Sc). We hypothesize that depletion of circulating endothelial progenitor cell (EPC) contributes to the development of vascular dysfunction in patients (pts) with Sc.
Method: We measured the brachial vascular function by vascular ultrasound to assess flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD). And circulating EPC (CD34/KDR+ and CD 133/KDR+) as determined by flow cytometry in 67 female Sc pts and 64 age-matched female controls.
Result: Compared to controls, Sc pts had a significantly lower CD34/KDR (0.99±0.50 vs. 1.49±1.46%, P=0.011) and CD133/KDR (0.29±0.36 vs. 0.54 ±0.36%, P<0.001) EPC. There was no significant difference in the FMD (5.23±2.85 vs. 5.95±4.62%, P=0.29) between them. However, Sc pts had a significantly lower NMD (19.15±10.17 vs. 22.86±9.40%) than controls, suggestive of impairment of endothelial independent dilation of brachial artery. Furthermore, the number circulating CD133/KDR EPC is positively correlated with NMD (r=0.25, P=0.006), but not FMD in pts with Sc.
Conclusions: This result of this study suggest that depletion of circulating EPC is associated with impairment of endothelial independent dilatation, likely related to smooth muscle dysfunction in pts with Sc.

P24.

Involvement of Sp1 Binding Sequences in Basal Transcription of the Rat Fibroblast Growth Factor-2 Gene in Neonatal Cardiac Myocytes

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Objective: Fibroblast growth factor-2 (FGF-2) plays a pivotal role during cardiovascular development and in the pathogenesis of cardiovascular disease. For understanding the molecular mechanisms pertain to these phenomena, it is important to study the FGF-2 promoter regions essential for gene transcription and the main transcription factors which are involved in FGF-2 promoter activation.
Methods and results: FGF-2 promoter-luciferase reporters were constructed by inserting a serials of fragments of the rat FGF-2 promoter, corresponding to -1266 to +267 bp or -1014 to +267 bp etc. from the transcriptional start site, into pGL3-basic. Neonatal rat cardiac myocytes were transfected with -1266 FGF-2p.Luc, -1014 FGF-2 p.Luc, -665 FGF-2 p.Luc, -372 FGF-2 p.Luc, -116 FGF-2 p.Luc, +60 FGF-2 p.Luc and pGL3-basic, respectively. The promoter activity was significantly increased 3.29-fold with -116 FGF-2 p.Luc as compared to control but not increased with +60 FGF-2 p.Luc. The proximal promoter region (-116/+59) of rat FGF-2 contains putative binding sites for the stimulating protein 1 (Sp1) transcription factors. Electrophoretic mobility shift assay established the existence of an atypical G-rich Sp1-binding element located between +13 to +23 bp form the transcriptional start site.
Conclusions: These data indicate that it may be the Sp1 which binds to a DNA element in the rat FGF-2 promoter and positively regulates transcription.

ABSTRACTS

Abstracts for Posters:

P25.

Construction and Function Indentification of Rat Endothelin Converting Enzyme-1c Eucaryotic Experession System in CHO Cells

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Objective: Since ET-1 is thought to play a crucial role in cardiac hypertrophy and level of which in myocardium mainly depends on ECE-1c, we constructed and identified the function of rat ECE-1c eucaryotic expression system in CHO cells.
Methods: Total RNA was extracted from SD rat arteria carotis communis tissue, then the rat ECE-1c gene was amplified by RT-PCR and cloned into the eukaryotic expression vector pCDNA3.1-myc-his-c. The recombinant vector was identified by restrictive enzyme analysis and sequencing. After stable transfection of CHO cells with the recombinant plasmid by lipofectamine2000, stable high experession monocell clones were obtained under selective growth conditions with G418. The ECE-1c expression level was identified with RT-PCR and Western blotting. The activity of ECE-1c was testified by ELISA with big ET-1 as substrate.
Results: The 2.3kb rat ECE-1c gene was successfully cloned from SD rat arteria carotis communis tissue. Result from restrictive enzyme analysis and sequencing showed that the rat ECE-1c gene was successfully inserted into pCDNA3.1-myc-his-c. Result from RT-PCR and Western blotting showed that the recombinant vector could express the rat ECE-1c in CHO cells. Result from ELISA showed the rat ECE-1c expressed in CHO cells possessed normal activity.
Conclusion: The successfully constructed eukaryotic expression system of rat ECE-1c could provide a drug screening target for cardiac hypertrophy therapy.

P26.

Endothelial Function of Pulmonary Vessels in COPD (Chronic Obstructive Pulmonary Disease) Patients – Functional and Molecular Studies

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Objectives: Chronic obstructive pulmonary disease (COPD) is one of the major killers in Hong Kong and world-widely. Structural and functional abnormalities have been demonstrated in the pulmonary circulation in COPD patients, including compromised pulmonary arterial endothelial function. The present study was designed to study endothelial function in COPD patients of different severity in both pulmonary arteries and veins with regard to the role of nitric oxide (NO) and non-NO-non-PGI_2 pathway.
Methods: Human lung tissue was obtained from patients suffering lung carcinoma undergoing lobectomy or pneumonectomy. Patients were grouped to mild COPD, severe COPD and control according to the preoperative pulmonary function test. Pulmonary arteries and veins (diameter 500 ~ 1000 μm) were normalized in a myograph and endothelium-dependent relaxation was evaluated by bradykinin (-10 ~ -6.5 LogM) with/without the presence of indomethacin (7 μM), N^G-nitro-L-arginine (300 μM), and oxyhemoglobin (20 μM) (n=8). eNOS protein expression was determined by Western blot.
Results: Endothelium-dependent relaxation was more significant in pulmonary arteries (82.2±4.3%) than in veins (64.6±2.4%) with the presence of non-NO-non-PGI_2 pathway in both arteries and veins. The bradykinin-induced relaxation was slightly decreased in arteries from mild (72.3±3.4%) and significantly decreased in severe COPD (39.3±8.4%, $p<0.001$) patients. Similar reduction was observed in pulmonary veins (mild: 51.2±2.2%, severe: $p<0.001$, 28.0±4.3%). Severe COPD abolished non-NO-non-PGI_2 pathway in the pulmonary vasculature. Phosphorylated eNOS (p-eNOS) protein expression in pulmonary arteries was significantly lower in COPD patients.
Conclusions: Both NO and non-NO-non-PGI_2 pathway are involved in the regulation of the vascular tone in the human pulmonary arterial and venous systems. The endothelium-dependent relaxation is impaired in COPD patients that involves both NO and non-NO-non-PGI_2 pathway and the severity of the impairment increases with the progress of the disease. The functional impairment of the endothelium is associated with a decreased eNOS expression.

ABSTRACTS

Abstracts for Posters:

P27.

Therapeutic Concentrations of Raloxifene Stimulate Increase in Nitric Oxide and Calcium in Endothelial Cells from Rat Small Mesenteric Artery

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Objective: Estrogen reduces myogenic responses in mammalian resistance arteries. Our previous study showed that raloxifene, a second-generation selective estrogen receptor modulator in therapeutic concentrations (0.3-10 nM) could dilate small mesenteric arteries which developed myogenic tone and the dilatation was sensitive to NOS inhibitor. The present study aims at examining whether raloxifene at therapeutically relevant concentrations could stimulate production of nitric oxide (NO) in endothelial cells isolated from Sprague-Dawley rat small mesenteric artery (MAECs) of both genders.
Methods and Results: Effects of raloxifene were determined on intracellular calcium concentration ($[Ca^{2+}]_i$) and NO level in MAECs by Fura-2 AM and 4-amino-5-methylamino-2', 7'-difluorofluorescein (DAF-FM) diacetate, respectively. Endothelial nitric oxide synthase (eNOS) phosphorylation at ser-1177 was detected by Western blot analysis. Addition of 3 nM of raloxifene resulted in increases in $[Ca^{2+}]_i$ and NO signal. Raloxifene-induced NO production was inhibited by acute treatment of 100 μM NG-nitro-L-arginine methyl ester (L-NAME, NOS inhibitor), but unaffected by ICI 182,780 (classic estrogen receptor antagonist). Moreover, Western blot analysis demonstrated that acute treatment with raloxifene increased the eNOS phosphorylation at ser-1177, without affecting the total eNOS content, which was insensitive to ICI 182,780.
Conclusion: The present results show that raloxifene at therapeutic concentrations elevates $[Ca^{2+}]_i$ and enhances the production of NO by increasing eNOS phosphorylation in MAECs. The causal link between the elevated $[Ca^{2+}]_i$ and the increased eNOS activity needs further study.
(Supported by RGC grant, CUHK Li Ka Shing Institute of Health Sciences and Focused Investment Scheme)

P28.

Effect of Diazoxide on Fas/FasL Protein Expressions in Rat Myocardium Suffered from Long-term Hypothermic Preservation

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The purpose of this study was to investigate the effect of diazoxide (DE) on Fas/FasL protein expressions in rat heart suffered from long-term hypothermic preservation. The isolated rat heart Langendorff model was used. The hearts were stored in 4°C Celsior solution for 8 h followed by 60 min of reperfusion. The recovery of rate-pressure product (RPP) was observed. Apoptotic cardiomyocytes were detected by TdT-mediated dUTP nick end labeling (TUNEL) technique. The expressions Fas/FasL proteins were also analyzed by immunohistochemical method. The results showed that: As compared with the control group, DE (30 μmol/L) could increase the recovery of RPP during reperfusion after 8 h of hypothermic preservation, reduce the percentage of apoptotic cells and the expression of Fas and FasL proteins in rat hearts suffered from 8 h of hypothermic preservation. The above effects of DE were attenuated by a mitoK_{ATP} channel inhibitor 5-hydroxydecanoate (5-HD). These results indicate that DE could alleviate rat myocardial injury induced by ischemia-reperfusion through reducing the expressions Fas and FasL proteins via opening of mitochondrial mitoK_{ATP} channel.
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