ABSTRACTS

Abstracts for Posters:

CP1.

AVE3085 RESTORES ENDOTHELIAL FUNCTION IN TYPE 2 DIABETIC *DB/DB* MICE THROUGH ENHANCEMENT OF ENOS EXPRESSION

WS Cheang,[1] WT Wong,[1] XY Tian,[1] Q Yang,[2] CW Lau,[1] GW He,[2] Y Huang[1]
[1]Li Ka Shing Institute of Health Sciences and Institute of Vascular Medicine, School of Biomedical Sciences, [2]Department of Surgery, The Chinese University of Hong Kong, Hong Kong

Objectives: Reduced nitric oxide (NO) bioavailability is associated with diabetes-related vasculopathy. The present study investigated whether AVE3085 can restore the impaired endothelial function in type 2 diabetic db/db mice through increasing endothelial NO synthase (eNOS) expression.
Methods: Aortae from C57BL/6J, eNOS$^{-/-}$ and *db/db* mice were cultured with AVE3085 (10 μM) overnight. Twelve-week-old *db/db* mice were orally administrated with AVE3085 (10 mg/kg/day) for 7 days. Vascular reactivity was measured in isomeric and isobaric myograph. Reactive oxygen species (ROS) levels in aortae were determined using a DHE fluorescence dye. NO productions in cultured aortic endothelial cells upon stimulation of A23187 were detected with a DAF fluorescence dye. Protein expression was detected by Western blotting.
Results: Culturing diabetic mouse aortae with AVE3085 overnight improved the acetylcholine-induced endothelium-dependent relaxations. Furthermore, AVE3085 prevented the high glucose (30 mM)-induced endothelial dysfunction and ROS over-production in C57BL/6J mice but not in eNOS$^{-/-}$ mice. NOS inhibitor L-NAME and transcription inhibitor actinomycin D abolished the effects of AVE3085. AVE3085 increased the eNOS expression but not the phosphorylation of eNOS at ser1177 in mouse aortae. AVE3085 also reversed the NO production in isolated endothelial cells. Chronic treatment with AVE3085 to db/db mice improved acetylcholine-induced relaxations in aortae and flow-mediated dilatations in mesenteric arteries, together with reduced oxidative stress.
Conclusion: The present study shows that AVE3085 improves endothelial function in type 2 diabetic mice through increasing NO bioavailability accompanied with reducing oxidative stress. Targeting eNOS and NO production may be useful to combat against endothelial dysfunction in diabetes.

CP2.

HEME OXYGENASE-1 INDUCTION BY HEMIN ENHANCES EDHF-MEDIATED RELAXATIONS IN THE MESENTERIC ARTERY OF THE SHR

Z Li, Y Wang, PM Vanhoutte
Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong

Objective: Heme oxygenase-1 (HO-1) is a crucial enzyme in regulation of reactive oxygen species (ROS) and heme degradation. Up-regulation of HO-1 lowers blood pressure and improves endothelial function in spontaneous hypertensive rats (SHR). However, it is unknown whether or not up-regulation of HO-1 by the pharmacological inducer hemin enhances endothelium-dependent hyperpolarizations (EDHF)-mediated relaxation. The present study was designed to investigate the effect of hemin on EDHF-mediated relaxations.
Methods: 36 weeks old SHRs were divided into a hemin treatment (50 mg/kg, intraperitoneal injection, 24 hours before sacrifice) and a control group. Mesenteric arteries were isolated for the measurement of isometric tension and protein expressions.
Results: Hemin treatment potentiated acetylcholine-evoked endothelium-dependent hyperpolarizations in the presence of L-NAME and indomethacin. The IK_{Ca} channel blocker TRAM-34 and the Na^+-K^+-ATPase channel blocker ouabain significantly impaired the hemin potentiated-EDHF relaxations, indicating the involvement of the IK_{Ca}–Na^+-K^+-ATPase pathway. Relaxations induced by the IK_{Ca} and SK_{Ca} channel activator NS309 were comparable in preparations from the hemin treatment and control groups. In preparations without endothelium, K^+ mediated-relaxations were significantly increased in arteries from hemin-treated rats. In addition, the protein expression of α2-Na^+-K^+-ATPase, but not IK_{Ca}, SK_{Ca} or Kir, was augmented by hemin treatment. These observations suggest that hemin treatment enhances the expression and activity of Na^+-K^+-ATPase. They exclude an involvement of SK_{Ca}–Kir pathway, gap junctions, BK_{Ca} channel, K_{ATP} channel, hydrogen peroxide or EETs in hemin-potentiated EDHF-mediated responses.
Conclusions: Up-regulation of HO-1 by hemin treatment enhances EDHF-mediated relaxations mainly because of an increased of expression and activity of Na^+-K^+-ATPase. The present findings suggest that HO-1 may be a potential therapeutic strategy for treating endothelial dysfunction in hypertension.

ABSTRACTS

Abstracts for Posters:

CP3.

INHIBITION OF NUCLEOSIDE TRANSPORTERS IN ENDOTHELIAL CELLS BY EMODIN

SYT Lin,[1] RWS Li,[1] ASM Sit,[1] YW Kwan,[2] GPH Leung[1]
[1]Department of Pharmacology and Pharmacy, The University of Hong Kong, [2]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

Nucleoside transporters play critical roles in endothelial cell functions. Nucleosides are precursor molecules for ATP, nucleic acids, coenzymes and signaling molecules. However, it is known that epithelial cells have a low capacity for de novo synthesis of nucleosides, and they therefore also depend on nucleoside absorption from the extracellular fluid (i.e. salvage pathway). In addition, nucleoside transporters are important in fine tuning the extracellular concentration of adenosine (a vasodilator and anti-inflammatory agent which is generated in ischemia and inflammation) in the vicinity of adenosine receptors. In endothelial cells, the major nucleoside transporters are equilibrative nucleoside transporter (ENT) 1, ENT2 and nucleoside/nucleobase transporter.

Emodin is a natural anthraquinone compound present in Chinese herbs and vegetables. Emodin possesses anti-cancer, antiviral, antimicrobial, hepatoprotective, anti-inflammatory and anti-angiogenic properties. The relaxing and anti-proliferative effect of emodin on vascular smooth muscle cells has also been recently reported. The objective of this study was to investigate the effects of emodin on nucleoside transporters.

The [^{3}H]adenosine uptake was measured in human umbilical vein endothelial cells (HUVECs). The results showed that emodin inhibited [^{3}H]adenosine uptake with and IC_{50} of 17.1±2.3 μM. Since adenosine is a substrate of ENT1, ENT2 and nucleobase/nucleoside transporters, further experiments were performed to differentiate the effects of emodin on these three transporters. [^{3}H]uridine uptake was measured instead because uridine is a substrate for ENT1, ENT2 but not nucleobase/nucleoside transporter. The data showed that emodin inhibited [^{3}H]uridine uptake in HUVECs with an IC50 of 51.4±5.6 μM. Besides, [^{3}H]uridine uptake was measured in the nucleoside-transporter-deficient PK15NTD cells that stably express cloned human ENT1 and ENT2. Emodin could inhibit ENT1 and ENT2 with IC50 of 33.8±1.7 μM and 18.5±3.0 μM, respectively. The inhibitory effects of emodin on nucleoside transporters can be washed out. Taken together, our data indicates that emodin is a general inhibitor which can block ENT1, ENT2 and nucleobase/nucleoside transporter reversibly. The inhibition of nucleoside transporters may account for the vasodilatory and antiangiogenic effects of emodin. Kinetic studies will be carried out to study whether emodin is a competitive or non-competitive inhibitor.

CP4.

INVOLVEMENT OF NADPH OXIDASE AND RENIN-ANGIOTENSIN SYSTEM IN TISSUE INFLAMMATION OF THE RAT ADRENAL MEDULLA DURING INTERMITTENT HYPOXIA

Y Liu, M L Fung
Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong

We have shown that intermittent hypoxia (IH) associated with recurrent apnea induces oxidative stress and inflammation in the adrenal medulla. However the pathogenic mechanism is not clear at present. We hypothesized that the expression of NADPH oxidase induced by renin-angiotensin system (RAS) plays a role in the tissue inflammation of the rat adrenal medulla in chronic IH. Adult Sprague-Dawley rats were exposed to air (normoxic (Nx) control) or IH treatment (8 hrs/day) which mimicked a severe recurrent sleep apneic condition for 14 days. Injections of apocynin, an inhibitor of NADPH oxidase, (25 mg/kg i.p.) or vehicle were performed daily before the IH treatment. The mRNA levels of NADPH oxidase subunits $p22^{-phox}$, NOX-2 and NOX-4 were examined by RT-PCR, the protein expressions of IL-6, TNF-α and COX-2 were examined by the ELISA kit and the protein expressions of NOX-4 and RAS components (AGT, AT1 and AT2) were examined by Western blot. Our results showed that the protein expression of IL-6, TNF-α and COX-2 were significantly higher in the IH group than that of the Nx and apocynin-treated hypoxic (AIH) group, suggesting that inhibition of NADPH oxidase attenuates IH-induced local inflammation in the rat adrenal medulla. The mRNA levels of $p22^{-phox}$, NOX-2 and NOX-4 were also increased significantly in the IH group, when compared with that of the Nx control and AIH group. In addition, the protein expression of NOX-4 was significantly more in the IH group than that of the AIH group. Furthermore, the protein expressions of AGT, AT2 and AT2 were increased in the IH group, indicating that the upregulation of NADPH oxidase may be induced by the increased RAS expression. In conclusion, we have shown that NADPH oxidase plays a pathogenic role in the IH-induced local inflammation in the rat adrenal medulla.

ABSTRACTS

Abstracts for Posters:

CP5.

STUDIES ON THE SIGNAL TRANSDUCTION MECHANISM FOR ACIDOSIS-INDUCED ATP RELEASE FROM L6 SKELETAL MYOCYTES

L Lu, J Tu,* GR Li, HJ Ballard
Department of Physiology & Institute of Cardiovascular Science & Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, and *Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China

ATP is an extracellular signalling molecule involved in the regulation of skeletal muscle blood flow as well as vascular responses including haemostasis and inflammation. We have previously shown that depression of pH using lactic acid treatment stimulated the efflux of ATP from cultured skeletal myocytes. The present study was undertaken to further explore the mechanism and regulation of ATP release from muscle.

Expression of channels that may have the capability to permit ATP efflux was investigated in cultured skeletal myocytes: RT-PCR showed that mRNA for the chloride channels, CFTR, CLCN-2, CLCN-3, CLCN-7, CACC, and VDAC, and the stretch-sensitive channels, CX40, CX43 and PX3 was expressed by the cells; Western blot further confirmed protein expression of CFTR and CX43. Protein expression for CLC-7 was not found, while protein expression for the DIDS-sensitive chloride channels, CACC and VDAC, was not tested, since the lactic-acid-induced ATP release was not inhibited by DIDS.

Accumulation of ATP in the medium surrounding the myocytes was measured using a luminescence assay. Incubation of the myocytes in a low pH medium containing lactic acid (10 mM) for 3 hours increased the extracellular ATP from 0.67±0.08 to 1.15±0.11 nM (n=36; P<0.001). Patch clamp studies showed that the whole cell chloride current was increased at low pH, suggesting that a chloride channel may be involved in the ATP efflux. The specific inhibitor of CFTR, $CFTR_{inh}$-172, abolished the increase in chloride conductance at low pH, and inhibited the lactic-acid-induced accumulation of ATP in the medium surrounding the cultured myocytes. Lactic acid treatment increased the intracellular cAMP from 3.2±0.3 to 7.1±1.0 nM, and the lactic-acid-induced increase in extracellular ATP was inhibited by the Protein Kinase A inhibitor, KT5720, suggesting that the PKA/cAMP pathway was involved in CFTR activation. Amiloride, an inhibitor of the Na/H exchanger, prevented the lactic-acid-induced increase in intracellular cAMP, whilst inhibitors of the Na/Ca exchanger, SN-6 and KB-R7943, inhibited the lactic-acid-induced accumulation of ATP in the medium surrounding the cultured myocytes.

We therefore propose the following signal transduction mechanism for the acidosis-induced efflux of ATP from muscle: operation of the Na/H exchanger, during the extrusion of excess H+ from the intracellular space, increases the intracellular Na; this drives the Na/Ca exchanger to extrude Na, creating a localised increase in Ca, which then activates adenyl cyclase, elevating the intracellular cAMP; this, in turn, activates Protein Kinase A to phosphorylate CFTR, and CFTR finally regulates the opening of the ATP release channel.

CP6.

CELLTHERAPY BEHIND THE ESSAY TUBES AND THE LAB COAT

EV Tonleu, XH Liu
Xi'an Jiaotong University School of Medicine, Department of Neurovascular Physiology Research; Catholic University of Central Africa, Public Health Department

Stem cell therapy has from decades been announced by scientist like the solution in to solve almost all the complex equations of the different fatal diseases that ravage the world. Nevertheless it is one of the most controversial matters of last decades as all the ethical issues which surround it, clearly influence the re-orientation of many research works generally away from the hEsc despite their acknowledged wide regenerative abilities. In march 2009 US president overturned the previous cessation of hEsc, but what about the rest of the world.
Objectives: So we decided in our work to turn the research over the outside world a giving the speech to society specifically third world population in order to have an idea of the perception of the research and also its real impact for clinics away from the acclaimed discovery institutions in this research field. What is the real understanding and the knowledge of society about stem cell? How do they really evaluate the progress made by the therapy?
Methods: We used four tools:

- An electronic grill composed of 4 questions at which the candidates should replied by yes or no
- Cell and molecular biology manual by Gerald Karp at the beginning of the third question to explain the concept to all the individuals.
- A questionnaire of two clinical cases situation in which they will identify oneself as at first a doctor having a critical case patient; in the second, as the relative.
- Basic calculation software Microsoft Excel

Group I: 30 professional heath patricians in public heath university
Group II: 20 students of 1st year, and 80 students including 2nd year, 3rd year, 4th year and 5th year Medical students of different ethnicities and religious background.
Group III: 160 students and citizens all over world: divided in group A and B.
Group A: 80 individuals' engineers, economists, politics and law related worker
Group B: 80 individuals' social workers, commercials and others

Results:

Groups		QUESTION 1	QUESTION 2	QUESTION 3	QUESTION 4
I		63.33%	6.667%	100%	36.6%
I	A	1.25%	1.25%	100%	16.25%
	B	28.75%	23.75%	85%	83.75%
II	C	76.25%	63%	67%	11.25%
	D	21.25%	3.75%	100%	95%

Conclusions: It seems that all the fights revolving around the stem cell therapy is stronger in the group of "deciders" [group A] while in general population is dying and but still keep the hope that medicine can make things change, but most of people seem to ignore that they are the one putting more and more barriers to all the research efforts. However, the blame is as well on the health professionals among which the progress of the medical science is ignored, and shadowed (case of the health workers in third world countries very skeptical about hEsc use). So, cell therapy research should it not take a step further by not only be emphasized to "the lab coat" in close up rooms but rather, take its own defense in the large court that is the world; well, considering 'sensibilization and population education about research

ABSTRACTS

Abstracts for Posters:

CP7.

ACTIVATION OF α1-ADRENERGIC RECEPTORS CAUSES THROMBOXANE-PROSTANOID RECEPTOR DESENSITIZATION IN THE AORTA OF THE SPONTANEOUSLY HYPERTENSIVE RAT

YZ Zhao, SWS Leung, PM Vanhoutte
Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong

Objective: In the aorta of male spontaneously hypertensive (SHR), but not in that of normotensive Wistar-Kyoto (WKY) rats, previous exposure to phenylephrine inhibits subsequent contractions to prostaglandin E_2 or prostaglandin $F_{2\alpha}$. The present experiments were designed to examine the mechanism underlying this inhibition.
Methods: Aortic rings of adult male SHR and WKY were suspended in organ chambers for the measurement of isometric tension. The ability of prostaglandin E_2, prostaglandin $F_{2\alpha}$, phenylephrine or U46619 to evoke endothelium-independent contractions was tested in aortic rings previously exposed to phenylephrine, norepinephrine, or phorbol 12,13-dibutyrate (PDBu). S18886 was used to partially block thromboxane-prostanoid (TP) receptor.
Results: In the SHR aorta, previous exposure to phenylephrine inhibited subsequent contractions to prostaglandin E_2, prostaglandin $F_{2\alpha}$ but not those to phenylephrine or U46619. When the SHR aorta was incubated with S18886, at a concentration that only partially blocked the TP receptors, pre-exposure to phenyleprhine caused a significant depression of the subsequent contraction to U46619. In the WKY aorta, there were no significant differences in contraction to prostaglandin E_2, prostaglandin $F_{2\alpha}$, phenylephrine or U46619 after previous exposure to phenylephrine. Previous exposure to PDBu or norepinephrine also inhibited subsequent contractions to prostaglandin E2 in the aorta of SHR but not in that of the WKY.
Conclusion: In the aorta of SHR but not that of the WKY, activation of α_1-adrenergic receptor causes TP receptor desensitization through phosphorylation of TP receptors by protein kinase C.