ABSTRACTS

Abstracts for Posters:

P1.

RESTORATION OF BLUNTED INSULIN RELEASE AND $[Ca^{2+}]_i$ CHANGES BY SIMVASTATIN OF ISOLATED PANCREATIC ISLETS OF LANGERHANS OF OBESE/DIABETIC (db^+/db^+) MICE

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Background: One of the factors responsible for Type 2 diabetes mellitus development is a decline in pancreatic islets β-cells secretory functions. Diabetic patients with dyslipidemia are now receiving HMG CoA reductase inhibitors (so-called statins). However, there is no consensus on whether statins consumption can improve diabetic conditions in patients.

Methodology: The pancreatic islets of age-matched (female; ~6 month-old) lean/control (db^+/m^+) and obese/diabetic (db^+/db^+) of C57BL/KsJ mice were isolated using collagenase; single pancreatic b-cells of both strains of mice were further isolated by trypsin. Effects of simvastatin (a HMG CoA reductase inhibitor) (10 nM, 24 hr) and L-phenylalanine (an allosteric activator of CaR) (10 mM, 24 hr) on protein expression of CaR, SNARE proteins (VAMP-2 and syntaxin) and glucokinase (GCK) were compared. Glucose (15 mM)- and carbachol (500 μM)-elicited $[Ca^{2+}]_i$ changes of single pancreatic islet β-cells, with and without simvastatin incubation, were measured. Glucose (5 and 15 mM)-induced insulin secretion (GIIS) was determined (ELISA).

Results: A lowered protein expression of CaR and a smaller magnitude of glucose (15 mM)- and carbachol (500 μM)-induced $[Ca^{2+}]_i$ changes were detected in pancreatic islets/pancreatic β-cells of db^+/db^+ mice compared to db^+/m^+ mice, which was restored by simvastatin and L-phenylalanine. An attenuated glucose (5 and 15 mM)-induced insulin release was measured in pancreatic islets of db^+/db^+ mice and the suppressed glucose (15 mM)-induced insulin release was partially restored by simvastatin and L-phenylalanine. A lowered expression of GCK (but not SNARE proteins) was detected in pancreatic islets of db^+/db^+ mice which was not modified by simvastatin.

Conclusions: The blunted GIIS of isolated pancreatic islets of db^+/db^+ mice is related to an attenuated CaSR expression and the redundancy of its associated $G_{q/11}$ receptor pathways. The "enhanced" protein expression of CaSR and the improvement of glucose-/carbachol-elicited $[Ca^{2+}]_i$ changes by simvastatin is probably responsible for the restoration of the attenuated GIIS of the pancreatic β-cells of db^+/db^+ mice.

Acknowledgements: This project was financially supported by GRF Grant (to YWK) (Reference number: 2140565).

P2.

INVOLVEMENT OF CFTR IN ATP RELEASE FROM CONTRACTING SKELETAL MUSCLE IN ANAESTHETISED RATS

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Contracting skeletal muscle releases ATP into the interstitial space where it is subsequently broken down to adenosine by the action of ecto-5'-nucleotidase. Both ATP and adenosine are vasodilators that contribute to the exercise hyperaemia. However, the mechanism for the release of ATP from muscle during exercise remains unknown. Cystic fibrosis transmembrane conductance regulator (CFTR) is a possible channel for ATP release: this study was performed to investigate whether CFTR was involved in the ATP release from muscle during exercise.

Experiments were performed in rats anaesthetised with sodium pentobarbitone and breathing spontaneously. A microdialysis probe was placed in one gastrocnemius muscle and samples of interstitial microdialysate were analysed by HPLC for the ATP breakdown products, AMP and adenosine. An electrode was placed on the sciatic nerve, which was stimulated to induce two bouts of muscle contractions, separated by a recovery period of 40 mins. In the test group of rats, KT5720, a protein kinase A inhibitor which prevents activation of CFTR, was administered between the two bouts of contractions; in the control rats, no drug was given.

In the control rats, interstitial adenosine increased from 0.57±0.08 to 1.61±0.40 μM during the first contraction bout and from 0.65±0.13 to 1.51±0.44 μM in the second contraction bout, whilst AMP increased from 0.40±0.06 to 1.14±0.29 μM during the first contraction bout and from 0.46±0.09 to 1.07±0.31 μM in the second contraction bout; neither the force nor the interstitial adenosine and AMP differed significantly between the two bouts of contraction. Administration of KT5720 did not alter the resting interstitial concentrations of adenosine or AMP, but reduced the contracting adenosine concentration from 1.81±0.21 to 0.90±0.16 μM and the contracting AMP concentration from 3.76±0.57 to 2.25±0.28 μM.

These results suggested that the CFTR may have been involved in the ATP release from skeletal muscle during exercise hyperaemia.

ABSTRACTS

Abstracts for Posters:

P3.

A NEWLY-DERIVED SMALL SYNTHETIC COMPOUND ALLEVIATED VENTRICULAR FIBRILLATION IN A PIG MODEL WITH CHRONIC MYOCARDIAL INFARCTION AS REVEALED BY OPTICAL MAPPING

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The electrophysiological hallmark of cells and tissues isolated from failing hearts is prolongation of action potential duration (APD), resulted from down-regulation of repolarizing K^+ currents and/or alterations in depolarizing Na^+ and Ca^{2+} currents, which predisposes the failing heart to lethal ventricular tachyarrhythmia (ventricular tachycardia (VT) and ventricular fibrillation (VF)). C11, a small synthetic Cl^- channel, exhibits membrane-repolarizing power. Therefore, we hypothesize C11 corrects the delayed repolarization and shortens APD at cellular level, thus modifying ventricular arrhythmogenic substrate at whole heart level. First, we demonstrated APD reduction upon C11 application (30 μM) at 37°C to isolated guinea pig ventricular cardiomoyocytes with patch-clamp experiments in whole cell configuration.

To examine whether C11 works in disease model, pig hearts with chronic myocardial infarction (MI) were optically mapped. Electrocardiograms (ECGs) and the optical mapping signals with optical timing maps displayed the attenuation of ventricular fibrillation (VF) to ventricular tachycardia (VT) in the presence of C11 (30 μM).

In conclusion, C11 alleviated VF in our *ex vivo* pig heart model with chronic myocardial infarction. Further investigation in the ionic properties of C11 will be of worthy to further dissect the underlying mechanism of function posing potential use of C11 in clinical prospect.

P4.

VASCULAR MODULATION EFFECT OF GINSENG EXTRACTS

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Background: It is known that endothelial damage is the primary cause of the vascular complications of diabetes mellitus. Moreover, the endothelial damage is usually not reversible.
Materials and methods: In the present study, we examine the effects of ginseng extracts (PPD and PPT) on acetylcholine-induced endothelium dependent vasorelaxation pre-contracted with phenylephrine. Studies were performed in adult Sprague-Dawley male rats and the responses were examined in vitro using isolated thoracic aortic rings.
Result: Severe impairment of vasorelaxation was found in diabetic group (62.4% of control in maximum dosage of ACh-induced vasorelaxation) while the groups fed with ginseng extracts restored the vasorelaxation (no significant difference with control). Blood profile exhibited that the ginseng extracts could not reduce blood glucose and blood cholesterol level caused by diabetes mellitus. However, ginseng extract PPD improved nitric oxide (in terms of nitrite) production stimulated by acetylcholine.
Conclusion: Ginseng may have vascular protective effect on diabetic condition.
Acknowledgement: The funding support is provided by the Collaborative Research Fund (HKBU 1/06C) of Research Grant Council of HKSAR.

ABSTRACTS

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P5.

NAHS RELAXES RAT CEREBRAL ARTERIES THROUGH INHIBITING L-TYPE CALCIUM CHANNEL

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Objectives: H_2S may act through the opening of ATP-sensitive K^+ channels or 4-aminopyridine-sensitive voltage-gated K^+ channels to cause vasodilatation, depending on the type of blood vessels. It is however unclear how H_2S acts on cerebral arteries. The present study investigates whether NaHS, a H_2S donor, inhibits voltage-sensitive Ca^{2+} channels and thus relaxes cerebral arteries.
Methods: Middle cerebral arteries of Sprague-Dawley rats were suspended in wire myograph or pressurized myograph for measurements of vascular reactivity under isometric or isobaric conditions. Single myocytes were enzymatically isolated from the rat cerebral arteries for measuring whole-cell L-type Ca^{2+} currents by a patch-clamp method. Ca^{2+} movement in isolated cerebral arteries was determined using a fluo-4 fluorescence dye under confocal microscope.
Results: NaHS relaxed both phenylephrine and 60 mM KCl pre-contracted rat cerebral arteries with the same potency. The relaxations were unaffected by several K^+ channel blockers, NOS inhibitor N^G-nitro-L-arginine methyl ester, nor cyclo-oxygenase inhibitor indomethacin. H_2S precursor L-cysteine induced dilatation in cerebral arteries, which was inhibited by cystathionine γ-lyase inhibitor DL-propargylglycine. NaHS concentration-dependently inhibited $CaCl_2$-induced contraction in Ca^{2+}-free Krebs solution. NaHS reduced the amplitude of L-type Ca^{2+} currents in isolated myocytes and directly inhibited Ca^{2+} influx in rat cerebral arteries. NaHS also caused relaxation of myogenic tone in cerebral arteries under pressurized condition.
Conclusions: NaHS causes relaxations, independent of K^+ channel activation, and suppresses $CaCl_2$-induced contractions in rat cerebral arteries. It reduced L-type Ca^{2+} currents in myocytes and inhibited Ca^{2+} influx in rat cerebral arteries. These suggest that H_2S relaxes cerebral arteries primarily through inhibiting Ca^{2+} influx via L-type Ca^{2+} channels.

P6.

EXPRESSION OF TRP CHANNELS IN ARTERIAL BARORECEPTOR NEURONS

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TRP channel is a superfamily of non-selective cation channels that can be divided into seven subfamilies: TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV. Many TRP isoforms have been reported to be sensors for diverse source of external and/or internal stimuli. Although still under debating, TRPC1, -C5, -C6 are suspected to be responsive to direct membrane stretch; TRPV1, -V4 are suggested be activated by stretch-induced cytoskeleton displacement and/or flow stimuli; TRPM4, -V4, -C6 are reported to be activated by stretch-sensitive cytosolic signaling molecules.

Arterial baroreceptors are the mechanosensor to detect blood pressure. Upon changes in arterial blood pressure, the baroreceptive nerve terminal on the blood vessel adventitia will be activated, resulting in action potentials which propagate to the cardiovascular control centre in the brain through its afferent nerve. However, the molecular identity of the baroreceptor mechanosensors is not well understood.

There are two major baroreceptors, aortic and carotid baroreceptors. In the present study, immunohistochemistry was employed to explore the expression of mechanosensitive TRP isoforms in the rat aortic baroreceptor. The results demonstrated that TRPC1, C5, C6, V4 are expressed in the aortic baroreceptor nerver terminal which is located on the aortic arch, along the nerve fibre (aortic depressor nerve) and in the ganglion region (nodose ganglion). Moreover, western blotting and RT-PCR using isolated rat nodose ganglion also showed the expression of some TRP channels. In summary, our study suggests that TRP channels could be mechanosensor involved in blood pressure detection in the arterial baroreceptor neurons.

ABSTRACTS

Abstracts for Posters:

P7.

ASSOCIATION OF A GENETIC VARIANT IN THE APOLIPOPROTEIN A5 GENE WITH THE METABOLIC SYNDROME IN CHINESE

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Introduction: We previously reported that the single nucleotide polymorphism (SNP) rs662799 (-1131T>C) in the apolipoprotein A5 gene (*APOA5*) was an important determinant of plasma triglycerides in both Hong Kong and Guangzhou Chinese. We, therefore, investigated the association of SNPs in *APOA5* with the metabolic syndrome (MetS) in the Hong Kong and Guangzhou Chinese.

Methods: MetS was defined according to the consensus criteria proposed jointly by several organizations in 2009. Five tagging SNPs were genotyped in 1330 unrelated subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study cohort with follow-up after a median interval of 6.4 years. 1952 subjects from the Guangzhou Biobank Cohort Study-Cardiovascular Disease Subcohort were used to replicate the findings.

Results: The minor allele of rs662799 was significantly associated with higher odds for the MetS in Hong Kong subjects at both baseline (OR=1.47, *P*=0.00082) and follow-up (OR=1.30, *P*=0.010). A similar association was found in Guangzhou subjects (OR=1.27, *P*=0.0041). In a pooled sample of Hong Kong subjects at follow-up and Guangzhou subjects, this SNP was also associated with HDL and LDL cholesterol (*P*<0.001 and 0.010 respectively). All these associations disappeared after further adjusting for plasma triglycerides (*P*>0.05). In a meta-analysis of 6 studies, the combined OR (95% CI) was 1.38 (1.25-1.52) for the TC + CC genotype compared to the TT genotype (*P*<0.00001).

Conclusion: The association of -1131T>C polymorphism in *APOA5* with the MetS was mainly due to its strong effect on plasma triglycerides. Further studies are needed to assess the utility of this genetic marker in risk stratification.

P8.

ROSIGLITAZONE UPREGULATES ENDOTHELIAL EXPRESSION OF ENDOTHELIN B RECEPTOR AND ATTENUATES ENDOTHELIN-1-INDUCED VASOCONSTRICTION

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Objectives: Thiazolidinediones improve insulin resistance and endothelial dysfunction. However, the mechanisms underlying the vasoprotective effects of thiazolidinediones remain to be fully elucidated. The present study aimed to investigate the molecular mechanism for the anti-vasoconstrictive effects of peroxisome proliferator-activated receptor-gamma (PPARgamma) ligand rosiglitazone in response to endothelin (ET)-1.

Methods: Mouse aortas were treated with rosiglitazone for 24 hours, and ET-1-induced vasoconstriction was assessed by wire myography. The results showed that rosiglitazone attenuated ET-1-induced contraction in mouse aortas; this effect was abolished by ET-B receptor (ET_BR) antagonist A192621, NO synthase inhibitor (L-NAME), and by the removal of endothelium. Western blotting, and immunohistochemistry showed that rosiglitazone upregulated expression of ET_BR in mouse aortas. Rosiglitazone also enhanced selective ET_BR agonist sarafotoxin 6c-induced vasodilatations in mesenteric resistance arteries which were abolished by L-NAME or A192621. In vivo treatment with rosiglitazone also attenuated the ET-1-induced vasoconstrictions and increased the ET_BR expression without affecting the expression of ET_AR in mouse aortas and mesenteric arteries.

Conclusions: These results demonstrated that rosiglitazone attenuated ET-1-induced vasoconstriction through the upregulation of endothelial ET_BR, which is a PPAR gamma direct target. (Supported by the NSFC and RGC)

ABSTRACTS

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P9.

CHARACTERIZATION OF MULTIPLE ION CHANNELS IN HUMAN INDUCED PLURIPOTENT STEM CELLS-DERIVED MESENCHYMAL STEM CELLS

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Our recent studies demonstrated that functional mesenchymal stem cells (MSCs) can be derived from human induced pluripotent stem cells (iPS) which can be used as an alternative source of stem cells for cardiac repair. However, transplantation of iPS-MSC with undesirable electrical ionic profile into human myocardium can be potentially lethal. Here, we characterized the electrophysiological properties of iPS-MSCs with comparison to that of bone-marrow (BM) derived MSCs, since human iPS-MSCs showed similar phenotype and expression of surface markers for MSCs as BM-MSCs.

The expression of various common ion channels for sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-) in human iPS-MSCs were examined by reverse transcription-polymerase chain reaction (RT-PCR). Those functional ion channels identified by RT-PCR were confirmed by whole-cell patch clamp technique. RT-PCR revealed the molecular identities (mRNAs) of some ion channels and their possible existence in human iPS-MSCs, including KCa.1.1 (responsible for the big conductance Ca^{2+}-activated K^+ current, BK_{Ca}), Kv10.1 (for the delayed rectifier K^+ current, IK_{DR}), Kir2.1 and Kir2.3 (for the inwardly-rectifying K^+ current, I_{Kir}), KCa3.1 (for the intermediate conductance Ca^{2+}-activated K^+ current, IK_{Ca}), Clcn3 (for the chloride current, I_{Cl}), SCN9A (for the tetrodotoxin-sensitive sodium current, $I_{Na.TTX}$), CACNA1C (for the nifedipine-sensitive L-type Ca^{2+} current, $I_{Ca.L}$) and Kv4.3 (for the transient outward potassium current, I_{to}), but not Kv1.4 and Kv4.2 (for I_{to}). Patch clamp experiments were conducted to verify the existence of functional ion channels, five types of currents (BK_{Ca}, IK_{DR}, I_{Kir}, IK_{Ca} and I_{Cl}) were found in human iPS-MSCs, but not the three ($I_{Na.TTX}$, $I_{Ca.L}$ and I_{to}) reported in BM-MSCs.

We conclude that although human iPS-MSC and BM-MSC showed similar phenotype, they have different ionic channel profile. The functional implication for these differences in the ionic profile merits further investigation.

P10.

EXPRESSION OF TRPC AND TRPM CHANNELS IN HUMAN ATRIAL MYOCYTES

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Generation of cardiac arrhythmias, especially human atrial fibrillation underlying mechanisms, is not fully understood. Recent studies have demonstrated that transient receptor potential (TRP) channels play important roles in the regulation of physiological and pathological cellular function. Little information is documented about TRP channels in human hearts. The present study was designed to investigate the expression of TRP channels in human atrial myocytes using whole-patch voltage clamp and molecular biological approaches. It was found that the previously reported background nonselecteve cation current was inhibited by the TRPC channel blocker La^{3+} in a concentration dependent manner (IC_{50}=46 μM), suggesting the contribution of TRPC channels. In addition, we recorded a currents that is sensitive to inhibition by divalent cations, e.g. Mg^{2+}, Ni^{2+}, Ba^{2+}, etc. The current is enhanced by removing intracellular Mg^{2+} or extracellular Mg^{2+} ion, but blocked by Ni^{2+} or Ba^{2+}. This divalent cation-sensitive current was inhibited by 2-aminoethoxydiphenyl borate (2-APB, IC_{50}=32 μM), increased when the bath medium pH was reduced from 7.3 to 4.0. These properties are similar to those of TRPM7 channels. RT-PCR and Western blot analysis revealed that mRNAs and proteins of TRPC1, TRPC3, and TRPM7 were significant in human atrial myocytes. These results demonstrate the novel information TRPC1, TRPC3, and TRMP7 channels are present in human atrial myocytes. Activation of TRP channels likely contribute to the genesis of human atrial fibrillation, and therefore TRP channels may be a target for the development of anti-atrial fibrillation approach.

ABSTRACTS

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P11.

ENDOTHELIAL NOS-INDEPENDENT RELEASE OF NITRIC OXIDE IN THE AORTA OF THE SPONTANEOUSLY HYPERTENSIVE RAT

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Objective: In the aorta of male spontaneously hypertensive (SHR), but not in that of normotensive Wistar-Kyoto (WKY) rats the endothelium inhibits the contraction to phenylephrine despite the presence of indomethacin (inhibitor of cyclooxygenase) and L-NAME (inhibitor of nitric oxide synthase (NOS). The present studies were designed to examine the mechanism underlying this endothelium-dependent inhibition in the SHR aorta.

Methods: Aortic rings, with and without endothelium, of male SHR and WKY of age (12 to 22 weeks and 38 to 48 weeks old) were suspended in organ chambers for the measurement of isometric tension. The preparations were incubated with indomethacin (10^{-5} M) and L-NAME (10^{-4} M) to eliminate the effect of endogenous prostanoids and nitric oxide (NO) produced by nitric oxide synthases (NOS), respectively. After incubation with carboxy-PTIO (nitric oxide scavenger, 3 x 10^{-4} M) for five minutes or ODQ (guanylyl cyclase inhibitor, 10^{-5} M) for 30 minutes, the rings were contracted with increasing concentrations (10^{-9}-10^{-6}M) of phenylephrine. Sodium nitrite (10^{-7}-$10^{-2.5}$M) was used to relax the rings during the contraction to phenylephrine.

Results: Contractions to phenylephrine were smaller in SHR aortae with endothelium than in those without endothelium of both either. The inhibitory effect if the endothelium was larger in preparations from 38-48 weeks than those from 12-22 weeks old SHR. The endothelium-dependent difference in contraction to the alpha-adrenergic agonist was prevented by carboxy-PTIO or ODQ. The contraction of aortic rings with and without endothelium of 12-22 weeks or 38-48 weeks old WKY were comparable were not affected by either carboxy-PTIO or ODQ. Sodium nitrite relaxed SHR aortae with or without endothelium to the same extent and this relaxation was eliminated by ODQ. Otherwise, the contraction evoked by KCl has the same difference in aorta of SHR or WKY with and without endothelium.

Conclusion: In the SHR aorta, there is an endothelium-dependent release of nitric oxide which is not produced by NOS. This NOS-independent nitric oxide release, which is only seen in preparations from hypertensive animals and augments with aging when endothelial dysfunction develops. Nitrite may be the source of the NOS-independent nitric oxide since its dilator effect is comparable in rings with and without endothelium of either normotensive or hypertensive animals.

P12.

ACTIVATION OF NICOTINIC RECEPTORS CONTRIBUTES TO ACETYLCHOLINE-INDUCED ENDOTHELIUM-DEPENDENT RELAXATIONS IN THE AORTA OF THE SPONTANEOUS HYPERTENSIVE RAT

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Acetylcholine causes both endothelium-dependent relaxations and contractions in the rat aorta. Both muscarinic (mAChRs) and nicotinic (nAChRs) acetylcholine receptors are expressed in endothelial cells. It is generally accepted that mAChRs [of the M_3-subtype] are responsible for both endothelium-dependent relaxations and contractions evoked by acetylcholine. To study the expressions of acetylcholine receptors in rat aorta, PCR primers for different subunits of nAChRs and mAChRs were designed. To study whether or not nAChRs are involved in endothelium-dependent relaxations evoked by the cholinergic transmitter, 36 weeks old spontaneous hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, and 64 weeks old WKY rats were used. Rings with endothelium were suspended in organ chambers for isometric tension recording. Quiescent rings were incubated with vehicle, mecamylamine (nAChRs inhibitor, 10^{-4}M), atropine (mAChRs inhibitor, 10^{-5}M), or mecamylamine plus atropine. The concentration of mecamylamine and atropine was the lowest concentration that gave the most significant inhibitory effect in organ chamber experiments. All the rings were incubated with indomethacin (non-selective cyclooxygenase inhibitor, 10^{-5} M) to prevent endothelium-dependent contractions. After 40 minutes of incubation, the rings were contracted with phenylephrine (10^{-5}M) and then relaxed with cumulatively increasing concentrations of acetylcholine (10^{-9} to 10^{-5}M). The PCR results revealed that α2, 3, 4, 5, 6, 7, 9, 10 subunits of nAChRs and M1-M5 subunits of mAChRs were expressed in 36 weeks old SHR aorta. The organ chamber experiments demonstrated that in both SHR and WKY aortae, acetylcholine-induced relaxations were similar in control and mecamylamine-treated rings, while the relaxations were significantly reduced in atropine-treated preparations. In rings of 36 weeks old SHRs, the remaining acetylcholine-induced relaxations in the presence of atropine approximated 50% of those observed in untreated control preparations, while in both 36 weeks and 64 weeks old WKY aortae the remaining response in atropine- treated preparations was minimal. The response to acetylcholine was nearly abolished in rings which had been incubated with mecamylamine plus atropine. Thus in the SHR and WKY aorta, mAChRs are mainly responsible for endothelium- dependent relaxations to acetylcholine under control conditions. However, when mAChRs are inhibited by atropine, nAChRs mediate relaxation to the cholinergic transmitter in the SHR but not the WKY aorta.

ABSTRACTS

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P13.

CALCITRIOL PROTECTS RENOVASCULAR FUNCTION OF OVARIECTOMIZED RATS THROUGH THE DOWN-REGULATION OF CYCLOOXYGENASE-2 AND TP RECEPTOR

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Exaggerated release of cyclooxygenase (COX)-derived contracting factors can result in an impaired vasodilatation via TP receptor activation. While Vitamin D may be beneficial to the cardiovascular function in addition to its classical action on calcium homeostasis, its effect on renal vasculature during estrogen deficiency is yet to be explored. The present study aims at investigating changes in the renovascular reactivity in ovariectomized rats and whether calcitriol, an active form of vitamin D, could reverse the altered vascular function. Changes in isometric tension of the intrarenal arteries from the sham and ovariectomized rats were recorded in microvessel myograph. Expression levels of relevant proteins were analyzed by Western blotting. Acetylcholine (ACh)-induced endothelium-dependent relaxations were impaired in renal arteries from ovariectomized rats while their endothelium-independent relaxations to sodium nitroprusside remained unchanged as compared with the sham control. The non-selective COX inhibitor, indomethacin (3 μM), improved the ACh-induced relaxations, and so did the selective COX-2 inhibitor celecoxib (3 μM) but not the COX-1 inhibitor sc-560 (10 nM). Antagonist of thromboxane-prostanoid (TP) receptor S18886 (0.3 μM) improved the relaxations. Western blot analysis showed that calcitriol reduced the elevated expression of COX-2 and TP receptor in renal arteries from ovariectomized rats. The present results suggest that the impaired endothelium-dependent relaxations in renal arteries of ovariectomized rats are possibly attributed by the release of COX-2-derived prostaglandin(s) that activate the TP receptor and that calcitriol normalizes the renovascular function at least in part through COX-2 and TP receptor down-regulation.

P14.

DOSE-RESPONSE RELATIONSHIP BETWEEN SMOKING, SMOKING CESSATION STATUS AND CAROTID ATHEROSCLEROSIS: THE GUANGZHOU BIOBANK COHORT STUDY-CVD

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Objective: To examine the dose-response relationship between smoking and quitting smoking status with carotid atherosclerosis in 959 relatively healthy Chinese men.
Methods: 959 men aged 50-85 years were randomly selected from phase III (2006-2007) of the Guangzhou Biobank Cohort Study into this cross-sectional study. Common carotid artery intima-media thickness (CCA-IMT) was measured by B-mode ultrasonography, and carotid arterial plaques were identified. Major cardiovascular risk factors, including fasting triglyceride, LDL- and HDL-cholesterol, glucose, and systolic and diastolic blood pressure were assessed.
Results: 1) Composition of the cases: 39.1% were non-smokers, 25.7% were former smokers and 35.2% were current smokers. The mean (95% confidence interval) carotid IMT was 0.78 (0.77-0.79) mm. 18.4% of the subjects had a carotid IMT ≥1.0 mm, while 34.1% had carotid plaque. 2) After adjusting for age, sex, physical activity, body mass index, fasting glucose, triglyceride, HDL-cholesterol, systolic and diastolic blood pressure, compared to never smokers, current smokers had significantly increased risk for thicker IMT and carotid plaque [odds ratio(OR)=1.82, 95%CI: 1.30-2.55 and OR=1.95, 95%CI:1.38-2.75, respectively, all P<0.001]. The risk for thicker IMT and carotid plaque increased with the increasing amount (cigarettes/day) and duration of smoking (years) as well with cigarette pack-years (P for trend all ≤0.01). 3) Compared to current smokers, after adjustment for cigarette pack-years and other potential confounders, the adjusted ORs (95% CI) for the duration following quitting for 1-9, 10-19 and 20+ years were 0.77 (0.47 to 1.26), 0.45 (0.26 to 0.79) and 0.37 (0.17 to 0.77) for the presence of CCA atherosclerosis, and 0.69 (0.43 to 1.12), 0.47 (0.27 to 0.82) and 0.45 (0.23 to 0.96) for the presence of carotid plaques, respectively.
Conclusion: An elevated risk with a clear dose-response relationship was found between cigarette smoking and carotid atherosclerosis. Smoking cessation was beneficial in attenuating the risk of carotid atherosclerosis associated with a dose-response relationship with quitting time.
Acknowledgement: This project was supported by grants from the National Natural Science Foundation of China (No. 30518001/CO301070202) and the Hong Kong Research Grants Council (No. N-HKU720/05)

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P15.

THE POTENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPS) DERIVED ENDOTHELIAL PROGENITOR CELLS (EPC) IN THERAPEUTIC ANGIOGENESIS

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Introduction: Experimental and clinical studies have shown that endothelial progenitor cells (EPCs) can enhance angiogenesis in ischemic hindlimb muscles and myocardium. However, the use of autologous EPCs transplantation isolated from patients' blood are limited by their number and proliferative potential. Human induced pluripotent stem cell (hiPS) is a potential alternative cell source for EPC generation due to their autology, high power of proliferation and pluripotency.
Methods: We characterize the phenotype and function of hiPS-derived EPCs and compare them with human endothelial cell line (HUVEC) and human embryonic stem cell derived EPC (H1-EPC). Donor specific Induced pluripotent stem cells were generated from their skin fibroblast in feeder free, serum free culture system and subsequently differentiated into EPCs.
Results: Two EPCs were generated (SIU1-EPC and IMR90-EPC) and have similar morphology as H1 HES derived EPC. Positive vWF, ∝SMA, Di-acetyl-LDL-DiI and Lectin staining was observed in both iPS-EPC and H1-EPC. Tube formation assay revealed similar potential in forming capillary with IMR90-EPC and SIU1-EPC as H1-EPC and HUVEC (13450±882, 15108±984.6 vs 14867.12±934.54 and 15349.59±1034.67 AU/well). Animal model showed that the iPS derived EPSs can have comparable potential that resume part of regional blood flow 28 days after left femoral artery occlusion (IMR90-EPC and SIU1-EPC 35.5±6.2%, 37.9±5.5% vs H1-EPC and HUVEC 38.5±4.5%, 32.8±2.8%).
Conclusions: Our results demonstrate that hiPS-derived EPCs resemble normal human endothelial cells with similar phenotypes and angiogenic function but unlimited proliferation capacity. These findings suggest that hiPS-derived EPC can be used as patient specific cell source in therapeutic angiogenesis.

P16.

TNNI3K, A NEW MAP KINASE GENE, COULD BE A MOLECULAR TARGET FOR THE TREATMENT AND DIAGNOSTIC AGENTS ON CARDIAC DISEASES

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Objectives: TNNI3K is a novel cardiac-specific and troponin I (cTnI)-interacting MAP kinase. Recently, we investigated the effects of TNNI3K on P19CL6-derived cardiomyogenesis in vitro, and results indicated that TNNI3K specifically interacted with cTnI by inhibiting cTnI phosphorylation and played important roles in cardiac myogenesis. The aim of this study is to evaluate whether TNNI3K overexpression can reduce myocardial ischemic injury in mouse cardiac infarction model and whether plasma TNNI3K levels can predict the occurrence of acute cardiac infarction (AMI) in patients or not.
Methods and Results: A myocardial infarction (MI) model of mice was made by ligation of left anterior descending branch of the coronary artery, and the differentiated P19CL6 cells (induced 4 days in 1% DMSO) with overexpressing TNNI3K was injected at four points, with 20 microliter per point, in the border zone surrounding the infarcted area. TNNI3K-overexpression attenuated ventricular remodeling, improved impaired UCG ejection fraction, left ventricular end-diastolic and end-systolic dimensions, decreased the infarct size when compared with the medium-only or the vector-only groups ($P<0.05$). To measure the circulating TNNI3K level in AMI patients, a polyclonal anti-human TNNI3K antibody was raised in rabbit by injecting recombinant TNNI3K and purified as IgG by using protein A-conjugated sepharose column. Standard curve gained by enzyme-linked immunosorbent assay (ELISA) was linear through the range from 0.011 to 11.00 ng/ml of TNNI3K protein. Plasma TNNI3K concentrations of healthy volunteers, acute renal failure, chronic heart failure and acute myocardial infarct patients measured by ELISA were 221.38±12.8 (n=21), 218.26±6.8 (n=6), 320.7±8.9 (n=18) and 2025.0±200.7 (n=18) ng/ml, respectively. Results indicated that circulating TNNI3K level in AMI patients was significantly higher than that in any other group ($p<0.001$). Specificity and sensitivity to differentiate AMI from the other two was evaluated using receiver operating characteristic curve analysis (MedCalc software) and 82.4% of specificity and 86.7% of sensitivity was gained by setting a cut-off value at 366 ng/ml.
Conclusions: Over-expressing the TNNI3K level would be a useful therapeutic approach for ischemic cardiac diseases, and measurement of plasma TNNI3K level may be a novel useful diagnostic tool for acute myocardial infarction. Using TNNI3K as a molecular target may be a new potential approach for developing remedies or diagnosis agents for ischemic cardiac diseases.

ABSTRACTS

Abstracts for Posters:

P17.

RUTAECARPINE RELAXES RAT MESENTERIC RESISTANCE ARTERIES VIA THE RELEASE OF ENDOTHELIUM-DERIVED NITRIC OXIDE AND NEUROGENIC CALCITONIN GENE-RELATED PEPTIDE

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Rutaecarpine, a bioactive alkaloid extracted from the dried fruit *Evodia rutaecarpa,* is widely used to treat hypertension. However, its effect on peripheral resistance arteries remains elusive. The present study characterizes the vascular action of rutaecarpine in resistance arteries. Alterations in isometric tension of the third-order Sprague-Dawley rat mesenteric resistance arteries were studied in myograph. Field stimulation was applied to trigger neurogenic relaxations. Real-time changes in intracellular calcium concentration ($[Ca^{2+}]_i$) in native mesenteric endothelial cells and NO production ($[NO]_i$) in primary cultured mesenteric endothelial cells were determined by confocal microscopy. Rutaecarpine caused potent relaxations which were partially inhibited by N^G-nitro-L-arginine methyl ester (NOS inhibitor), ODQ (guanylate cyclase inhibitor) or in rings without endothelium. Rutaecarpine-induced relaxations were attenuated by CGRP receptor antagonist, CGRP (8-37) or depletion of CGRP from sensory nerves by capsaicin. Field stimulation-induced relaxations were eliminated by CGRP (8-37) and capsaicin. Rutaecarpine only partially relaxed mesenteric arteries of eNOS knockout mice to an extent similar to the L-NAME-treated arteries from the wild type mice, and the relaxations in eNOS knockout mice were abolished by CGRP (8-37). Rutaecarpine stimulated rises in both endothelial cell $[Ca^{2+}]_i$ and $[NO]_i$. The present results demonstrate that rutaecarpine exerts potent vasodilatory effects in resistance arteries by (1) increasing eNOS activity and calcium-dependent NO formation in endothelial cells, and (2) stimulating the neurogenic release of capsaicin-sensitive CGRP which causes NO-independent relaxations. The concomitant neurovascular effects in the resistance arteries are likely to account for the reported blood pressure-lowering action of rutaecarpine.

P18.

CALCIUM HANDLING IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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#contribute equally to this work

Although human induced pluripotent stem cells (hiPSCs) has brought optimism and excitement to the field of cardiac regenerative medicine due to its unquestioned potential to differentiate into cardiomyocytes, many questions remain to be answered before clinical application can be contemplated. The crucial first step is the characterization of the functional properties of hiPSC-derived cardiomyocytes. However, the calcium handing properties, the key process for excitation-contraction coupling, has not been studied. In fact, the functional immaturity in calcium homeostasis may at best result in poor graft-host integration, and at worst lead to potential lethal arrhythmias, thus raising important safety issues for regenerative purposes. This Here, we studied the calcium homeostasis of hiPSC-derived cardiomyocytes in comparison with H7 hESC-derived cardiomyocytes with fluorescence confocal microscopy. Cardiac differentiation of hiPSCs and hESCs were induced with standard mesoderm induction protocol. Beating outgrowth suggestive cardiomyocytes were first appeared on day 15. Compared with differentiated hESCs, differentiated hiPSCs appeared to have a lower expression level of a panel of cardiac specific genes including Nkx2.5, α-MHC and β-MHC. In addition, hiPSC-derived cardiomyocyes exhibited an more immature calcium handling property in comparison with hESC-derived cardiomyocytes with a significantly smaller amplitude and slower maximal upstoke velocity (Vmax upstroke) in the spontaneous calcium transients as well as caffeine induced calcium transient suggestive poorly developed sarcoplasmic reticulum function. Consistently, the expression level of the a few key calcium-handling proteins including sodium-calcium exchanger (NCX-1) and sacroplasmic recticulum Ca^{2+}-ATPase (SERCA) as well as the sarcoplasmic recticulum (SR) junctional candidate, triadin (Trd), were significantly lower in hiPSC in comparison with hESCs.
Conclusions: Taken collectively, hiPSCs exhibited immature calcium handling properties, which may hamper its potential for future clinical application.

ABSTRACTS

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P19.

HEMIN RESTORES THE IMPAIRED ENDOTHELIUM-DEPENDENT VASODILATATION IN DIABETIC *db/db* MICE THROUGH PI3K/Akt PATHWAY

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Heme oxygenase (HO) catalyzes heme into carbon monoxide, free iron and biliverdin. A large amount of data demonstrates that HO-1, an inducible isoform of HO, can exert vaso-protective effects. However, such vascular benefit in diabetic vasculopathy remains largely unknown. The present study investigates the impact of HO-1 induction on endothelial dysfunction in type 2 diabetic *db/db* mice. Diabetic *db/db* and non-diabetic *db/m+* mice were treated with hemin (a potent inducer of HO-1) for two weeks or vehicle. The aortae were isolated and suspended in myograph for force measurement and levels of marker proteins were determined by the Western blotting method. Two-week hemin administration restored the impaired endothelium-dependent relaxation to acetylcholine in *db/db* mice, which could be reversed by co-treatment with HO-1 inhibitor SnMP. The effect of hemin could also be blocked by 24 h-culture with PI3K inhibitor Wortmannin. In addition, 24 h-culture with bilirubin improved the impaired endothelium-dependent relaxation to acetylcholine in *db/db* mice. Finally, hemin treatment enhanced the eNOS and Akt phosphorylation level in the vascular wall. The present study provides clear evidence for the protective role of HO-1 induction against endothelial dysfunction in a mouse model of diabetes. (Supported by Hong Kong GRF and CUHK Focused Investment Scheme)

P20.

MELATONIN ATTENUATES INTERMITTENT HYPOXIA INDUCED OXIDATIVE STRESS AND TISSUE INFLAMMATION IN RAT ADRENAL MEDULLA

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Patients with sleep-disordered breathing suffer from intermittent hypoxia (IH). Previous studies have shown that chronic exposure to IH associated with recurrent apnoea induces oxidative stress and pathophysiological changes in the cardiovascular system. The adrenal medulla is responsive to acute hypoxia and plays an important role in the cardiovascular response to hypoxia. Yet, the chronic effect of IH on the adrenal medulla is currently undefined. We hypothesized that the free radical scavenger melatonin can attenuate the IH-induced oxidative stress and local injury and inflammation in the adrenal medulla. Adult Sprague-Dawley rats were exposed to air or IH mimicking a severe condition of sleep apnoea for 14 days. An intraperitoneal injection of melatonin (10 mg/kg) or vehicle was given daily prior to the IH treatment. The adrenal medulla was harvested for the measurement of markers for oxidative stress, malondialdhyde (MDA) and nitrotyrosine, and for the histological analysis of macrophages infiltration and TUNEL staining for apoptosis. Also, the protein expression of iNOS was examined by western blot. Our results showed that the MDA level was significantly increased in the IH group, when compared with the Nx control and melatonin-treated IH group. Image analysis also showed significantly more percentage area of the adrenal medulla with positive immunostaining of NTR than that of the Nx group and the melatonin-treated IH group. In addition, macrophage marker ED1-immunoreactivity was remarkable and the protein expression of iNOS was also significantly increased in the IH group, suggesting a local inflammation induced by IH in the adrenal medulla. Moreover, there was an increase in the number of apoptotic cells in the adrenal medulla of IH rats, and the increase was ameliorated in the IH group treated with melatonin. In conclusion, our results support the hypothesis that IH-induced oxidative stress is involved in the local inflammation and apoptosis in the adrenal medulla. The antioxidant melatonin can effectively attenuate the IH-induced tissue injury and inflammation in the rat adrenal medulla.

ABSTRACTS

Abstracts for Posters:

P21.

N-ACETYL-L-CYSTEINE REDUCES HIGH GLUCOSE-INDUCED MITOCHONDRIAL ROS GENERATION AND CORTICAL F-ACTIN CYTOSKELETON LEVELS IN PANCREATIC ISLETS β-CELLS OF OBESE/DIABETIC MICE

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Background: Failure of insulin secretion associated with β-cell dysfunction caused by glucotoxicity is a key factor in the development and progression of type 2 diabetes mellitus (T2DM). The beneficial effects of anti-oxidants consumption on treating T2DM remains to be determined. Therefore, in this study, we tested the hypothesis that L-NAC (an antioxidant) incubation, in vitro, restores the insulin secretory dysfunction via the modulation of mitochondrial ROS generation and cortical F-actin cytoskeleton levels of pancreatic islets b-cells of obsess/diabetic (*db+/db+*) mice (an animal model for human T2DM research).

Experimental approaches: The pancreatic islets of age-matched (female; ~6 month-old) lean/control (*db+/m+*) and obese/diabetic (*db+/db+*) of C57BL/KsJ mice were isolated using collagenase; single pancreatic β-cells of both strains of mice were further isolated by trypsin. Effects of L-NAC (20 mM; 24-hr incubation) on mitochondrial ROS generation, insulin release/content and cortical F-actin cytoskeleton levels of pancreatic islets/single β-cells (bathed in normal (5 mM) and high (15 mM) glucose culture medium) of both strains of mice were evaluated and compared.

Results: A higher level (the basal and high glucose-induced) of mitochondrial ROS was detected in single pancreatic β-cells of *db+/db+* mice, compared to *db+/m+* mice. L-NAC (20 mM, 24 hr incubation) significantly attenuated high glucose-induced mitochondrial ROS generation in single pancreatic β-cells of (*db+/db+*) mice. A consistently lower level of insulin release (in response to 15 mM glucose) and insulin content was measured in isolated pancreatic islets of *db+/db+* mice, and L-NAC incubation significantly enhanced glucose-induced insulin release and the insulin content of pancreatic islets of both species. A consistently higher level of cortical F-actin cytoskeleton was observed in single pancreatic β-cells of *db+/db+* mice (irrespective of glucose levels in the culture medium) which was markedly attenuated by L-NAC. No apparent change of cortical F-actin cytoskeleton level, in response to L-NAC, was observed in single pancreatic β-cells of *db+/m+* mice.

Conclusions: Our results demonstrate that L-NAC incubation is effective in suppressing high glucose-induced oxidative stress and attenuating cortical F-actin cytoskeleton levels which are probably responsible for the observed enhancement of insulin release/content of pancreatic β-cells of obese/diabetic (*db+/db+*) mice.

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P22.

ERGOTHIONEINE PROTECTS ENDOTHELIAL CELLS FROM OXIDATIVE STRESS

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Ergothioneine is a chemical abundant in mushroom. It possesses strong antioxidant properties by removing toxic radical species or chelating metal ions. However, these antioxidant properties are mainly studied in simple cell-free systems. In addition, unlike other water soluble antioxidants, ergothioneine is cell membrane impermeable and requires the specific carrier OCTN1 to be internalized. This OCTN1 is not ubiquitously expressed in all tissues. Therefore, their relevance to the actual function of ergothioneine in the body has been questioned.

In this study, we aimed to investigate whether or not ergothioneine can enter endothelial cells and protect endothelial cells against cellular damage induced by oxidative stress. Human brain microvascular endothelial cells (HBMECs) were used in this study. The results of RT-PCR demonstrate the expression of OCTN1 in HBMECs. Consistently, [^{3}H]ergothioneine could be taken up by HBMECs through a sodium-dependent and transporter-dependent system. MTT assay shows that the viability of HBMECs was decreased when the cells were incubated in medium containing 25 mM (which mimics hyperglycemic condition in diabetes) instead of 5 mM glucose. Interestingly, ergothioneine (in a concentration as low as 10 nM) was able to reduce the detrimental effect of 25 mM glucose. In addition, 1 mM ergothioneine could reduce the cytotoxic effect of 600 nM hydrogen peroxide on HBMECs.

In conclusion, our findings suggest that ergothioneine can be taken up by endothelial cells, probably through OCTN1. Besides, ergothioneine is a potential agent that can protect endothelial cells against oxidative stress.

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P23.

BLACK TEA POLYPHENOLS IMPROVE ENDOTHELIAL FUNCTION IMPAIRED BY HOMOCYSTEINE IN RAT AORTAS

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Objectives: Homocysteine is a well-known risk factor of cardiovascular disease. Hyperhomocysteinemia is found in patients with atherosclerosis and myocardial infarction. Homocysteine also impairs endothelium-dependent vasodilatation. Tea consumption is known to reverse endothelial dysfunction in patients with cardiovascular disease. This study aims at investigating whether homocysteine-induced endothelial dysfunction involves endoplasmic reticulum (ER) stress, and whether black tea polyphenols can improve endothelial function impaired by homocysteine.
Methods: Vasoreactivities of rat aortas were assessed in wire myograph. Protein expressions were examined by Western blotting.
Results: Homocysteine impairs endothelium-dependent relaxations (EDRs) of rat aortas in a concentration-dependent manner. ER stress alleviator sodium 4-phenylbutyrate (PBA) increases EDRs impaired by Homocysteine. A mixture of theaflavins (TE) and theaflavin-3,3'-digallate (TF3) also improves EDR impaired by Homocysteine. In addition, Western blotting showed homocysteine increased eIF2α phosphorylation in rat aortic endothelial cells, which was inhibited by PBA, and theaflavins.
Conclusions: The present study provides evidences for the possible involvement of ER stress in homocysteine-induced endothelial dysfunction, it also suggests that theaflavins improve endothelial function impaired by homocysteine.

P24.

ROLE OF JUMI EXTRACTION IN ACUTE RENAL FAILURE RATS

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Objective: The purpose of this study is to investigate the effect of jumi extraction on acute renal failure (ARF) rats: role of thyrosine hydroxylase (TH) in hypothalamus paraventricular (PVN) and kidney.
Methods: Male SD rats were randomly divided into four groups: control group, ARF group, Jumi extraction group and ARF+ Jumi extraction group. Glycerol-induced acute renal failure in rats was employed. Malondialdehyde (MDA) and reduced glutathione hormone (GSH) in renal cortex homogenate were measured by commercial kits. Electron microscope were used to examine the pathological changes. Meanwhile the expressions of TH in kidney were evaluated by immunohistochemistry.
Results: ARF rats administrated orally with 2 mL of normal saline for 48 h showed a significant increase in MDA (P<0.05), but GSH markedly decreased (P<0.05), when compared with that in control group. After treatment of ARF rats with jumi extraction for 48 h, MDA were significantly decreased (P<0.05), but GSH markedly increased (P<0.05), and the severity of tubular necrosis was alleviated when compared with that in ARF group. Immunohistochemistry showed obvious increase of thyrosine hydroxylase-immunoreactivity (TH-IR) in PVN and kidney in ARF group (P<0.05), but TH-IR was further enhanced in ARF+ Jumi extraction group (P<0.05).
Conclusion: The results indicated that jumi extraction could have a strong renal protective effect against ARF. TH in PVN and kidney may contribute to renal protective effect of jumi extraction against ARF.

P25.

RATE-DEPENDENT BLOCK OF HKV1.5 CHANNELS AND THE MOLECULAR DETERMINANT BY THE NATURAL FLAVONE ACACETIN

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We have recently demonstrated that the natural flavone acacetin is an atrial-selective compound that inhibits ultra-rapid delayed rectifier potassium current (I_{Kur}) and transient outward potassium current (I_{to}) in human atrial myocytes, and also acetylcholine-activated potassium current ($I_{K.ACh}$). It increased atrial effective refractory period and effectively prevented atrial fibrillation (AF) in anesthetized dogs without prolonging QT interval of ECG. The present study was designed to determine whether the I_{Kur} block of acacetin is rate- and/or use-dependent, and the molecular determinant of the channel block in HEK 293 cells expressing hKv1.5 channels (coding I_{Kur} in human atrial myocytes). It was found acacetin exhibited open channel block of hKv1.5 channels at 3 μM, and both closed and open channel block at 10 μM. Block of hKv1.5 channels by acacetin was use- and frequency-dependent, and the IC_{50} of acacetin for inhibiting hKv1.5 was reduced by increasing the depolarization rate from 3.5 μM at 0.2 Hz to 3.1, 2.9, 2.1, and 1.7 μM respectively at 0.5, 1, 3, and 4 Hz. The mutagenesis study showed that the hKv1.5 mutant V505A and I508A in the S6-segment remarkably reduced the channel block by acacetin (IC_{50}, 28.7 μM for V505A and 19.4 μM for I508A). These results demonstrate the novel information that acacetin blocks both closed and open channels of hKv1.5 by binding to the S6 domain of hKv1.5 channels. The use- and rate-dependent blocking property of hKv1.5 by acacetin indicates that this natural compound could exert a strong suppressive effect on atrial fibrillation in man.

ABSTRACTS

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P26.

CONTRAST OF MEAN ARTERIAL BLOOD PRESSURE, HEART RATE AND AORTIC TENSION INDUCED BY CLP & LPS SEPTIC SHOCK IN RATS

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Objective: To observe the differences of mean arterial blood pressure (MABP), heart rate (HR) and thoracic aorta tension induced by two septic shock models in rats and explore the possible mechanism.
Methods: we used cecal ligation and puncture (CLP) 20 hours and lipopolysaccharide (LPS) 6 hours to establish septic shock in rats. The carotid artery was cannulated and connected to a pressure transducer to determine mean arterial blood pressure (MABP). Ventricular dynamic parameters were determined following intraventricular cannulation via the carotid artery, including heart rate (HR), left ventricular developed pressure (LVDP), maximal rise/fall velocity of ventricular pressure (±dP/dtmax). Isolated thoracic rings were mounted on an organ bath and the tension of the vessel was recorded.
Results: (1) The mortality was 65.2% (30/46) in CLP shock rats, but no rats dead in LPS shock rats (0/24); (2) The two models showed significant decrease in MABP and HR, and CLP model's decrease more(P<0.01) (CLP decrease 55.7% and 71.5%, LPS decrease 41.5% and 58.3%); (3) Constriction by high K^+ (60 mmol/L) or 10^{-6} mol/L phenylephrine (PE) in endothelium-intact aortic rings were all decrease, and LPS model's decrease more (P<0.01) (CLP decrease 22.8% and 26.4%, LPS decrease 70.1% and 72.9%). And constriction by high K^+ or PE in endothelium-denuded aortic rings had the similar decrease.
Conclusion: The ventricular-dynamic parameters and vasoconstriction responsiveness of aorta were all decrease in two septic shock models in rats. CLP model's decrease more in the ventricular-dynamic parameters, though LPS model's decrease more in vasoconstriction responsiveness of aorta.

P27.

TELMISARTAN PROMOTES RELAXATIONS BY INCREASING PPARγ-DEPENDENT NITRIC OXIDE PRODUCTION IN MOUSE MESENTERIC ARTERIES

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Telmisartan robustly activates peroxisome proliferator-activated receptor-γ (PPARγ) besides serving as an angiotensin II type 1 receptor (AT_1R) blocker. It has been suggested that the activation of PPARγ exerts beneficial effects on the vascular function. However, the PPARγ agonistic effect of telmisartan on resistance arteries is unclear. The present study aimed at investigating whether telmisartan promotes relaxations in mouse mesenteric resistance arteries by stimulating the expression and activity of endothelial nitric oxide synthase (eNOS), which in turn augments nitric oxide (NO) production via the PPARγ-dependent mechanism. Second-order mesenteric arteries from male C57BL/6J were isolated and cultured overnight with telmisartan, afterwhich changes of their isometric tension were measured in myographs. Expression and activation of relevant proteins were analyzed by Western blotting. Real time changes in intracellular NO level in human endothelial cells was monitored by confocal microscopy. Telmisartan (10 μM) enhanced acetylcholine (ACh)-induced endothelium-dependent relaxations, which were inhibited by a PPARγ antagonist, GW9662 (300 nM). Telmisartan up-regulated GW9662-sensitive eNOS expression and phosphorylation, which were absent in PPARγ knockout (KO) mice. Additionally, telmisartan increased the basal and ACh-stimulated NO production, and both were prevented by GW9662. Taken together, the present results indicate that telmisartan enhances endothelium-dependent relaxations in resistance arteries, which is mediated through PPARγ-dependent elevation of NO production as a result of the increased eNOS expression and activity, possibly independent of AT_1R blockade. PPARγ agonistic activity of telmisartan may offer additional vascular benefits in addition to the known AT_1R antagonistic effect.

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P28.

ROLE OF AMPK AND HMG COA REDUCTASE IN SIMVASTATIN-MEDIATED INSULIN RELEASE FROM PORCINE ISOLATED PANCREATIC ISLETS OF LANGERHANS

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Background: Diabetes mellitus (DM) is a medical disorder characterized by a persistent hyperglycemia especially after meals. In DM patients, there is a deficiency/absence of insulin release. The ability of HMG CoA reductase inhibitors (statins) to decrease the incidence of diabetes in patients with dyslipidemia has also been documented. However, the underlying mechanisms involved for the beneficial effects of the uses of statins in treating DM have not been elucidated in details.

Methods: Fresh porcine pancreatic islets of Langerhans were isolated from porcine pancreases. Then, isolated islets were incubated with or without simvastatin (a commonly used HMG CoA reductase inhibitor) in medium with different glucose levels (5 and 25 mM). The protein expression of HMG CoA reductase, p-HMG CoA reductase-Ser^{871}, AMPK and p-AMPK-Thr^{172} was determined. Insulin release was measured using ELISA kits.

Results: The biochemical existence of HMG CoA reductase was demonstrated in pancreatic islets of Langerhans, and the expression of HMG CoA reductase was glucose (5 mM and 25 mM)-dependent (~ 40% increase). Simvastatin (10 μM, 24 hr incubation) caused a decrease in HMG CoA reductase expression irrespective of glucose levels. There was no apparent change of p-HMG CoA reductase expression caused by both glucose levels except with simvastatin (10 μM, with 25 mM glucose). An increase of p-AMPK-Thr^{172}, but not AMPK, expression was detected under high glucose conditions which was reduced by simvastatin. Simvastatin elicited a concentration (0.01, 0.1 and 10 μM)-dependent insulin release (3.6-fold and 8.5-fold increase with 0.1 and 10 mM simvastatin, respectively) from isolated islets bathed in 5 mM glucose medium. No apparent change caused by simvastatin was observed under high glucose (25 mM) conditions.

Conclusions: Our results demonstrate the biochemical existence of HMG CoA reductase in porcine's pancreatic islets. Simvastatin elicited a concentration-dependent insulin release under normal glucose conditions which is related to an inhibition of p-AMPK expression.

Acknowledgements: Provision of fund to Ms. Lori FY Kwok (summer of 2010) by the School of Biomedical Sciences (Faculty of Medicine, CUHK) is acknowledged. This project was financially supported by GRF Grant (to YWK) (Reference number: 2140565).

P29.

MONOAMINE OXIDASE-A-MEDIATED GENERATION OF REACTIVE OXYGEN SPECIE BY 5-HYDROXYTRYPTAMINE IN HUVECS

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Background: 5-Hydroxytryptamine (5-HT), a potent vasoactive neurotransmitter, after released is terminated at the nerve terminals mainly via enzymatic metabolism such as monoamine oxidases (MAOs), resulted in the generation of different metabolites (e.g. 5-HIAA, 5-HTOL and H_2O_2). In HUVECs, generation of H_2O_2 and the role(s) of MAOs in response to 5-HT challenge is unknown.

Methodology: Cultured human umbilical vein endothelial cells (HUVECs) (passages: 4-6) were used. ROS generation elicited by 5-HT in the absence or presence of L-NAME (an eNOS inhibitor) was evaluated using H_2DCF fluorescence dye by flow cytometry. Mitochondrial H_2O_2 levels were estimated by MitoTracker Red (reduced form) (a selective fluorescence probe for mitochondrial H_2O_2 measurement) using confocal laser scanning microscope. The participation of a particular isoform of MAOs (MAO-A and MAO-B) was evaluated using selective MAO inhibitors (MAO-A: clorgyline; MAO-B, selegiline).

Results: In the presence of L-NAME (100 μM), 5-HT consistently elicited a concentration (0.3, 0.6, 1, 3 and 10 μM)- and time (10-30 min)-dependent increase in ROS generation. Antimycin A (10 μM, a positive control) caused ROS generation in HUVECS as by 5-HT. 5-HT-induced ROS generation was sensitive to clorgyline (10 μM) and citalopram (10 μM, a selective 5-HT transporter inhibitor) but not to selegiline (10 μM) or GR127935 (10 μM, a 5-HT$_{1B/1D}$ receptor antagonist). 5-CT (a 5-HT analogue), 5-HIAA (10 μM), 5-HTOL (10 μM) and acetylcholine (10 μM) failed to cause ROS generation.

Conclusions: Our result clearly demonstrates that 5-HT elicits mitochondrial ROS production (a 5-HT$_{1B/1D}$ receptor-independent manner) probably via MAO-A-mediated 5-HT metabolism in HUVECs.

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ABSTRACTS

Abstracts for Posters:

P30.

GENDER-SPECIFIC RELAXATION OF FLAVONOIDS IN RAT MESENTERIC ARTERIES

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Flavonoids are considered as plant-derived estrogens. Estrogen shows different vascular effects in male and female. This study examined whether or not there is a gender difference in the vascular effects of flavonoids. The effects of genistein, daidzein, quercetin and luteolin on nitric oxide (NO)- and endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxations to acetylcholine were studied in mesenteric arteries of 8- and 28-week-old male and female Sprague-Dawley rats. EDHF-mediated relaxation was greater in 8-week-old female than in age-matched male rat mesenteric arteries. This gender difference was not observed in the presence of genistein, quercetin and luteolin. NO-mediated relaxations were not different between 8-week-old male and female rat mesenteric arteries. Luteolin caused a greater enhancement in NO-mediated relaxations in male than in female. In 28-week-old male and female rat mesenteric arteries, daidzein, quercetin and luteolin impaired EDHF-mediated relaxation in male mesenteric arteries. Gender difference was not observed in NO-mediated relaxation in 28-week-old rat mesenteric arteries, with or without flavonoids. The data suggests that genistein, quercetin and luteolin produce beneficial vascular effects in young male rats. However, EDHF responses were impaired by daidzein, quercetin and luteolin in older male rats. Therefore, the vascular effects of flavonoids are affected by both gender and age.

P31.

GLP-1(9-36)AMIDE AND EXENDIN-4 PROTECT ISOLATED CARDIOMYOCYTES FROM ADULT RATS AGAINST ISCHEMIA-REPERFUSION INJURY THROUGH DIFFERENT PATHWAYS

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Background: Glucagon-like peptide-1 (GLP-1), usually recognized as a gut hormone with potent plasma glucose-lowering action, is rapidly cleaved by the widely expressed dipeptidyl peptidase-4 enzyme at the N terminus to generate GLP-1 (9-36) amide. More recently, both GLP-1, its analogues exendin-4 (Exe-4) and GLP-1(9-36) amide have been showed cardioprotective actions in a number of experimental models. However, the mechanisms underlying the actions of GLP-1 and GLP-1 (9-36) amide remain poorly understood.
Objective: To investigate the signaling pathways of GLP-1 (9-36) amide and Exe-4 (both in the concentration of 1 nM) protecting the isolated cardiomyocyte from adult rats against ischemia-reperfusion (IR) injury.
Methods: When administered, the agents were presented for 10 min before 25 min simulated ischemia (glucose oxidase-catalase oxygen-scavenging system) and the whole reperfusion period.
Results: After 5 minutes reperfusion, both GLP-1 (9-36) amide and Exe-4 strongly reduced trypan blue-sensitive cells (from 53.57±7.68% in IR group to 39.29±3.02% and 41.53±6.73%, $P<0.01$). This cell death-limiting effect of GLP-1(9-36) amide was abolished by endothelial nitric oxide synthase (eNOS) inhibitor L-NAME, MEK1/2 inhibitor U0126 or protein kinase A (PKA) inhibitor H89, but not by GLP-1 receptor antagonist exendin (9-39), phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 or wortmannin. In contrast, such effect of Exe-4 was abolished by exendin (9-39), L-NAME, or LY294002 and wortmannin. All of the inhibitors alone had no effect on IR cardiomyocytes.
Conclusion: The results indicate that separate actions for GLP-1 (9-36) amide *vs.* GLP-1 analogues Exe-4 against ischemia-reperfusion death in cardiomyocytes isolated from adult rats, and reveal that the cardioprotective effect of GLP-1 (9-36) amide is independent on GLP-1 receptor, but through MEK, eNOS and PKA signaling pathways, while Exe-4 exerts cardioprotective effect through GLP-1 receptor, PI3K and eNOS signaling pathways.

ABSTRACTS

Abstracts for Posters:

P32.

ADVANCED GLYCATION END PRODUCT INDUCES ENDOTHELIAL DYSFUNCTION IN MOUSE AORTAS THROUGH MITOCHONDRIAL REACTIVE OXYGEN SPECIES

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Objectives: Increased production of advanced glycation end-products (AGEs) contributes to endothelial dysfunction in diabetes. The present study examines whether AGE could exert a direct vascular action and the possible mechanisms involved.
Methods: Aortas from *db/m*$^+$ mouse were treated with AGE-BSA at 10 μg/ml for 24 hours in the absence or presence of pharmacological inhibitors. Wire myograph was used to assess changes in the vascular reactivity of arteries. Production of mitochondria-derived reactive oxygen species (ROS) was determined using MitoSOX fluorescence under confocal microscope. Western blot was used to assay the p47 level in *db/m*$^+$ mouse aortas after AGE treatment with or without inhibitors.
Results: The present study shows that AGEs reduced acetylcholine-induced endothelium-dependent relaxations (EDRs) in aortas from *db/m*$^+$ mice and AGE-induced impairment of EDRs was reversed by co-incubation with AGE inhibitor aminoguanidine, NADPH oxidase inhibitor DPI and apocynin, mitochondrial ROS scavenger mitoQ, and protein kinase C inhibitors GF 109203X and chelerythrine. AGE treatment increased the mitochondrial ROS production in native endothelium of intact mouse aortas and in primary mouse aortic endothelial cells, which were inhibited by DPI, apocynin, mitoQ, and ROS scavenger Tiron+DETCA. Western blot data show that a p47 up-regulation by AGE was reversed by aminoguanidine, DPI, apocynin, mitoQ, and also PKC inhibitors.
Conclusions: The present study suggests that AGEs impair endothelial function through NADPH oxidase- and PKC-dependent increase of mitochondrial ROS, which may contributes to diabetes-related vascular dysfunction. (supported by Hong Kong GRF)

P33.

IN VITRO SYNERGISTIC BONE ANABOLIC EFFECTS OF 1,25α DIHYDROXYVITAMIN D_3 AND CU409B1 IN RAT OSTEOBLASTS

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Background: Osteoporosis is a disease of bones with increased risk of bone fracture because of reduced bone mineral and disrupted bone micro-architecture. The disease is mainly due to increased osteoclasts activity and/or reduced osteoblasts activity. Vitamin D_3 (Vit. D_3) is one of the medications for osteoporosis as it enhances Ca^{2+} absorption from diets. Previous findings in our laboratory demonstrated that CU409B1 (patent pending on chemical structure) improved rats osteoblasts differentiation. In this study, we tested the hypothesis that a combination of CU409B1 and Vit. D_3 (at the lowest plasma concentration, 10 nM) provided synergistic bone anabolic effects.
Methods: Primary rat osteoblasts were isolated from Sprague Dawley (SD) rats (female, 6 weeks old) (sacrificed using an over-dose of pentobarbital). Iliac crests collected were immediately immersed in PBS (for 10 min) supplemented with 10X antibiotics, and bone marrow was removed and the crests were washed thoroughly twice with plain low glucose-DMEM (LG-DMEM). Trabecular bones were harvested, cut into small pieces, and immersed in LG-DMEM (10% fetal bovine serum with 1X antibiotics) for cultured in a humidified incubator (37°C and 5% CO_2). Cells reached over 90% confluence were treated with either 1,25α dihydroxyvitamin D_3 (10 nM, the active form of Vit. D_3), CU409B1 (10 nM) or a combination of Vit. D_3 (10 nM) plus CU409B1 (10 nM) for 7 days before subjecting to analysis of various biomarkers. mRNA was isolated and subjected to quantitative real-time reverse transcription polymerase reaction (qRT-PCR) to determine the levels of osteogenesis-related mRNAs, including alkaline phosphatase (ALP), bone morphogenetic protein 2 (BMP2), osteopontin (OPN), osteocalcin (OCN), pro-collagen type I, and Runt-related transcription factor 2 (RunX2). The extent of osteogenesis was determined by measuring the activity of extracellular ALP and Alizarin Red staining of Ca^{2+} deposits.
Results: At day 7, a combination of CU409B1 (10 nM) and 1,25α dihydroxyvitamin D_3 (10 nM) significantly enhanced the mRNA levels of ALP, OCN, BMP2, and pro-collagen type I (P<0.05) whereas no apparent change was observed in osteoblasts treated with either CU409B1 (10 nM) or 1,25α dihydroxyvitamin D_3 (10 nM) alone. However, no significant change of ALP activity and calcium deposition by all drug treatments was observed.
Conclusions: Our results demonstrate that a combination of CU409B1 and 1,25α dihydroxyvitamin D_3 treatment (7 days) have osteogenesis effects. Current study is underway to elucidate the cellular signaling pathways involved.
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