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Carotid Intima-Media Thickness is Superior to other Non-Invasive Functional and Structural Vascular Assessments in Risk Stratification of Subjects with Established Cardiovascular Disease

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LAU ET AL.: ***Carotid Intima-Media Thickness is Superior to other Non-Invasive Functional and Structural Vascular Assessments in Risk Stratification of Subjects with Established Cardiovascular Disease.*** **Objective:** *In patients with known cardiovascular disease (CVD), risk scores based on traditional risk factors of atherosclerosis do not apply and alternative methods of risk stratification are needed. We aim to compare the prognostic values of a range of commonly used non-invasive vascular assessments in a group of patients with known CVD. Incremental values of surrogate markers used in combination would also be assessed.* **Methods:** *We determined the brachial endothelial function (flow-mediated dilatation (FMD), nitroglycerin-mediated dilatation (NMD)), carotid artery atheroma burden (carotid mean maximum intima-media thickness (mmIMT) and plaque), ankle-brachial index (ABI) and arterial stiffness (brachial-ankle pulse wave velocity (baPWV)) in 387 patients with known CVD or equivalent (139 patients with iscahemic stroke (ISS), 130 patient with coronary artery disease (CAD), 100 patients with diabetes without CAD or ISS and 19 patients with both CAD and ISS). Patients were followed-up at 25±6 months and presence of a major adverse cardiovascular event (MACE) was documented.* **Results:** *During the follow-up period, a total of 46 MACEs occurred. Carotid mmIMT was significantly greater in patients with MACEs (1.18±0.29 mm versus 1.07± 0.31 mm, P=0.027) but there were no significant differences in FMD, NMD, carotid plaque prevalence, ABI or baPWV in subjects with or without MACEs. Kaplan-Meier curve analysis revealed a significantly greater number of adverse events in patients with a mmIMT>1.2 mm (P<0.0001 by log rank test) or baPWV>1445 cm/s (P=0.005). Univariate analysis with Cox proportional hazards modeling identified mmIMT>1.2 mm, baPWV>1445 cm/s, ABI≤1.1, NMD≤13.5%, age and oral nitrate use as positive predictors of MACEs (all P<0.1). However, multivariate analysis revealed that out of all non-invasive vascular assessments, only mmIMT>1.2 mm (Hazards ratio 2.49, 95% confidence interval 1.15-5.39, P=0.020) was an independent predictor of MACEs. Furthermore, patients with a combined impairment of mmIMT and baPWV was associated with an increased risk of MACE compared with those with impairment of either marker alone (P=0.007 by log rank test) but did not provide significant incremental benefit for MACE prediction compared with mmIMT or baPWV alone (P=0.11 and P=0.053 by comparing 2 receiver operating characterisitic curves).* **Conclusions:** *Amongst a range of non-invasive vascular assessments of atherosclerosis, carotid IMT provides the best predictive value for MACEs in patients with established CVD or equivalent.*

Cardiovascular adverse event, carotid intima-media thickness, risk prediction

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摘要

目的：對心血管疾病患者，基於經典動脈粥樣硬化危險因素的危險評分並不適用，需要另類的危險程度分級方法。本文旨在比較心血管疾病患者中幾種常用非創傷性血管評估指標的診斷意義並評估替代標誌組合的增量價值。**方法：**測定387例確診心血管疾病或類似疾病患者（其中139例缺血性中風（ISS）、130例冠脈疾病（CAD）、100例無CAD或ISS的糖尿病、19例CAD合併ISS）的肱動脈內皮功能（流量介導的血管舒張功能FMD）、硝酸甘油介導的血管舒張（NMD）、頸動脈粥樣硬化負荷（頸動脈平均最高中層內膜厚度mmIMT和斑塊）、踝臂指數（ABI）及動脈硬度（肱動脈–踝動脈脈搏波傳導速度baPWV），並隨訪25±6月的主要不良心血管事件（MACE）。**結果：**隨訪期間共發生46次MACE。發生MACE組的頸動脈mmIMT明顯增加（1.18±0.29 mm versus 1.07±0.31 mm, P=0.027）。但有無MACE兩組間FMD、NMD、頸動脈斑塊發生率、ABD或baPWV皆無顯著差異。Kaplan-Meier 曲線分析顯示mmIMT>1.2 mm（時序檢驗P<0.0001）或 baPWV>1445 cm/s（P=0.005）患者發生不良事件的次數更多。Cox比率危險模型單因素分析定義mmIMT>1.2 mm、baPWV>1445 cm/s、 ABI≤1.1、NMD≤13.5%、年齡及口服硝酸鹽爲MACE 的正性預測因數（所有P < 0.1）。然而，多因素分析顯示所有非創傷性血管評價指標中，僅有mmIMT>1.2 mm 是MACE的獨立預測因數（危險比率 2.49, 95% 可信區間1.15-5.39, P=0.020）。此外，mmIMT 和baPWV同時減少患者較單一指標減少患者的MACE危險性增加（時序檢驗P=0.007），但是沒有證據表明兩者同時減少較單一指標減少對MACE預測具有顯著增值效益（兩組受者運行特徵曲線P=0.11、P=0.053）。**結論：**確診心血管疾病患者的非創傷性血管動脈粥樣硬化評價指標中，頸動脈IMT對MACE具有最佳預測意義。

關鍵詞：心血管不良事件　頸動脈中層內膜厚度　危險預測

Introduction

Various types of risk assessment scores, such as the Framingham Risk Score have been used for risk stratification of subjects without established cardiovascular diseases (CVD). However, these risk scores are not applicable for patients with established CVD. Recently, various types of surrogate markers of atherosclerosis, including vascular endothelial function, carotid intima-media thickness (IMT) and plaque burden, ankle-brachial index (ABI) and arterial stiffness have been increasingly used for the prediction of cardiovascular events. Several studies have shown that these markers correlate with the extent and degree of atherosclerosis,[1-10] and have prognostic values in the prediction of cardiovascular-related events.[11-18] Indeed, recent clinical guidelines have recommended the use of these markers for risk stratification of asymptomatic subjects.[19,20] However, the relative clinical values of these different surrogate markers in patients with established CVD or equivalent remain unclear.

The purpose of this study was to compare the prognostic implications of several surrogate markers of vascular assessment including brachial endothelial function, carotid atheroma burden, ABI and arterial stiffness in the prediction of cardiovascular events. Furthermore, the potential incremental prognostic values of these surrogate makers used in combination will be assessed.

Materials and Methods

Subjects

The study population comprised of 387 consecutive patients with a known history of coronary artery disease (CAD), ischaemic stroke (ISS) or diabetes mellitus (DM) (CAD equivalent).[21] The presence of CAD was defined by a documented history of an acute coronary event (myocardial infarction or unstable angina), previous percutaneous coronary intervention, coronary artery bypass grafting surgery or positive myocardial perfusion scan. ISS was defined as a neurological deficit of sudden onset that persisted for more than 24 hours, corresponded to a vascular territory in the absence of primary haemorrhage, was not explained by other causes (trauma, infection, vasculitis), and was confirmed by computerised axial tomography or magnetic resonance imaging of the brain. Patients with haemorrhagic stroke, suspected cardioembolic

stroke and stroke due to other causes were excluded. DM was defined as a serum fasting glucose of ≥7.1 mmol/L or on oral hypoglycaemic agents or insulin injection therapy. The study was approved by the institutional review board, and all subjects gave written informed consent.

Study Design

All patients were prospectively recruited between July 2005 and June 2006 from our medical outpatient clinics. Baseline demographic data, cardiovascular risk factors and cardiovascular medications at the time of recruitment were documented. Cardiovascular risk factors, including tobacco smoking, DM, hypercholesterolaemia, hypertension, body-mass index and family history of CVD in first-degree relatives younger than 55 years of age were assessed. Hypertension was defined as either resting systolic or diastolic blood pressure ≥140/90 mmHg at two different times or on anti-hypertensive medications. Hypercholesterolaemia was defined as a fasting total serum cholesterol level of ≥4.9 mmol/L or on lipid-lowering medications. Body-mass index was calculated as weight in kilograms divided by the square of height in metres. Smoking status was recorded as either smoker (past and current) or non-smoker.

Vascular Assessments

Vascular ultrasound examinations for brachial endothelial function, carotid IMT and presence of carotid plaque were evaluated through a standard B-mode ultrasound examination with the use of a 7.5 MHz linear array transducer and a high resolution ultrasound system (Agilent Sonos 5500, Philips, Andover, Massachusetts, USA) as described previously.[22,23] Measurements of ABI and arterial stiffness were performed using a commercially available device based on oscillometric method (VP-2000, Colin Corporation, Komaki, Japan).

A single experienced operator, who was blinded to the identity of the study subjects performed all the vascular ultrasound examinations. Another experienced operator, also blinded to the identity of the study subjects, operated on the VP-2000 and obtained the ABI and arterial stiffness parameters.

Brachial Endothelial Function

Patients were studied in the fasting state and vasoactive medications were withheld for 12 hours before the scans. Longitudinal brachial artery diameter was obtained at rest, and then during flow-mediated dilation (FMD), induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 50 mmHg above systolic blood pressure for 5 minutes. The cuff was then released and serial imaging of the brachial artery was recorded for 5 minutes. After another 5 minutes of rest, 400 μg of sublingual nitroglycerin via a spray was administered. The brachial artery diameter was measured again, 4 minutes after administration of nitroglycerin. FMD was defined as the percentage change in brachial artery diameter between 1 minute after cuff deflation and that on the baseline scan. Nitroglycerin-mediated dilatation (NMD) was defined as the percentage change in brachial artery diameter 4 minutes after administration of nitroglycerin and that on the baseline scan. All digital images were stored on optical diskettes for subsequent off-line analysis using a computer workstation (EchoPAC, GE Medical, Wisconsin, USA). The brachial artery diameter was measured by a single operator and an average value from three consecutive measurements was calculated. The intra-observer correlation coefficient for FMD was 0.90 and the intra-observer correlation coefficient for NMD was 0.86 (2 repeated measurements in 20 randomly chosen subjects).

Carotid Intima-Media Thickness and Plaque

Carotid IMT was determined by measuring manually the distance between the lumen-intima and media-adventia border of the vascular wall using electronic calipers. Each ultrasonic scan was performed in the anterior, lateral and posterior projections of the right and left carotid arteries. Three IMT measurements were made on the near and far wall of the common carotid arteries, carotid bifurcation and internal carotid arteries. The mean maximum IMT (mmIMT) was used for analysis and was calculated by averaging the values of maximum IMT measured from 12 pre-selected segments of the carotid arteries. Presence of carotid plaque was defined as an endoluminal protrusion of the arterial lumen of at least 0.5 mm or 50% of the

surrounding IMT value or demonstrates an IMT of >1.5 mm.[24] The intra-observer correlation coefficient for mmIMT was 0.97 (2 repeated measurements in 20 randomly chosen subjects).

Ankle-Brachial Index and Arterial Stiffness

Subjects were studied under supine resting conditions in a quiet and temperature-controlled room for the measurements of ABI and arterial stiffness. Pneumatic pressure cuffs with oscillometric pressure sensors were wrapped tightly around both arms and both ankles. Electrocardiographic electrodes were attached onto both wrists and a phonocardiogram was placed at the left second intercostal space, at the margin of the sternum. After ensuring that the patients have rested for 15 minutes, a fully automatic data acquisition would begin. Pressure waveforms of the brachial and posterior tibial arteries were recorded. Based on the height of the patient, the device estimated the path lengths from the brachial artery to posterior tibial artery. Brachial-ankle PWV (baPWV) was calculated as the path length divided by the corresponding time interval (cm/s). The right and left baPWV were averaged and the resulting value selected as the representative baPWV.

The pneumatic pressure cuffs over the arms and ankles enabled simultaneous measurement of systolic blood pressure of the 4 limbs. The right-and left-sided ABI were calculated as the ankle systolic blood pressure divided by the brachial systolic blood pressure measured from right and left side, respectively. The right and left ABI were averaged and the resulting value selected as the representative ABI.

The intra-observer correlation coefficient for ABI was 0.85 and the intra-observer correlation coefficient for baPWV was 0.98 (2 repeated measurements in 20 randomly chosen subjects).

Clinical Outcome

All patients were followed-up in our clinic every 3-4 months. Clinical data of all patients were retrieved from the medical records and subsequently during the most recent clinic visit. Major adverse cardiovascular event (MACE) was defined as death due to cardiovascular causes, acute coronary syndrome, heart failure hospitalisation, symptom driven revascularisation procedures (carotid endarterectomy, percutaneous coronary intervention or coronary artery bypass graft surgery) or ISS. Cardiovascular death was defined as death due to lethal cardiac arrhythmias, myocardial infarction, heart failure, fatal stroke or unexplained sudden death. Myocardial infarction was defined as the presence of chest pain and/or elevation of creatine kinase >2 times the upper limit of normal, with or without new ST-segment elevation (>0.1 mV) in at least 2 contiguous leads. Unstable angina was defined as hospitalisation because of angina pectoris that occurred at rest and that was associated with ECG changes. ISS was defined as clinical and radiological evidence of stroke without intracranial haemorrhage. Heart failure hospitalisation was defined as hospitalisation because of heart failure as diagnosed by using the modified Framingham criteria.[25,26]

Statistical Analysis

Continuous variables were presented as mean±1 standard deviation. Categorical data were presented as frequencies and percentages. Statistical comparisons between groups were performed with Student's t test for continuous variables and Chi-squared test for categorical variables. Correlations between vascular assessment variables were evaluated by calculating the Pearson's correlation coefficient. Receiver operating characteristic (ROC) curves were constructed and the areas under the curve (AUC) as well as the cut-off values of the vascular assessments with optimal sensitivity and specificity were obtained. The standard error (SE) of the AUC was quoted. Cumulative event rates were calculated according to Kaplan-Meier method and log rank test. Cox regression analysis was used to determine the clinical predictors of MACE. Variables with $P<0.1$ in the univariate analysis were entered into a multivariate analysis model to identify the independent predictors for MACE.

All calculations were performed with use of SPSS 15.0 software with the exception of construction and comparison of the ROC curves which were calculated using the MedCalc 8.2.1.0 software. A P value <0.05 was considered to be statistically significant.

Results

Clinical Characteristics

The clinical characteristics of the study population are summarised in Table 1. Their mean age was 66.4±10.4 years and 251 were men (65%). Among them, 139 patients (36%) had ISS, 130 (34%) had CAD, 100 (26%) had DM without CAD or ISS, and 18 (5%) had both CAD and ISS. Furthermore, 268 (69%) patients had hypertension, 210 (54%) had DM and 245 (63%) had hypercholesterolaemia.

Relationships between Vascular Assessment Parameters

As shown in Table 2, there were only weak correlations among different vascular assessment parameters. Parameters of endothelial function including FMD and NMD were negatively correlated with carotid mmIMT (r=-0.12 and r=-0.21 respectively) and baPWV (r=-0.14 and r=-0.17 respectively) (all *P<0.05*). Carotid mmIMT was negatively correlated with ABI (r=-0.31) and positively correlated with baPWV (r=0.20) (all *P<0.001*).

Table 1. Clinical characteristics of the study population

Characteristic	All (N=387)	With MACE (N=46)	Without MACE (N=341)	P-value
Age, years	66.4±10.4	72.0±7.2	65.6±10.6	<0.0001
Males, n (%)	251 (65)	30 (65)	221 (65)	0.92
Body-mass index, kg/m^2	25.4±3.6	25.8±3.5	25.4±3.6	0.43
Blood pressure, mmHg				
Systolic	138.5±20.4	144.0±22.8	137.7±20.0	0.093
Diastolic	76.1±10.1	77.5±12.2	76.0±9.8	0.46
Hypertension, n (%)	268 (69)	33 (72)	235 (69)	0.38
DM, n (%)	210 (54)	29 (63)	181 (53)	0.20
Hypercholesterolaemia, n (%)	245 (63)	31 (67)	214 (63)	0.14
Smoking, n (%)	167 (43)	21 (46)	146 (43)	0.36
Family history of CVD, n (%)	23 (6)	3 (7)	20 (6)	0.87
Biochemistry analysis				
Total cholesterol, mmol/L	4.62±0.90	4.59±1.05	4.62±0.89	0.86
LDL, mmol/L	2.64±0.77	2.57±0.94	2.65±0.76	0.60
HDL, mmol/L	1.33±0.35	1.34±0.32	1.32±0.35	0.69
Triglyceride, mmol/L	1.43±0.82	1.47±0.84	1.43±0.82	0.79
Blood glucose, mmol/L	6.13±2.04	6.71±2.77	6.08±1.93	0.18
Medications				
Beta-blocker, n (%)	130 (34)	20 (43)	110 (32)	0.17
Calcium channel blocker, n (%)	117 (30)	15 (33)	102 (30)	0.94
ACEI/ARB, n (%)	190 (49)	25 (54)	165 (48)	0.27
Nitrate, n (%)	98 (25)	20 (43)	78 (23)	0.001
Aspirin, n (%)	229 (59)	25 (54)	204 (60)	0.32
Statin, n (%)	214 (55)	28 (61)	186 (55)	0.75

Abbreviations: MACE=major adverse cardiovascular event; DM=diabetes mellitus; CVD=cardiovascular disease; LDL=low-density lipoprotein; HDL=high-density lipoprotein; ACEI=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker

Clinical Outcomes

During a mean follow-up of 25±6 months (range 1 month-30 months), 46 MACE were observed in 46 patients (11.9%), including 6 cardiovascular deaths, 6 acute coronary syndromes (2 unstable angina, 3 non-ST elevation myocardial infarctions and 1 ST elevation myocardial infarction), 7 heart failure hospitalisations, 14 revascularisation procedures (all percutaneous coronary interventions) and 13 ISS.

As shown in Table 1, patients with MACE were significantly older (72.0±7.2 years versus 65.6±10.6 years, *P<0.0001*) and were more likely to be treated with an oral nitrate (43% versus 23%, P=0.001). There was nonetheless no significant differences in the two groups in terms of gender, proportion with hypertension, hypercholesterolaemia or DM, smoking or family history of CVD (all *P>0.05*).

Results from different vascular assessment parameters are shown in Table 3. Patients with MACE had significantly greater mmIMT (1.18±0.29 mm versus 1.07±0.31 mm, *P=0.027*) than those without MACE. However, there were no significant differences in FMD, NMD, carotid plaque prevalence, ABI nor baPWV between patients with or without MACE.

ROC curves were constructed to obtain the diagnostic values as well as optimal cut-off values of the different vascular assessment parameters (Table 4). All these parameters had good negative predictive values (90-98%) but poor positive predictive values (13-23%). These cut-off points were then used for subsequent Kaplan-Meier and Cox regression analysis. Kaplan-Meier analysis revealed that subjects with a raised mmIMT>1.2 mm (*P<0.0001*, Figure 1a) and increased baPWV>1445 cm/s (*P=0.005*, Figure 1b) were associated with the occurrence of MACE during follow-up. In contrast, FMD>2.1% (*P=0.10*), impaired

Table 2. Pearson correlation coefficients between vascular assessment parameters

	FMD	NMD	mmIMT	ABI
NMD	0.41**			
mmIMT	-0.12*	-0.21*		
ABI	0.10	0.10	-0.31**	
baPWV	-0.14*	-0.17**	0.20**	-0.10

Abbreviations: FMD=flow-mediated dilatation; NMD=nitroglycerin-mediated dilatation; mmIMT=mean maximum intima-media thickness; ABI=ankle-brachial index; baPWV=brachial-ankle pulse wave velocity
* *P<0.05*, ** *P<0.001*

Table 3. Vascular assessment parameters of study population

Vascular assessment parameters	All (N=387)	With MACE (N=46)	Without MACE (N=341)	P-value
FMD, %	2.94±2.47	3.25±2.12	2.90±2.53	0.34
NMD, %	13.76±6.49	12.16±5.29	14.00±6.63	0.053
mmIMT, mm	1.09±0.31	1.18±0.29	1.07±0.31	0.027
Carotid plaque, n (%)	239 (62)	30 (65)	209 (62)	0.25
ABI	1.08±0.10	1.06±0.13	1.08±0.10	0.18
baPWV, cm/s	1771±414	1845±410	1764±416	0.27

Abbreviations: MACE=major adverse cardiovascular event; FMD=flow-mediated dilatation; NMD=nitroglycerin-mediated dilatation; mmIMT=mean maximum intima-media thickness; ABI=ankle-brachial index; baPWV=brachial-ankle pulse wave velocity

NMD≤13.5% (*P=0.052*), the presence of carotid plaque (*P=0.43*) and decreased ABI≤1.1 (*P=0.062*) were not associated with the occurrence of MACEs.

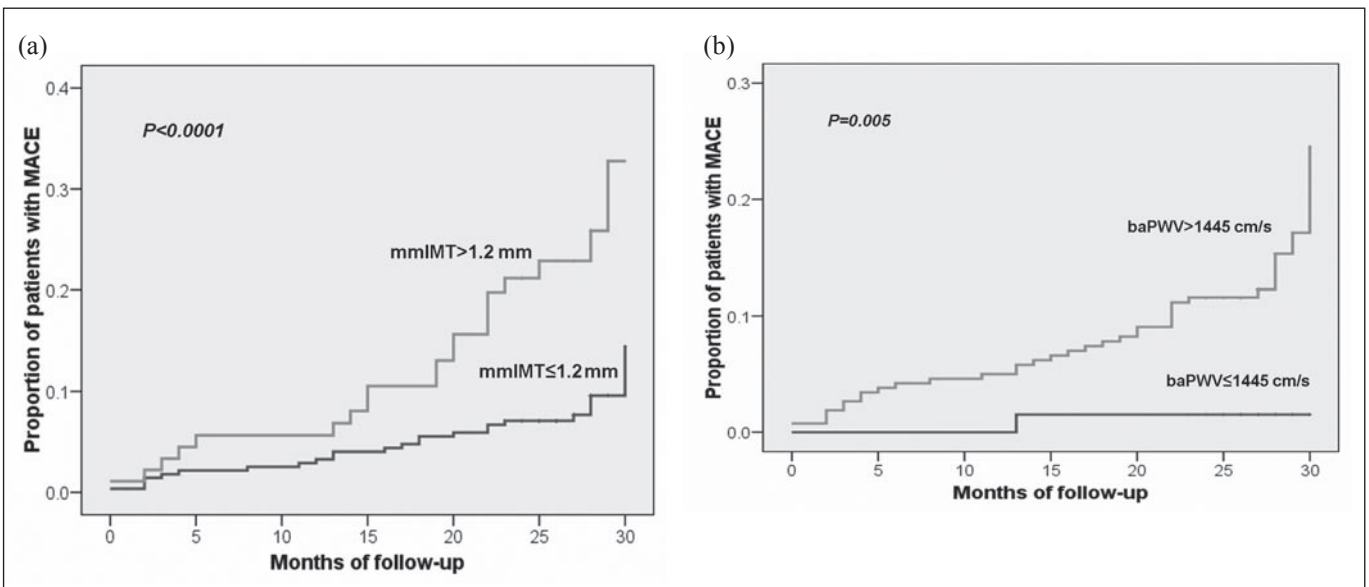
Univariate Cox regression analysis calculated that NMD≤13.5%, mmIMT>1.2 mm, ABI≤1.1, baPWV>1445 cm/s, age and oral nitrate use were positive predictors of MACE (Table 5, all *P<0.1*). Multivariate Cox regression analysis then revealed that mmIMT>1.2 mm (Hazards ratio [HR] 2.49, 95% confidence interval [CI] 1.15-5.39, *P=0.020*) and oral nitrate use (HR 2.37, 95% CI 1.14-4.94, *P=0.021*) were independent predictors for MACE.

The incremental value of using mmIMT and baPWV together for prediction of MACE was examined.

Table 4. Diagnostic values of various vascular assessments according to specified cut-off values

Marker	AUC (SE)	P-value	Cut-off values	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive predictive value, %	Negative predictive value, %
FMD	0.56±0.05	0.27	>2.1%	72.5 (56.1-85.4)	41.5 (35.9-47.2)	14.0	92.0
NMD	0.58±0.05	0.10	≤13.5%	71.8 (55.1-85.0)	43.8 (38.0-49.6)	14.4	92.2
mmIMT	0.63±0.05	0.005	>1.2 mm	46.5 (31.2-62.3)	78.9 (74.1-83.2)	22.5	91.8
Carotid plaque	0.53±0.05	0.54	-	69.8 (53.9-82.8)	36.1 (30.9-41.6)	12.6	90.1
ABI	0.56±0.05	0.20	≤1.1	85.7 (71.4-94.5)	30.2 (25.1-35.7)	14.3	93.9
baPWV	0.55±0.05	0.32	>1445 cm/s	97.2 (85.4-99.5)	21.4 (16.8-26.5)	13.1	98.4

Abbreviations: AUC=area under curve; SE=standard error; CI=confidence interval; FMD=flow-mediated dilatation; NMD= nitroglycerin-mediated dilatation; mmIMT=mean maximum intima-media thickness; ABI=ankle-brachial index; baPWV= brachial-ankle pulse wave velocity



Abbreviations: MACE= major adverse cardiovascular event; mmIMT= mean maximum imtima-media thickness; baPWV= brachial-ankle pulse wave velocity

Figure 1. *Kaplan-Meier curves for the development of MACE in patients with (a) mmIMT>1.2 mm or ≤1.2 mm and (b) baPWV> 1445cm/s or ≤1445cm/s*

Table 5. Cox regression analyses for MACE in patients with CVD

Risk variable	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
FMD>2.1%	1.74 (0.87-3.50)	0.12		
NMD≤13.5%	1.97 (0.98-3.97)	0.058	1.17 (0.54-2.55)	0.69
mmIMT>1.2 mm	2.88 (1.58-5.25)	0.001	2.49 (1.15-5.39)	0.020
Carotid plaque	1.30 (0.68-2.49)	0.43		
ABI≤1.1	2.23 (0.94-5.30)	0.069	1.14 (0.43-3.04)	0.79
baPWV>1445 cm/s	10.04 (1.37-73.4)	0.023	6.76 (0.90-50.67)	0.063
Age	1.07 (1.03-1.10)	<0.0001	1.03 (0.99-1.08)	0.16
Male gender	1.09 (0.59-2.00)	0.78		
Body-mass index	1.04 (0.96-1.12)	0.39		
Hypertension	1.43 (0.68-2.98)	0.35		
DM	1.63 (0.88-3.01)	0.12		
Smoking	1.28 (0.70-2.34)	0.43		
Family history of CVD	1.01 (0.31-3.27)	0.98		
Beta-blocker	1.70 (0.91-3.16)	0.10		
Calcium channel blocker	1.29 (0.68-2.46)	0.43		
ACEI/ARB	1.44 (0.78-2.74)	0.27		
Nitrate	1.97 (1.08-3.61)	0.008	2.37 (1.14-4.94)	0.021
Aspirin	0.84 (0.44-1.60)	0.59		
Statin	1.15 (0.63-2.08)	0.65		

Abbreviations: MACE=major adverse cardiovascular event; CVD=cardiovascular disease; HR=hazards ratio; CI=confidence interval; FMD=flow-mediated dilatation; NMD=nitroglycerin-mediated dilatation; mmIMT=mean maximum intima-media thickness; ABI=ankle-brachial index; baPWV=brachial-ankle pulse wave velocity; ACEI=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker

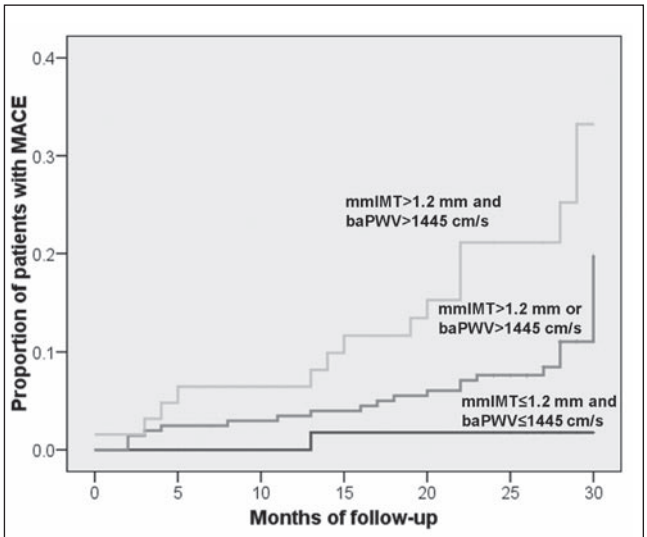
In Kaplan-Meier analysis, either increased mmIMT or baPWV was associated with an increase risk of MACE compared with patients with a normal mmIMT and baPWV (*P=0.045*, Figure 2). Furthermore, patients with both increased mmIMT and baPWV were associated with a further increase risk of MACE compared with those with increased either marker alone (*P=0.007*, Figure 2). Nevertheless, the combination of mmIMT and baPWV (AUC=0.67) did not provide significant incremental benefit for MACE prediction compared with mmIMT (AUC=0.63, *P=0.11*) or baPWV (AUC=0.55, *P=0.053*) alone.

Discussion

The results of this study demonstrated that in a population with established CVD or equivalent, increased mmIMT or baPWV were associated with increased risk of MACEs upon follow-up. However, multivariate analysis revealed that only an increased mmIMT>1.2 mm was an independent predictor for MACE and that subjects with mmIMT>1.2 mm were associated with a 2.5-fold increased risk of developing MACEs. In contrast, parameters of brachial endothelial function (including FMD and NMD) and ABI did not

have significant prognostic values. Although patients with both increased mmIMT and baPWV had a significantly greater risk of developing MACEs, the combined use of both parameters did not significantly increase the prediction value for adverse clinical outcome.

Carotid IMT has been shown to be one of the most promising non-invasive surrogate markers of predicting clinical outcomes in patients with CVD. Indeed, both the American Heart Association and the Society for Heart Attack Prevention and Eradication have recommended the use of carotid IMT as a surrogate marker for risk stratification in asymptomatic subjects.[19,27] A recent meta-analysis which included 37,000 individuals also concluded that carotid IMT was a strong predictor of future vascular events.[14] The results of the present study confirmed this finding and demonstrated that carotid IMT has the best prognostic value among different vascular surrogate makers in a patient cohort with established CVD. Whilst previous studies have identified an increased mmIMT>1 mm as a predictor for CVD events in the general population,[5] the optimal cut-off value of mmIMT in the prediction of MACE identified in the present study was >1.2 mm. This is likely because of the patient population included in this study had underlying CVD and thus their baseline IMT values would be expectedly much higher than the general population. As a result, a higher cut-off value of >1.2 mm for carotid mmIMT was required to identify patients at greatest risk of developing a recurrent major cardiovascular event.



Abbreviations: MACE= major adverse cardiovascular event; mmIMT= mean maximum imtima-media thickness; baPWV= brachial-ankle pulse wave velocity

Figure 2. *Kaplan-Meier curves for the development of MACE in patients with 1) both mmIMT>1.2 mm and baPWV>1445 cm/s, 2) either mmIMT>1.2 mm or baPWV>1445 cm/s and 3) both mmIMT≤1.2 mm and baPWV≤1445 cm/s.*

Previous studies have shown that the presence of carotid plaque is also a good predictor for CVD.[28] Based on the definition of carotid plaque from the Mannheim Carotid Intima-media Thickness Consensus,[24] we failed to demonstrate the predictive value of the presence of carotid plaque for MACE in patients with CVD. A more detailed assessment of carotid plaque by measuring the plaque area or even plaque volume may provide a more sensitive and better assessment of the burden of atherosclerosis, and thus improve its prognostic value.[29,30]

Arterial stiffness, as measured by baPWV has been suggested as a promising marker to predict adverse events in a high risk population. Previous studies have shown that increased arterial stiffness was associated with an increased risk of CAD and stroke.[31-34] Furthermore, increased arterial stiffness has been identified as an independent predictor of all-cause and cardiovascular mortality in hypertensive patients.[17] It has been postulated that an increased arterial stiffness is closely correlated with left ventricular hypertrophy, arterial wall thickening and atherosclerosis which could potentially contribute to the higher risk of adverse clinical outcomes in patients with CVD.[35-37] In this study, patients with higher baPWV had a significantly increased risk of MACEs. However, multivariate analysis failed to demonstrate baPWV as an independent predictor for MACEs in patients with CVD. The use of ABI, another structural assessment of the peripheral vascular system, also did not predict the occurrence of MACEs in this study. Indeed, the use of ABI as a surrogate marker is rather complicated as both low

(<1.0) or high (>1.4) ABI are associated with the presence of peripheral vascular disease.[38] Therefore, ABI is a less sensitive marker to predict MACEs in patients with established CVD as such subjects might have both low and high ABI values.

Endothelial dysfunction represents an early phenomenon of atherosclerosis preceding structural changes and clinical manifestations.[39] In low risk subjects without established CVD, the presence of endothelial dysfunction have been shown to predict the occurrence of adverse clinical events.[23,40] However, assessment of endothelial function appeared to have limited prognostic value in high risk subjects and patients with established CVD.[20,41,42] The results of this study supported these findings and demonstrated that brachial FMD did not predict MACEs in our patient cohort with established CVD or DM. In contrast, NMD, a marker which reflects the ability of vascular smooth muscle relaxation, has previously been shown to be impaired in subjects with cardiovascular risk factors as well as in patients with established CAD.[43,44] In this study, patients with an impaired NMD was associated with an increased risk of developing MACEs. Nevertheless, impaired NMD was not an independent predictor for MACE. On the other hand, the use of oral nitrate was shown to be an independent predictor for MACE. Although the reason remains unclear, it is possible that oral nitrate was more likely prescribed in patients with more severe CAD or it may be related to the potential adverse effects attributed to the long-term use of oral nitrate.[45]

Conclusions

The results of this study suggested that the measurement of carotid IMT as a non-invasive structural marker of atherosclerosis provides the best predictive value for MACEs in patients with established CVD or equivalent, and the combined use of vascular surrogate markers did not further improve their predictive value. Nevertheless, whether the regression of carotid IMT in those higher risk patients can reduce the risk of MACEs remains unclear.

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Institute of Cardiovascular Science and Medicine
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The University of Hong Kong

The Twelfth Annual Scientific Meeting

December 13-14, 2008
Hong Kong Convention and Exhibition Centre
Hong Kong

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PROGRAMME

13 DECEMBER 2008 (SATURDAY)

08:00-09:00 Registration

09:00-09:30 **Symposium: "Heart Failure-Causes and Management of a Modern Epidemic"**
Chairmen: Dr TF Tse, Prof Y Huang
IL 1 Heart Failure in China: Prevalence and Current Treatment - *Prof P Ding*

09:30-10:00 Coffee Break, Poster Viewing and Booth Visit

10:00-12:00 **Young Investigator Award (poster presentation)**
(supported by Sun Chieh Yeh Heart Foundation)

12:00-12:45 *Chairmen: Dr CS Chiang, Prof SSM Chung*
IL 2 Molecular Basis of Heart Failure and Cardiac Arrhythmias: Cause and Cures - *Prof A Marks*

12:45-13:45 **Lunch**

13:45-14:30 **Lunch Lecture (Sponsored by Servier Hong Kong)**
Chairman: Dr KLF Lee
IL 3 Ivabradine: From Angina Pectoris to Heart Failure - *Dr G Lerebours*

14:30-14:45 **Opening Ceremony**

14:45- 16:15 *Chairmen: Prof HF Tse, Prof PM Vanhoutte, Dr CO Pun*
IL 4 Heart Failure: a Vascular Disease? - *Prof V Richard*
IL 5 From Myocardial Infarction to Heart Failure: Mechanisms and Management - *Prof T Lüscher*

16:15-16:45 Coffee Break, Poster Viewing and Booth Visit

16:45-17:45 **Case-Based Interactive Session on the Treatment of Heart Failure**
Chairmen: Prof PM Vanhoutte, Dr YK Lau, Dr EMC Chow

17:45-18:15 Annual General Meeting

14 DECEMBER 2008 (SUNDAY)

09:00-11:00 **Young Investigator Award (oral presentation)**
Chairmen: Prof TM Wong, Dr HJ Ballard

11:00-11:30 Coffee Break, Poster Viewing and Booth Visit

11:30-12:00 **Symposium: "New frontiers in Cardiovascular Medicine"**
Chairmen: *Prof RYK Man, Prof GW He*
IL 6 Endothelial Protective Effect of Nucleoside Uptake Inhibitors - *Dr GPH Leung*
IL 7 Update on Stem Cell Research in Cardiology - *Dr DCW Siu*

12:00-14:30 **Meeting the Expert Luncheon: Case Based Tutorial of Guideline for the Treatment of Hypertension**
(jointly organized with Hong Kong College of Cardiology, sponsored by AstraZeneca)
Chairmen: Prof CP Lau, Dr CH Cheng

Panel members: Dr AWK Chan, Dr PC Fong, Dr SK Li

1430-1530 **Symposium: "Recent Advances in the Management of Cardiovascular Risk Factors"**
Chairmen: Prof YF Cheung, Dr Chris Wong, Dr KT Chan
IL 8 Latest Advances in Lipid Lowering Therapy - *Prof HF Tse* (Sponsored by AstraZeneca)
IL 9 Addressing Obesity and the Metabolic Syndrome - *Dr AWK Tso*

1530-1600 Coffee Break, Poster Viewing and Booth Visit

1600-1730 **Symposium: "Evidence-Based Research of Traditional Chinese Medicine"**
Chairmen: Dr ML Fung, Dr ST Lau
IL 10 Unravelling the Mystery of TCM: Application of Molecular Cell Signalling Technologies - *Prof ASY Lau*
IL 11 Berberine as a Potential Drug for the Treatment and Prevention of Vascular Dysfunction in Obesity and Diabetes - *Dr A Xu*
IL 12 A Compound from Chinese Medicinal Herb Provides a Potential Cure for Atrial Fibrillation - *Dr GR Li*

1730-1800 **Young Investigator Awards Ceremony and Closing remarks**
Prof TM Wong, Prof CP Lau

ABSTRACTS

Abstracts for Invited Lectures:

IL1.

HEART FAILURE IN CHINA: PREVALENCE AND CURRENT TREATMENT

Philip YA Ding
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Heart failure (HF), a major cause of morbidity and mortality, is reported to consume 2% to 3% of the total health care costs in industrialized countries. The age-adjusted mortality for HF patients is four to eight times that of the general population, comparable to that of cancer diseases in the same age groups. The predominant causes of HF are hypertension and coronary heart disease (CHD). Other identified risk factors for HF include left ventricular (LV) hypertrophy, valvular heart disease, diabetes mellitus, cigarette smoking, obesity, and dyslipidemia. Population-based studies to date have shown different results concerning the relative importance of these risk factors. In the past decade, considerable knowledge has been gained regarding the pathophysiology of HF in the experimental setting, small clinical samples, and larger population-based studies. New mechanisms, such as insulin resistance, inflammation, and oxidative stress, have been investigated. Epidemiologic studies revealed that these mechanisms are largely established in the general population.

Based on the 2001 Chinese national survey of cardiovascular diseases and risk factors study, a total of 15,518 adults were collected from 10 provinces of China (5 in the North and 5 in the South) with an equal distribution from urban and rural populations. The prevalence of chronic heart failure (CHF) was 0.9%, 0.7% and 1.0% for the general population, the males and females, respectively. Females with CHF were more frequent than males ($p<0.05$). CHF prevalence was 0.4%, 1.0%, 1.3% and 1.3% in the 35-44, 45-54, 55-64, and 65-74 years of age, respectively. The prevalence rate of CHF increased substantially with aging. The figure of prevalence of CHF was 1.4% and 0.5% in northern and southern population, respectively; and the figure was 1.1% and 0.8% in urban and rural population, respectively. The risk of CHF was higher in northern than in southern China and was higher in urban than in rural area.

Over the last two decades, major advances have occurred in the treatment of heart failure patients. Randomized clinical trials showed that ACE-inhibitors, angiotensin receptor blockers, β-blockers, and aldosterone antagonists as well as mechanical circulatory support devices can reduce morbidity and mortality in patients with heart failure. Guidelines have been established to help physicians in reaching a clinical decision - making this a rapidly evolving field. In China, most physicians are increasingly encouraged to apply the most updated treatment guidelines in their practice. However, there is a considerable proportion of heart failure patients who do not receive evidence-based treatment. Several factors may explain the reported under-utilization of evidence-based treatment such as lack of knowledge and technical expertise of the physicians, economic restraints, and family wishes from patients.

In conclusion, among cardiovascular diseases, heart failure is becoming an important issue of public health in China.

IL4.

HEART FAILURE: A VASCULAR DISEASE?

V Richard
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Although heart failure (HF) is by definition a disease mostly affecting the cardiomyocytes, there is also growing evidence that this disease has important consequences for the vasculature, and especially impacts the vascular endothelial cells, both at the coronary and peripheral levels.

At the coronary level, HF markedly alters coronary endothelial function and especially the release of endothelium-derived factors such as nitric oxide (NO). Given the role of this factor as a regulator of vasodilator tone, but also as an important inhibitor of platelet aggregation and leukocyte function, it is likely that such an impaired NO production not only will contribute to increased vasoconstriction, but also will favour thrombosis and atherosclerosis, thus increasing the risk of (re)infarction and aggravating HF. In parallel, the development of HF is associated with a progressive reduction of coronary arteriolar and capillary density (due at least in part to impaired coronary angiogenesis). Such reduced vascular density, and the resulting impaired cardiac perfusion is now recognized as a critical determinant of the aggravation of HF and of cardiac decompensation.

At the peripheral level, there is growing evidence that HF also profoundly impairs physiological NO production, notably at the level of small peripheral arteries. Such impairment is especially important since it will contribute to peripheral vasoconstriction and will potentiate the effects of the major vasoconstrictor systems known to be activated in HF (i.e. the renin-angiotensin, endothelin and sympathetic systems). This results in an increased peripheral vascular resistance which augments the afterload of the heart, ultimately aggravating HF. The impaired peripheral production of NO appears to be the consequence of interaction between mechanical (e.g. chronic decrease in blood flow), neurohumoral (e.g. angiotensin) and inflammatory (e.g. reactive oxygen species and TNFα) influences.

Importantly, endothelial dysfunction in HF can be reduced by various pharmacological approaches, including 'classic' treatments of HF (e.g. angiotensin converting enzyme inhibitors such as perindopril) and more recent treatments (e.g. heart rate lowering agents such as ivabradine). In addition, recent therapies have emerged and appear promising in the treatment of HF-induced endothelial dysfunction, including modulators of endothelial phosphorylation pathway (e.g. using tyrosine phosphatase inhibitors), which may provide new clinical opportunities for the treatment of HF.

ABSTRACTS

Abstracts for Invited Lectures:

IL6.

ENDOTHELIAL PROTECTIVE EFFECT OF NUCLEOSIDE UPTAKE INHIBITORS

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Inflammation of endothelium is closely associated with the development of atherosclerosis. It has been well established that interstitial level of adenosine is increased during inflammation. Adenosine possesses anti-inflammatory property so it may serve to protect tissues from injury and potentially slow down the progression of atherosclerosis. The increase in extracellular adenosine level at the sites of inflammation is mainly due to the up-regulation of ecto-5' nucleotidase by phosphoinositide 3-kinase (PI3K)-dependent pathways. The ecto-5' nucleotidase catalyses the conversion of extracellular adenosine nucleotides into adenosine.

Patients with diabetes mellitus suffer greater morbidity from atherosclerosis. Glucose *per se* does not affect ecto-5' nucleotidase but it increases the expression levels of equilibrative nucleoside transporters (ENTs) via mitogen-activating protein kinase (MAPK)-dependent pathways. It is speculated that the increase in ENTs may reduce the availability of adenosine in the vicinity of adenosine receptors, thereby attenuating the anti-inflammatory effect of adenosine in diabetes.

The anti-inflammatory effect of adenosine can be potentiated pharmacologically by ENT inhibitors, of which nitrobenzylmercaptopurine ribonucleoside (NBMPR) and dipyridamole are the representatives. No oral hypoglycemic agents can inhibit ENTs except troglitazone. Unfortunately, this thiazolidinedione has been withdrawn from the market. Screening of anti-hypertensive agents has revealed that ENTs are sensitive to dihydropyridine-type calcium channel antagonists, particularly nimodipine, which can inhibit ENTs in nM range. Those calcium channel antagonists are non-competitive inhibitors of ENTs, probably working through the reversible interactions with allosteric sites. In vitro study has demonstrated that nimodipine and nifedipine can reduce the lipopolysaccharide-induced interleukin-8 release in endothelial cells. This effect is independent of their inhibitory effects on calcium channels but is more likely to be due to the blockade of ENTs. Therefore, while treating vascular diseases such as hypertension and angina pectoris, dihydropyridines may have an additional beneficial effect in attenuating endothelial inflammation and hence, ameliorating atherosclerosis and its complications.

IL11.

BERBERINE AS A POTENTIAL DRUG FOR THE TREATMENT AND PREVENTION OF VASCULAR DYSFUNCTION IN OBESITY AND DIABETES

Y Wang,[1] Y Huang,[2] Karen SL Lam,[1] Chi-Wai Lau,[2] Paul M Vanhoutte,[3] A Xu[1,3]
[1]Department of Medicine, The University of Hong Kong; [2]Department of Physiology, The Chinese University of Hong Kong; [3]Department of Pharmacology, The University of Hong Kong, Hong Kong SAR, China

Introduction: Vascular disorder is a common soil for many deadly diseases and is one of the most common complications observed in Type 2 diabetes. Endothelial dysfunction, characterized by impaired vasodilation, is a key event that links obesity, diabetes, hypertension and vascular diseases. The AMP-activated protein kinase (AMPK) plays a key role in endothelial cell, including protection of cells from apoptosis, inhibition of inflammation and stimulation of angiogenesis. The objectives of this study are to evaluate the protective effect of berberine, an alkaloid purified from traditional Chinese medicine, against hyperglycemia-induced cellular injury, endothelial dysfunction, and to investigate the potential role of AMPK pathway in this process.

Method: Berberine was tested for its effect on the production of nitric oxide (NO), activation of eNOS as well as the association of eNOS with heat shock protein(HSP)90 and AMP-activated protein kinase (AMPK) in human umbilical vein endothelial cells (HUVEC). The effect of berberine on vascular reactivity was examined on aortic rings isolated from rats. In addition, berberine was also evaluated for its actions on inhibiting production of intracellular ROS, apoptosis and NF-κ B activation under hyperglycemia circumstance.

Results: In cultured endothelial cells and blood vessels, berberine dose-dependently enhanced eNOS phosphorylation and promoted the association of eNOS with heat shock protein (HSP)90, leading to an increased production of nitric oxide (NO). Furthermore, berberine attenuated high glucose-induced generation of reactive oxygen species (ROS), cellular apoptosis, NF-κappaB activation and expression of adhesion molecules, thus suppressing monocyte attachment to endothelial cells. In rat aortic rings, berberine elicited endothelium-dependent vasodilatations and alleviated ROS-mediated endothelial dysfunction. These beneficial effects of berberine on the endothelium were abolished by either pharmacological inhibition of AMP-activated protein kinase, or adenovirus-mediated overexpression of a dominant negative version of AMPK.

Conclusions: The present results demonstrate that berberine protects against endothelial injury and enhances NO-dependent vasodilatation through activation of the AMPK/eNOS signaling cascade. Berberine or its derivatives may be useful for the treatment and/or prevention of endothelial dysfunction associated with diabetes.

ABSTRACTS

Abstracts for Invited Lectures:

IL12.

A COMPOUND FROM CHINESE MEDICINAL HERB PROVIDES A POTENTIAL CURE FOR ATRIAL FIBRILLATION

GR Li
Departments of Medicine and Physiology, The University of Hong Kong, Hong Kong SAR, China

Atrial fibrillation (AF) is the most common form of cardiac dysrhythmia. It increases the risk of death, congestive heart failure, and embolic phenomena including stroke. AF is believed to be a lifetime risk in an aging population. Antiarrhythmic drug therapy remains the principal approach for suppressing AF and its recurrence. Class III anti-arrhythmic agents are effective in treating AF, but have major limitations, such as inducing severe ventricular arrhythmia (i.e. long QT syndrome). A key objective among the current strategies for suppressing AF is the development of new Class III antiarrhythmic agents that preferentially affect atrial rather than ventricular electrical parameters. Pharmaceutical researchers have been focusing on developing selective inhibitors of the human atrial I_{Kur} or hKv1.5 channels, which is present in atrium, but not in ventricles of the human hearts. However, there is no such drug commercially available yet. We have been investigating traditional Chinese medicine to find selective I_{Kur} blockers for the treatment of atrial fibrillation. By collaborating with Shanghai Institute of Materia Medica, Chinese Academy of Science, we have recently found that the natural flavone acacetin from the Chinese medicine *Xuelianhua* selectively inhibited human atrial ultra-rapid-delayed rectifier K^+ current (I_{Kur}) and transient outward K^+ current (I_{to}), and prolonged action potential duration in human atrial myocytes. The compound blocked acetylcholine-activated K^+ current; however, it had no effect on other currents in guinea pig cardiac myocytes. In anesthetized dogs, acacetin prolonged atrial effective refractory period (ERP) after intraduodenal administration without QTc prolongation, whereas the clinical drug sotalol prolonged atrial ERP, and ECG QTc interval. In addition, acacetin prevented AF induction in anesthetized dogs. Our study demonstrates that acacetin targets to human atrial repolarization currents and is an atrial selective agent. It effectively prevents AF induction in anesthetized dogs after intraduodenal administration, indicating that oral acacetin is a promising atrial-selective agent for the treatment of AF. Therefore, the natural compound acacetin from the traditional Chinese medicinal herb *Xuelianhua* provides a potential cure for atrial fibrillation.

ABSTRACTS

Abstracts for Oral Communications:

OC1.

CONTRACTILE RESPONSE TO HYPOXIA IN THE PORCINE CORONARY ARTERY

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The contractile response to hypoxia has been studied in the lung, and is termed hypoxic pulmonary vasoconstriction. However this response is also observed in coronary arteries. The present study investigated the mechanism underlying this contractile response in isolated porcine coronary arteries. Isometric tension was measured in rings with or without endothelium. In quiescent preparations, the contractile response to hypoxia was only observed in rings with endothelium and was abolished by indomethacin and terutroban, which shows the involvement of cyclooxygenase products and TP receptor activation, respectively, in this phenomenon. In contracted preparations, the hypoxic response was also endothelium-dependent, but was abolished by L-NAME and ODQ, suggesting the involvement of cyclic GMP. Assay of the cyclic GMP content showed no change upon exposure to hypoxia in preparations with and without endothelium. These experiments suggest that these hypoxic coronary contractions depend on more than one signaling pathway.

OC2.

HEIGHTENED SYSTEMIC OXIDATIVE STRESS CRITICALLY ACCELERATES WORSENING ATHEROSCLEROSIS IN THE LATE CARDIOVASCULAR CONTINUUM

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Background: Both increased oxidative and inflammatory stresses are implicated in atherogenesis. However, little is known about their role in atherosclerotic progression in patients already at the advanced cardiovascular continuum.
Objective: To investigate the impact of oxidative and inflammatory stress on the progression of carotid atherosclerosis in patients with established ischemic stroke.
Methods: A total of 43 consecutive patients (mean age 65.7±8.8 years; male 70%) with primary or recurrent ischemic stroke (>6 months) were recruited from our medical outpatient clinics. High resolution ultrasound (Agilent Sonos 5500, Philips, USA) was used to assess burden of carotid atherosclerosis in terms of maximum intima-media thickness (mIMT). Serum malodialdehyde (MDA) and high-sensitivity C-reactive protein (hsCRP) were respectively measured as markers of systemic oxidative and inflammatory stress.
Results: These patients showed a mean mIMT of 2.25±0.98 mm. Serum MDA (Pearson r=0.32, *P=0.035*) and hsCRP (Pearson r=0.41, *P=0.007*) were both positively associated with mIMT. Adjusting for potential confounders by multivariate model (age, gender, hypertension, diabetes mellitus, hyperlipidemia, smoking history, use of aspirin/statins/antihypertensives and body-mass index), each 1 μM increase in serum MDA independently predicted increase in mIMT by 0.79 mm (95%CI [0.23-1.36], *P=0.008*). Furthermore, each 1 mg/L increase of hsCRP was independently predictive of increase in mIMT by 0.06 mm (95%CI [0.01-0.12], *P=0.017*). Hyperlipidemia and diabetes accounted for IMT increase by 0.56 mm (95%CI [0.04-1.08], *P=0.037*) and 0.53 mm (95%CI [0.01-1.05], *P=0.046*) respectively.
Conclusions: This study demonstrated that systemic oxidative stress strongly accelerates secondary progression of carotid atherosclerosis in patients with established ischemic stroke, independent of and above all conventional risk factors including systemic inflammation. This suggests that effective reduction of oxidative stress should be a major therapeutic target in patients at the advanced cardiovascular continuum.

ABSTRACTS

Abstracts for Oral Communications:

OC3.

CONTROL OF ADENOSINE FORMATION BY VASCULAR ENDOTHELIAL CELLS

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Introduction: Adenosine is formed extracellularly by ecto-5'-nucleotidase (5'N) during exercise vasodilation. It has been suggested that vascular endothelial cells act as a source of adenosine during hypoxia. However, it is not known whether the 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 vascular endothelial cells were capable of forming adenosine intracellularly in sufficient quantities for it to diffuse out into the extracellular space.
Methods: Vascular endothelial cells from hindlimb muscles of young male SD rats were isolated and purified. In the first series of experiments, cells were homogenised, and the cytosolic- and ecto-5'Ns were separated by differential centrifugation. 5'N in the cell homogenates was assayed at different pH values, as previously reported (Le & Ballard, 2007). Enzyme kinetic parameters, such as *Vmax*, *Km* and *Kcat* were calculated using the Eadie-Hofstee equation. In the second series of experiments, the cultured primary endothelial cells were exposed to normoxia or hypoxia (19% or 2% O_2) or pH 6.0 for 24 hours. The effects of hypoxia or low pH on the accumulation of adenosine in the culture medium were determined using HPLC.
Results: The highest value of *Vmax* occurred at pH 7.5 for ecto-5'N and at pH 7.0 for cytosolic-5'N, but the *Vmax* for the cytosolic-5'N was 2-3 times higher than that for the ecto enzyme across the pH range 6.0-8.0. The *Km* for both enzymes decreased with pH. Thus, *Kcat* for both enzymes was increased at low pH. Accumulation of adenosine in the medium surrounding the cultured endothelial cells was not changed greatly by a reduction in the pH to 6.0, but it was increased by around 50% following 24 hours exposure to hypoxia; preliminary data suggest that AOPCP, an inhibitor for ecto-5'N, did not significantly decrease the formation of adenosine in hypoxia.
Conclusions: Cytosolic-5'N has a higher activity than ecto-5'N, which is the opposite of the situation in muscle cells, suggesting that adenosine may be formed intracellularly in vascular endothelial cells. Furthermore, hypoxia significantly increased the adenosine formation by primary endothelial cells, confirming the capability of vascular endothelial cells to form adenosine during hypoxia.
Reference: GY Le, HJ Ballard. Properties of Adenosine-Metabolising Enzymes Extracted from Vascular Endothelial Cells. J HK Coll Cardiol 2007;15(2):85.

OC4.

ADIPOCYTE-FATTY ACID BINDING PROTEIN (A-FABP) IS PRESENT IN THE ENDOTHELIUM OF MALE APOLIPOPROTEIN E-KNOCKOUT MICE

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Adipocyte-fatty acid binding protein (A-FABP) modulates inflammatory responses in macrophages. A-FABP has been detected in cell cultures derived from porcine regenerated endothelium. These findings suggest a role for A-FABP in the formation of foam cells and atherosclerotic plaques. The present experiments were designed to investigate the presence (or not) of A-FABP and endothelial function in aortae of 8,12 and 16 weeks old male C57 (strain: B6.129P2) apolipoprotein E-knockout ($ApoE^{-/-}$) mice. A-FABP was detected by immunohistochemical staining in the endothelial layer of the aorta at 12, but not 8 weeks. Endothelium-dependent relaxations were measured in a myograph and compared to those obtained in aortae of C57 wild type mice. The relaxations to acetylcholine were reduced significantly in the $ApoE^{-/-}$ mice from 8 weeks on while those to the calcium ionophore A23187 were diminished significantly only from 12 weeks on. The endothelium-independent relaxation in response to the nitric oxide donor sodium nitroprusside was not affected in $ApoE^{-/-}$ mice. In conclusion, A-FABP was detected in male atherosclerotic-prone C57 $ApoE^{-/-}$ mice since the age of 12 weeks. Endothelial dysfunction was observed as early as at 8 weeks of age to judge from the reduced endothelium-dependent relaxations to acetylcholine. The dissociation between the absence of A-FABP and the presence of endothelial dysfunction at 8 weeks suggests that the presence of A-FABP at an older age is a consequence rather than a cause of the insufficient production of nitric oxide.

ABSTRACTS

Abstracts for Oral Communications:

OC5.

GENISTEIN ACUTELY POTENTIATES ACETYLCHOLINE-INDUCED RELAXATION THROUGH A G-PROTEIN COUPLED PATHWAY IN SPONTANEOUSLY HYPERTENSIVE RATS

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Objectives: Genistein, a phytoestrogen rich in soy beans and soy products, was reported to be a vasorelaxant. This study examined the receptor and related signaling pathways in the rapid vascular actions of genistein.
Methods: Isometric tension was measured in isolated aortic rings from 32-weeks-old male spontaneously hypertensive rats (SHR).
Results: Acute exposure to genistein at 10 μM, a concentration with no direct relaxation effect, potentiated acetylcholine (ACh)-induced relaxation and reduced ACh-induced contraction in the presence of L-NAME (100 μM). Both actions were insensitive to 10 μM actinomycin D (transcription inhibitor) and 10 μM cycloheximide (translation inhibitor). The potentiation of ACh-induced relaxation by genistein in the absence or presence of indomethacin was inhibited by 10 μM NF023 and 10 μM GP antagonist-2A, the selective G_i and G_q α-subunit antagonists, respectively, but not by 10 μM NF449, a selective G_s α-subunit antagonist. Interestingly, NF023, NF449 and GP antagonist-2A did not alter the inhibitory effect of genistein on ACh-induced contraction. To further elucidate the mechanism of the vascular response given by genistein, the involvement of G-proteins was inspected in A23187-induced relaxation and contraction. NF023 and GP antagonist-2A, but not NF449 inhibited the potentiating effect of genistein on A23187-induced relaxation in the presence of indomethacin. Reduction of A23187-induced contraction by genistein was unaffected by all three G-protein inhibitors.
Conclusion: These results demonstrate that rapid vascular actions of genistein in modulating ACh-induced relaxation and contraction responses in SHR are mediated by non-genomic pathways. $G\alpha_i$ and $G\alpha_q$, but not $G\alpha_s$, were involved in the potentiating effect of genistein in ACh and A23187-induced relaxations, but none were involved in the inhibitory effect of genistein in ACh and A23187-induced contractions. Involvement of G-proteins in the enhancement of ACh-induced relaxation by genistein suggests that genistein exerts its effect through a putative G-protein coupled phytoestrogen receptor.

OC6.

ESTROGEN SUPPRESSES THE CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II THUS CONFERRING CARDIOPROTECTION

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Estrogen confers cardioprotection by down-regulating β_1-adrenoceptor and suppressing the expression and activity of protein kinase A. We hypothesized that estrogen may also protect the heart by suppressing the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), another signaling messenger activated by β_1-adrenoceptor via the Gs protein that enhances apoptosis. We first determined the expression of CaMKII in the heart of sham operated rats and ovariectomized rats with and without estrogen replacement. Both CaMKIIδ and phosphorylated CaMKII were up-regulated in the heart from ovariectomized rats, which was restored to normal by estrogen replacement. We then determined the injury and contractile responses to ischemic insult with or without β-adrenoceptor stimulation with isoprenaline (10^{-7}M) in isolated perfused hearts and isolated ventricular myocytes. The infarct size and lactate dehydrogenase release from the heart in response to ischemic insult were significantly greater after ovariectomy. Similarly the cardiac contractility, the amplitude of the electrically induced intracellular Ca^{2+} transient, which is directly correlated to the shortening of the myocyte, and TUNEL-positive cells, were also greater in the ovariectomized rats upon ischemia/reperfusion in the presence or absence of isoprenaline. Most importantly, the responses to ischemic insult in ovariectomized rats were reversed not only by estrogen replacement, but also by blockade of CaMKII with a selective inhibitor, KN93 (2.5 μM). The observations indicated that estrogen confers cardioprotection by suppressing the CaMKII. The CaMKII isoform involved may be CaMKIIδ. The effect of estrogen on CaMKII is independent of β-adrenoceptor in addition to its effect of down-regulating the receptor.

ABSTRACTS

Abstracts for Oral Communications:

OC7.

ACUTE SIMVASTATIN INHIBITS THE IKATP CHANNELS OF PORCINE CORONARY ARTERY SMOOTH MUSCLE CELLS

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Statins (3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase inhibitors) have been shown to provide beneficial effects on cardiovascular system. Compared to the cholesterol-lowering properties, the effects of simvastatin (a commonly-used statin) on ion channel gatings of coronary artery smooth muscle cells have not been fully explored. In porcine isolated coronary artery, the cromakalim (10 nM-10 μM)- and pinacidil (10 nM-10 μM)-induced relaxation was inhibited by simvastatin (3 and 10 μM). In single cells of human left internal mammary artery and porcine coronary artery, simvastatin (1, 3 and 10 μM) suppressed the cromakalim (10 μM)- and pinacidil (10 μM)-mediated opening of the whole-cell IK_{ATP} channels, and it was eradicated by okadaic acid (100 nM). Simvastatin (10 μM) and AICAR (1 mM) elicited a compound C (1 μM)-sensitive [^{3}H]-deoxy-glucose uptake and an increase in $[ATP]_i$ of the coronary arterial cells. Simvastatin caused a time- and concentration-dependent increase in phospho-AMPKα-Thr^{172} and phospho-PP2A-Tyr^{307}. Simvastatin-induced phospho-PP2A-Tyr^{307} was eradicated by okadaic acid, ryanodine, KN93, phloridzin (1 mM), ouabain (10 μM), in $[glucose]_o$-free and in $[Na^+]_o$-free conditions. The enhanced phospho-AMPKα-Thr^{172} expression was abolished by compound C, ryanodine (100 μM), KN93 (10 μM) and in $[Ca^{2+}]_o$-free conditions. Acute simvastatin enhances glucose uptake and ATP formation which resulted in an inhibition of PP2A-Tyr^{307}. PP2A inhibition favored the subsequent Ca^{2+}/CaMKK-mediated AMPKα-Thr^{172} phosphorylation and thus inhibition of the vascular IK_{ATP} channels opening.

Acknowledgements: This project was financially supported by RGC Earmarked Grants of Hong Kong (Project code: 2140565).

OC8.

POLYOL PATHWAY CONTRIBUTES TO THE IMPAIRMENT OF CALCIUM HOMEOSTASIS IN POST-ISCHEMIC REPERFUSED RAT HEARTS

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A number of studies have shown that the polyol pathway contributes to ischemia-reperfusion (I/R)-induced myocardial infarction due to depletion of ATP. However, whether this glucose metabolic shunt also contributes to I/R-induced cardiac contractile dysfunction is not clear. In the present study, we show that post-ischemic contractile functions of the isolated perfused hearts was improved by pharmacological inhibition of aldose reductase (AR) or sorbitol dehydrogenase (SDH), two enzymes in the polyol pathway. I/R-induced contractile dysfunction is most likely due to impairment in Ca^{2+} signaling as indicated by lower amplitude of the Ca^{2+} peak, longer time to reach the peak, and slower return to base level. All these abnormalities were significantly ameliorated by treatment with AR or SDH inhibitors. Furthermore, we show that inhibition of AR or SDH protected the activity of SERCA and RyR, two of the key players in Ca^{2+} signaling mechanism that regulates cardiac contraction, from I/R-induced inactivation. During I/R polyol pathway probably contributes to the inactivation of SERCA and RyR by decreasing the level of GSH and increasing the level of superoxide.

ABSTRACTS

Abstracts for Oral Communications:

OC9.

A CENTRAL ROLE OF PKC_{δ} AND ACTIVATION OF P38 MAPK AND ERK1/2 PATHWAYS IN ANGIOTENSIN II-MEDIATED UPREGULATION OF COX-2 IN ENDOTHELIAL CELLS

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High circulating angiotensin II (Ang II) levels were reported in patients with hypertension and other vascular complications, while cyclooxygenase-2 (COX-2) has been regarded as the culprit of vascular inflammation and found to be localized in atherosclerotic plaque. Although both Ang II and COX-2 are associated with vascular inflammation and remodeling, it remains elusive whether COX-2 plays a direct role as a downstream event in mediating Ang II-induced vascular pathogenesis. The present study aimed at investigating the relations between Ang II stimulation and COX-2 expression and intracellular signaling pathways linking these two pro-inflammatory factors. Primary endothelial cells were freshly cultivated from thoracic aorta of Sprague Dawley rats. The expression level and activation of relevant proteins with and without drug treatment were examined by Western blot analysis. COX-2 expression was augmented with increasing Ang II concentration (3-100 nM), and it reached a maximum (>15-fold increase compared with control) after an 8-hour incubation with 100 nM Ang II. Ang II type 1 receptor (AT1R) blocker (losartan) and RNA synthesis inhibitor (actinomycin-D) inhibited such upregulation. Of the well-known transcriptional pathways tested, only the inhibitors of p38 MAPK and ERK1/2 (SB 202190 and PD 98059, respectively) significantly decreased the COX-2 expression, each producing ~ 50% reduction. Co-treatment with SB 202190 and PD 98059 caused further reduction, suggesting a joint mediation through p38 MAPK and ERK1/2. Although these signaling molecules are known to be redox-sensitive, inhibitors of reactive oxygen species (ROS) failed to alter the COX-2 upregulation. By contrast, PKC inhibitor (GF109203X), and particularly the specific PKC_{δ} inhibitor (rottlerin), but not PKC_{α} inhibitor (Go 6976), prevented both the phosphorylation of ERK1/2 and COX-2 expression. The pivotal role of PKC in Ang II-induced COX-2 expression was further supported by the stimulatory effect of a phorbol ester (phorbol 12-myristate 13-acetate, PKC activator) on COX-2 expression, which was again inhibited by GF109203X and rottlerin. The present results suggest an essential role of PKC_{δ} and subsequent activation of p38 MAPK and ERK1/2 in Ang II-mediated COX-2 upregulation and this response did not involve ROS. Since elevated Ang II and COX-2 induction serve as prerequisites for vascular complications, this study provides an important molecular basis for further elucidation of how altered COX-2- derived products participate in vascular inflammation (Supported by GRF 465308, CUHK Focused Investment Scheme and Li Ka Shing Institute of Health Sciences).

OC10.

CHRONIC INTERMITTENT HYPOXIA ELEVATES OXIDATIVE STRESS AND IMPAIRS CALCIUM HOMEOSTASIS IN RAT CARDIOMYOCYTES

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Objectives: This study examined the hypothesis that chronic intermittent hypoxia aggravates oxidative stress and deteriorates calcium (Ca^{2+})-handling in rat cardiomyocytes.
Methods: Adult male 2-month old Sprague-Dawley rats were daily administered with melatonin (MIH, 10 mg/Kg/day of body weight, i.p.) or vehicle (VIH, 2% ethanol in normal saline) and exposed to intermittent hypoxia (inspired oxygen alternating from 21 to 5±0.5% oxygen per minute for 8 hr/day) for 4 weeks. Levels of malodialdehyde (MDA) and the mRNA expression of anti-oxidant enzymes in the rat hearts were measured respectively by colorimetric study and reverse transcription-PCR. Changes in sarcoplasmic reticulum (SR) Ca^{2+}-handling were measured by spectrofluorometric study with isolated fura-2-loaded cardiomyocytes; also differences in protein levels and activities of SR-Ca^{2+} handling proteins were determined by Western blot and $^{45}Ca^{2+}$ flux study in the ventricular myocytes.
Results: The ratio of heart/body weight and the level of MDA were significantly increased in the VIH group compared with the normoxic control and MIH group. In addition, the mRNA levels of catalase and Mn-superoxide dismutase in the VIH group were much lower than that of the normoxic and MIH groups. Furthermore, spectroflurometric studies indicated that decreases in SR-Ca^{2+} content and the Ca^{2+}-overloading induced by metabolic inhibition/anoxia were remarkable in VIH cardiomyocytes compared to the normoxic control. Also, protein levels and activities of SR Ca^{2+}-ATPase and sarcolemmal Na^{+}-Ca^{2+} exchanger were markedly reduced in VIH cardiomyocytes. Moreover, these Ca^{2+} handling impairments in the cardiomyocyte were significantly less in the MIH group than that of the VIH group.
Conclusions: Our results demonstrate that chronic intermittent hypoxia, simulating severe levels of obstructive sleep apnea in patients, induces oxidative stress that could cause the impairment of Ca^{2+} handling in the cardiomyocyte leading to cardiac injury.

ABSTRACTS

Abstracts for Posters:

P1.

A COMPARATIVE STUDY ON THE ISOLATED PANCREATIC b-CELLS OF OBESE (+*DB*/+*DB*) AND LEAN (+*DB*/+*M*) MICE

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Pancreas plays an important role in glucose homeostasis, insulin secretion and the development of diabetes mellitus (DM). So far, most type 2 DM studies were performed on single pancreatic islets and/or β-cells of normal animals incubated in a specially-designed culture medium that "mimicked" the hyperinsulinemic/hyperglycemic conditions. However, a successful isolation of single, viable β-cells from animal models for human DM research (e.g. obese/diabetic (+*db*/+*db*) mice) has not been reported. In addition, a comparison of the pharmacological responses of single pancreatic β–cells of the obese/diabetic and lean mice is unknown. In this study, age-matched (female; ~6 month-old) non-diabetic (+*db*/+*m*) (~30 g) and diabetic (+*db*/+*db*) (~60 g) mice were used. Islets of Langerhans of both species were successfully isolated and the single viable b-cells were harvested. The basal $[Ca^{2+}]_i$ level and the $[Ca^{2+}]_i$ change in response to ionomycin (2.5 μM) challenge were significantly smaller in the β-cells of +*db*/+*db* mice, compared to +*db*/+*m* mice. Immunofluorescent analysis revealed a lesser degree of insulin staining in the β-cells of +*db*/+*db* mice, compared to +*db*/+*m* mice. An enhanced outward K^+ current amplitude in response to isopimaric acid (10 μM, a BK_{Ca} channel opener) was recorded in the β-cells of +*db*/+*m* mice. In contrast, isopimaric acid did not alter/suppress the K^+ current amplitude in the β-cells of +*db*/+*db* mice. Thus, our results demonstrate that the β-cells of +*db*/+*db* mice respond differently which may have significant clinical implications.

Acknowledgements: This project is financially supported by RGC Earmarked Grant of Hong Kong (Project code: 2140565).

P2.

24-HOUR EFFECTS OF BISOPROLOL/HYDROCHLOROTHIAZIDE COMBINATION COMPARED WITH VALSARTAN IN CHINESE HYPERTENSIVE PATIENTS

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Background and aim: Diuretic-based combination represents an alternative option for first-line antihypertensive therapy. We compared the efficacy of β1-blocker bisoprolol with hydrochlorothiazide (Lodoz) with valsartan on 24-hour ambulatory blood pressure in Chinese patients.

Methods: After a placebo run-in, 23 hypertensive patients were randomized to open-label treatment with once daily Lodoz 2.5 (2.5mg bisoprolol/6.25mg hydrochlorothiazide) or valsartan 80 mg. Dosage was up titrated every 4 weeks until patients reached target blood pressure (BP). Twenty-four-hour ambulatory (A) BP monitoring was conducted at weeks 0, 8 and 16. Twenty-four-hour ABP were analysed by time-structured cosinor which involved the least square fit of a 24-hour cosine curve, estimation of a rhythm-adjusted mean (MESOR), and measures of the extent and timing of predictable change within a day (circadian amplitude and acrophase). BP response rate was defined as the percentage of patients with clinic diastolic (D) BP ≤90mmHg or ≥10mmHg decrease after treatment. Control rate was percentage of patients with DBP ≤90mmHg after treatment.

Results: Both Lodoz and valsartan significantly reduced clinic, 24-hour as well as night-time and day-time BP. Both response and control rates in the Lodoz group were 100% compared to 72.7% ($p>0.05$) and 63.6% ($p=0.02$) in the valsartan group. The Lodoz group showed significantly greater reductions in rhythm-adjusted mean of 24-hour DBP (MESOR-DBP) and daytime DBP than the valsartan group ($p<0.05$). The MESOR-BP was decreased by 20.84/12.99mmHg in Lodoz group as compared with 14.33/7.66mmHg in valsartan group. The Lodoz reduced day-time BP by 17.81/10.64mmHg and night-time BP by 22.95/14.65 mmHg, whilst valsartan lowered day-time BP by 11.24/3.93 and night-time BP by 15.47/10.60 mmHg.

Conclusion: We concluded that the low dose combination of bisoprolol and hydrochlorothiazide was more effective than valsartan for 24-hour blood pressure control in terms of MESOR-DBP and day-time DBP.

ABSTRACTS

Abstracts for Posters:

P3.

INTERMEDIATE CONDUCTANCE CA^{2+}-ACTIVATED K^+ CHANNELS IN PORCINE CORONARY ENDOTHELIUM UNDER HYPOXIC EXPOSURE

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Objectives: The importance of endothelial intermediate conductance Ca^{2+}-activated K^+ channels (IK_{Ca}) in endothelial function has been revealed by the involvement of these channels in the function of endothelium-derived hyperpolarizing factor (EDHF) and nitric oxide (NO). Endothelial dysfunction is observed in many pathological conditions, including hypoxic / ischemic states. Little is known about the effect of hypoxia on the activity of IK_{Ca} and therefore, this study was designed to assess whether the electrophysiological property of IK_{Ca} is altered under hypoxic exposure.
Methods: Endothelial cells (ECs) were enzymatically isolated from porcine coronary arteries. Primary cultures of ECs were used for patch-clamp study. Hypoxia (PO_2: 25-40 mmHg, 10 min) was elicited in a sealed chamber bubbling with 95%N_2-5%CO_2. Whole-cell IK_{Ca} currents were compared in ECs with or without exposure to 1-hr hypoxia.
Results: Hypoxic exposure markedly reduced whole-cell K^+ current (21.3± 1.1 pA/pF to 9.8±1.5 pA/pF, at 100 mV, $P<0.05$) and the current activated by IK_{Ca} /SK_{Ca} activator 1-EBIO (from 31.8±3.3 pA/pF to 19.2±2.8 pA/pF, $P<0.05$). The inhibitory effect of IK_{Ca} blocker TRAM-34 on the current was more significant in the normoxic group than that in the hypoxic group. IK_{Ca} current was reduced from 12.2±2.0 pA/pF to 1.9±0.4 pA/pF after hypoxic exposure ($P<0.05$).
Conclusions: Hypoxia reduces the activity of endothelial IK_{Ca}. This may be an important mechanism underlying the endothelial dysfunction under hypoxic / ischemic conditions.
Acknowledgments: This study was supported by Hong Kong RGC grant (CUHK4651/07M) and CUHK direct grants 2041388 & 2041384.

P4.

THE EFFECT OF AGING AND HYPERTENSION ON ENDOTHELIAL-DERIVED HYPERPOLARIZING FACTOR (EDHF)-MEDIATED RESPONSES IN SPONTANEOUSLY HYPERTENSIVE RATS

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Aging is accompanied by endothelial dysfunction due to the reduced bioavailability of nitric oxide (NO) and endothelium-derived hyperpolarizing factors (EDHF). EDHF-mediated vasodilatation is present in aged or hypertensive animals. However, it appears impaired in animals when both conditions are combined. The present study examined the hypothesis that the release of EDHF is augmented to compensate for a reduced bioavailability of NO during the early stages of endothelial dysfunction, but that its release is reduced as endothelial dysfunction progresses. Endothelium-dependent relaxations to acetylcholine (Ach) were obtained in superior mesenteric arteries isolated from spontaneously hypertensive rats (SHR) at week 15, week 36, week 60 and week 72, using age-matched Wistar Kyoto (WKY) rats as controls. The contribution of EDHF to these relaxations was identified as the residual response in the presence of the cyclooxygenase inhibitor, indomethacin, and the NO synthase inhibitor, L-NAME.
Inhibition of the EDHF signaling cascade alone with a combination of TRAM-34 and UCL 1684 did not affect ACh-induced relaxations in both SHR and WKY rats at all ages. The NO-mediated relaxation was comparable in arteries of both SHR and WKY at all ages. However, in the combined presence of indomethacin and L-NAME, the ACh-mediated relaxation was reduced in arteries of 36 weeks old SHR and abolished at 60 weeks. In arteries of 60 weeks old WKY there was a reduction of the relaxation to acetylcholine. This EDHF-mediated response was not affected in the presence of the gap junction inhibitors, carbenoxolone and GAP-27, but, was inhibited in the presence of the Na^+/K^+-ATPase inhibitor, ouabain. These findings suggest that under control conditions NO can fully account for endothelium-dependent relaxations in mesenteric arteries of SHR and WKY rats. The endothelium releases EDHF to compensate for NO in the presence of L-NAME in young SHR and WKY rats. However, the ability of the endothelium to release EDHF is impaired by aging. In addition, the present results suggest that the Na^+/K^+ pump but not gap junctions, are involved in the actions of EDHF in the mesenteric artery of the rat. The expression and/or activity of these proteins may be reduced by aging, thus leading to an impaired compensatory EDHF-mediated relaxation in the absence of NO.

ABSTRACTS

Abstracts for Posters:

P5.

TISSUE-TYPE PLASMINOGEN ACTIVATOR: A POSSIBLE CANDIDATE OF ENDOTHELIUM-DERIVED HYPERPOLARIZATION FACTOR?

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It is well established that endothelial cells release endothelium-derived hyperpolarizing factor (EDHF), which dilates blood vessels. However, the identity of EDHF has not yet been clearly understood. Tissue-type plasminogen activator (t-PA) is a serine enzyme secreted by endothelial cells. It plays a critical role in the regulation of hemostasis by converting plasminogen into plasmin which initiates fibrinolysis and restricts propagation of the clot beyond the site of vascular injury. Besides, t-PA is used in the treatment of acute myocardial infarction because of its fibrinolytic property. Interestingly, both t-PA and EDHF are released from endothelial cells upon the stimulation by bradykinin and substance P, and the release occurs in response to the increase in intracellular calcium concentration. The aim of the present study was to investigate the vasodilatory effect of t-PA and whether this effect could account for the EDHF-dependent vasodilation. The results of tissue bath study showed that exogenously addition of t-PA elicited dilation of porcine coronary arteries in a dose-dependent manner, with an EC_{50} value of 0.008 ug/ml. The vasodilatory effect of t-PA was the same when the endothelial cells were removed, suggesting that t-PA-induced vasodilation was endothelium-independent. However, different from EDHF, the vasodilatory effect of t-PA was not affected by the pretreatment with tetraethylammomiun (a non-selective potassium channel blocker) and iberiotoxin (a large conductance calcium-activated potassium (BK_{Ca}) channel blocker). To study the EDHF response, porcine coronary arteries were pretreated with L-NAME (which blocks nitric oxide synthesis) and indomethacin (which blocks prostacyclin synthesis) before dilation by bradykinin. Our results showed that plasminogen activator inhibitor-1 (PAI-1), an inhibitor of t-PA, did not change the EDHF response. In addition, enzyme-linked immunosorbent assay (ELISA) study revealed that the endogenous release of t-PA from porcine coronary arterial endothelial cells, upon the stimulation by bradykinin and substance P, was in a negligible level and was far below the concentration which could cause vasodilation. We therefore conclude that t-PA can dilate porcine coronary arteries. However, our data do not support the hypothesis that t-PA is a candidate of EDHF.

P6.

INHIBITION OF THE RELAXATION TO CNP BY ENDOTHELIUM-DERIVED NITRIC OXIDE IN THE PORCINE CORONARY ARTERY

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C-type natriuretic peptide (CNP) is produced by endothelial cells and has been proposed as an endothelium-derived hyperpolarizing factor (EDHF). The present experiments were designed to define the mechanism underlying relaxations to CNP in coronary arteries and to determine the role of the endothelial cells in the response. Porcine coronary arteries rings were studied in organ chambers for isometric tension recording. Concentration-relaxation curves to CNP were obtained during contractions to prostaglandin $F_{2\alpha}$ or endothelin-1. The experiments were performed in the absence or presence of the inhibitor of nitric oxide synthase ω-nitro-L-arginine methyl ester hydrochloride (L-NAME), the inhibitor of cyclooxygenase indomethacin, the TP-receptor antagonist S18886 the inhibitor of soluble guanylyl cyclase ODQ, or a cell permeable analog of cyclic GMP (cGMP). In rings with, but not those without endothelium, the relaxation to CNP was potentiated by L-NAME. Incubation with ODQ and cGMP did not affect the response to CNP. Likewise indomethacin and S18886 did not affect the relaxation. These experiments suggest that in porcine coronary arteries endothelium-derived NO reduces the relaxation caused by CNP. This effect of NO is not related to inhibition of soluble guanylyl cyclase or the production of vasoconstrictor prostaglandins.

ABSTRACTS

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P7.

PPARγ AGONIST ROSIGLITAZONE AMELIORATES ENDOTHELIAL DYSFUNCTION IN TYPE II DIABETIC (DB/DB) MICE

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Peroxisome-proliferator-activated receptor (PPAR) agonists have been shown to exert beneficial effects against vascular disorders including hypertension and atherosclerosis. PPARγ activation also modulates glucose and lipid metabolism associated with type II diabetes. The present study investigated whether chronic PPARγ activation by rosiglitazone could ameliorate endothelial dysfunction in an animal model of type II diabetes, db/db mice. db/db mice were treated with rosiglitazone or vehicle for 6 weeks. Plasma glucose was monitored during the treatment. Plasma metabolic parameters including insulin, lipid, and adiponectin were determined. Vascular reactivity in isolated blood vessels was studied in myograph. Protein expressions were detected by Western blotting. Rosiglitazone treatment significantly improved endothelium-dependent relaxations in aortas, renal arteries, and resistant mesenteric arteries of db/db mice. Western blotting results demonstrate a down-regulation of angiotensin type I receptor (AT_1R) and nitrotyrosine. Rosiglitazone also increased the phosphorylation of eNOS at Ser^{1177} and phosphorylation of 5'AMP-activated protein kinase (AMPK) at Thr^{172} without affecting total eNOS or AMPK level. Rosiglitazone significantly improved glucose tolerance, reduced plasma insulin, total cholesterol, and triglyceride level. Moreover, rosiglitazone increased the plasma adiponectin level. 12 hrs treatment of adiponectin in organ culture improved endothelial function in db/db aortas, and increased phosphorylation of eNOS and AMPK. Taken together, the present results show that the vasoprotective effect of rosiglitazone in type II diabetes is mediated through reducing oxidative stress and increasing NO bioavailability, which may be related to adiponectin-AMPK signaling cascade. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme)

P8.

LACTIC-ACID-INDUCED ATP RELEASE FROM RAT SKELETAL MUSCULAR L6 CELLS IS MEDIATED BY CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) CHANNELS

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Background and Objective: We had previously shown that lactic-acid-infusion increased the interstitial ATP concentration of perfused rat skeletal muscle in-vivo, which could be inhibited by $CFTR_{inh\text{-}172}$, a specific inhibitor of CFTR. A stable rat skeletal muscular L6 cell line was used in this study to confirm that skeletal muscle cells (rather than nerve or vascular cells) were responsible for the acidosis-induced ATP release; the role of CFTR in mediating the ATP efflux during lactic acid incubation was confirmed using RNA interference (RNA_i) technology.
Methods: L6 cells transfected with or without siRNA for the *Cftr* gene were incubated with 10 μM lactic acid in the culture medium for 3 hours at 37°C. At the end of the incubation period, the cells were collected and blotted for CFTR expression by western blotting technique. HPLC was used to measure the ATP and adenosine concentrations of the collected bathing medium.
Results: CFTR expression was significantly increased in L6 cells incubated with 10 μM lactic acid, compared to the cells left untreated. Both CFTR siRNA(s), which target different sequences of the *Cftr* gene, suppressed CFTR expression in L6 cells. In L6 cells without *Cftr* gene silencing, 10 μM lactic acid incubation significantly increased extracellular ATP and adenosine concentrations to a similar extent; however when the CFTR was suppressed by CFTR siRNA, the lactic-acid-incubation-induced increases in ATP and adenosine outputs were prevented, suggesting that the increased ATP was released from L6 cells through CFTR channels during lactic acidosis, and this ATP was extracellularly converted to adenosine to increase the extracellular adenosine concentration.
Conclusions: These data confirmed that skeletal muscle cells were the source for the increased extracellular ATP during a lactic acid challenge; and this ATP efflux was mediated by CFTR channels.

ABSTRACTS

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P9.

CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 INVOLVES IN ENDOTHELIUM-DEPENDENT CONTRACTIONS IN THE AORTA OF THE SPONTANEOUSLY HUPERTENSIVE RATS

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Phospholipase A2, a regulatory enzyme found in most mammalian cells, catalyzes membrane phospholipids to arachidonic acid. There are two major cytosolic types of the enzyme, calcium-dependent (cPLA2) and calcium-independent (iPLA2) phospholipase A2. Calcium plays a crucial role in endothelium-dependent contractions. The present study investigated whether or not iPLA2 plays a role in such responses in the aorta of the spontaneously hypertensive rat (SHR). In endothelial cells, iPLA2 was densely distributed. At 10 μM, selective iPLA2 inhibitor, bromoenol lactone (BEL), at the concentration of, abrogated endothelium-dependent contractions induced by both acetylcholine and A23187. At 5 μM, only the contractions induced by acetylcholine were inhibited. Incubation with arachidonic acid methyl ester together with BEL restored the contractions. Same results can be obtained from the measurement of the release of the prostacyclin. Store-operated calcium channel (SOC) was also proved to be involved in the contraction process by using a SOC inhibitor, SKF96365. Thus, iPLA2 plays substantial role in generating endothelial-derived contracting factors and both calcium-dependent and -independent pathways areinvolved in the process.

P10.

EFFECTS OF DEXMEDETOMIDINE IN THE MESENTERIC ARTERY AND THE AORTA OF ENDOTOXIN INDUCED RAT

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Introduction: Dexmedetomidine is an anesthetic agent, with known α_2 adrenergic effects, used both in humans and in animals. The present experiments were designed to compare the vascular effects of dexmedetomidine in rat arteries during normal and septic conditions.
Methods: Ten weeks old male normal Sprague Dawley rats were used. The mesenteric arteries and thoracic aortae were dissected and suspended into 5 ml organ chambers for isometric tension recording. In some experiments, endotoxin from E-coli lipopolysaccharide (O55:B5) was injected 10 mg/kg intraperitoneally 2 or 24 hours before the start of experiments.
Results: In the mesenteric arteries, dexmedetomidine caused concentration-dependent relaxations (with a maximal response averaging 50%). It induced smaller relaxations in the rat aorta (maximal response averaging 10%). At concentrations above 100 nM, the relaxation was reverted to a concentration-dependent contraction in the mesenteric arteries. In the presence of L-NAME (a nitric oxide synthase inhibitor) and after the removal of the endothelium, the drug-induced relaxation was abolished in mesenteric arteries. The secondary contraction was reduced when prazosin (α_1 adrenergic antagonist) was added to the bath solution. After endotoxin administration, the relaxations in mesenteric arteries were reduced to around 20% in both 2 and 24 hours treatment durations. Endotoxin did not affect the magnitude of the secondary contraction, which were still abolished in the presence of prazosin.
Conclusions: The vascular effects of dexmedetomidine depend on the vascular bed studied. The vasodilatation caused by dexmedetomidine is nitric oxide and endothelium-dependent in the rat mesenteric artery. This relaxation can be reduced during sepsis, while α_1 adrenoceptors are responsible for the secondary contraction at higher concentrations of dexmedetomidine.

ABSTRACTS

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P11.

RENIN INHIBITION IMPROVES ENDOTHELIAL FUNCTION IN SPONTANEOUS HYPERTENSIVE RATS

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Aliskiren is the first orally effective renin inhibitor approved for the treatment of hypertension. The present study aims to investigate whether direct renin inhibition by aliskiren could improve the impaired endothelial function and NO bioavailability in spontaneous hypertensive rats (SHRs). SHRs and Wistar Kyoto rats (WKYs) were treated with vehicle (control) and aliskiren for 8 weeks. Blood pressure was monitored biweekly. Changes in vascular reactivity in isolated aortas and renal arteries were studied in organ bath and myograph. Protein expression of endothelial nitric oxide synthase (eNOS), angiotensin II type 1 receptor (AT_1R) and nitrotyrosine were detected by Western blot analysis. Vascular superoxide production was measured by dihydroethidium (DHE) staining. Blood pressure lowering effect of aliskiren was prominent in SHRs but not in WKYs. Aliskiren treatment improved endothelial function in SHRs by restoring the impaired endothelium-dependent relaxations to acetylcholine and decreasing the exaggerated endothelium-dependent contractions to acetylcholine in the presence of L-NAME. DHE staining and western blot on nitrotyrosine revealed the vascular superoxide anions and peroxynitrite levels were significantly lower in arteries of aliskiren-treated SHRs. The present results also demonstrate that treatment with aliskiren can restore the phosphorylation of eNOS at ser^{1177} without affecting the total eNOS level, as well as decrease the protein expression of AT_1R in SHR arteries. By contrast, aliskiren has minimal effects on WKYs. Taken together, the present study provides novel evidence demonstrating that direct renin inhibition can effectively protect endothelial function in hypertensive rats by augmenting NO bioavailability, which supports the therapeutic benefit of aliskiren in patients with hypertension. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme)

P12.

IMPROVED ENDOTHELIAL FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS STUDY OF ENDOTHELIAL NITRIC OXIDE SYNTHASE ENHANCER

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Objectives: Endothelium-derived nitric oxide (NO) plays a pivotal role in maintaining vascular homeostasis. NO-deficiency has been demonstrated in many cardiovascular disorders, including hypertension. We studied whether AVE3085, a newly developed endothelial nitric oxide synthase (eNOS) transcription enhancer, improved endothelial function in spontaneously hypertensive rats (SHR) under both acute and chronic conditions.
Methods: The isometric force study was performed with rat aortas. Protein expression of eNOS and phosphorylated eNOS (p-eNOS) was determined by Western blot. In chronic study, AVE3085 was administrated by oral gavage once daily for 4 weeks before thoracic aortas were removed. Rats receiving vehicle (5% methylcellulose) daily for 4 weeks served as control. Endothelium-dependent relaxation of rat aortas was induced by acetylcholine (ACh) in phenylephrine-precontraction (n=8).
Results: In the acute study, pretreatment with AVE3085 (10 μM) for 2 hr markedly increased the ACh-induced relaxation in the aorta of SHR (50.2±4.5% vs. 26.9±4.4%, P<0.05). eNOS and p-eNOS protein expression were significantly higher in the SHR treated with AVE3085. Four- week oral feeding of AVE3085 dramatically reduced the blood pressure in the SHR rats (170.0±4.0 vs. 151.8±1.8 mm Hg, P<0.001) with response to ACh augmented (50.1±3.4% vs. 19.7±7.0%, P<0.05) in the aorta. Compared with SHR without AVE3085 treatment, removal of endothelium and L-NAME pretreatment both enhanced phenylephrine-induced contraction in AVE-treated animals (P<0.05).
Conclusions: The present study demonstrated that AVE3085 improved the NO-related endothelial dysfunction in SHR and the functional improvement is associated with the upregulation of eNOS gene expression.
Acknowledgements: This study was supported by Hong Kong RGC grant (CUHK 4651/07M) and DUHK direct grants 2041388 &2041384.

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P13.

BONE MORPHOGENIC PROTEIN 4 INDUCES ENDOTHELIAL CELL APOPTOSIS

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Expression of bone morphogenic protein 4 (BMP4) can be induced by disturbed flow and oxidative condition in endothelial cells. BMP4 stimulates the production of reactive oxygen species (ROS) and causes endothelial cell dysfunction, leading to some inflammatory diseases, such as atherosclerosis. However, the molecular mechanism of BMP4-induced endothelial cell apoptosis was not fully understood. In this study, we investigated the signaling pathway of BMP4-induced apoptosis in primary cultured rat aortic endothelial cells (RAECs). Flow cytometry and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) were used to detect apoptosis. Protein expressions of caspase-3 and pro-caspase-3 were detected by Western blot. The production of superoxide anions was detected by dihydroethidium (DHE) staining. BMP4 induced a time- and concentration-dependent endothelial cell apoptosis through the release of intracellular superoxide anions and activation of caspase-3. SB 202190 (p38 MAPK inhibitor), SP 600125 (JNK inhibitor) but not PD 98059 (ERK inhibitor) inhibit the increase in expression of caspase-3 which demonstrates the involvement of p38 MAPK and JNK signaling in BMP4-induced apoptosis. In conclusion, the present study demonstrates that release of superoxide induced by BMP4, a novel proinflammatory factor, in RAECs may trigger p38 MAPK and JNK signalings which then activate caspase-3 and cause endothelial cell apoptosis. (Supported by GRF and CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme).

P14.

MULTIPLE ION CHANNEL BLOCK OF CHLOROFORM IS INVOLVED IN ITS ARRHYTHMOGENIC EFFECT

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Objective: Although chloroform is no longer used in clinic as an anesthetic due to its acute intoxication leading to lethal arrhythmias as well as depression of the central nervous system, it is still widely used in industrial production as organic solvent, and involved in the cases of suicide and homicide. The present study was designed to investigate the electrophysiological basis of the arrhythmogenic effect of chloroform.
Methods: Whole-cell patch clamp technique was employed to study effects of chloroform on inward rectifier K^+ channel (Kir2.1), human cardiac ether-a-go-go related (hERG) K^+ gene, Nav1.5, or pacemaker gene (HCN2) stably expressed in HEK 239 cells. Isolated rat heart was also used in this study.
Results: Chloroform showed obvious arrhythmogenic effect in isolated rat heats at a concentration of 10 mM. Although it (10 mM) had no effect on human cardiac Kir2.1 channels, chloroform inhibited the pacemaker HCN2 channel and human cardiac I_{Kr} (i.e. hERG) channels in a concentration-dependent manner, with IC_{50} of 4.57 μM and 4.29 μM respectively. The inhibition of hERG channel was recovered to 78.3% on washout. In addition, chloroform suppressed human cardiac Nav1.5 currents to 75.5%, 52.4%, and 17.2% of control at 5, 10 and 15 mM, respectively.
Conclusion: These results demonstrate that chloroform blocks multiple cardiac ion channels, I_{Kr}, I_{Na}, and the pacemaker HCN2, which likely account at least in part for the chloroform-induced lethal arrhythmias. These findings may be helpful in seeking effective treatment of acute chloroform intoxication.

P15.

PROTECTIVE EFFECT OF APIGENIN ON NEURONS AGAINST CEREBRAL ISCHEMIA/REPERFUSION INJURY VIA REGULATING THE ACTIVITY OF ATPASE

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Objectives: To determine whether apigenin, the main component of Flos Chrysanthemi, protects neurons against cerebral ischemia/reperfusion injury and its underlying mechanism.
Methods: Primary cultured hippocampal neurons were prepared from newborn Sprague-Dawley rats, and the model of oxygen-glucose deprivation/reperfusion (2 h/24 h) was used. Neuronal viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and cell injury was evaluated by lactate dehydrogenase (LDH) leakage rate. The percentage of apoptotic cells was measured by using Hoechst 33258 staining. Adult male Sprague-Dawley rats were subjected to four-vessel-occlusion for 10 min followed by reperfusion for 24 h, and the activities of Na^+, K^+-ATPase and Ca^{2+}, Mg^{2+}-ATPase were measured by spectrophotometry.
Results: Oxygen-glucose deprivation/reperfusion decreased the cell viability and increased LDH leakage rate and percentage of apoptotic cells. Compared with oxygen-glucose deprivation/reperfusion group, apigenin (1-100 μmol/L) treatment significantly increased the cell viability, decreased LDH leakage rate and the percentage of apoptotic cells in a dose-dependent manner. Apigenin (200 $mg \cdot Pkg^{-1}$, *i.p.*) markedly inhibited the decrease of ATPase activities induced by global cerebral ischemia/reperfusion.
Conclusion: Apigenin protects neurons against ischemia/reperfusion-induced cell injury via, at least partly, regulating the activity of ATPase.

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P16.

INVOLVEMENT OF ION CHANNELS IN PROLIFERATION OF HUMAN CARDIAC FIBROBLASTS

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Objective: Cardiac fibroblasts play a central role in the maintenance of extracellular matrix in the normal heart and as mediators of inflammatory and fibrotic myocardial remodeling in the injured and failing heart. Excessive fibroblast proliferation and increase in the extra-cellular matrix will increase myocardial stiffness and cause ventricular dysfunction and subsequent heart failure. Our previous study demonstrated that four types of ionic currents, I_{KDR} (voltage-gated delayed rectifier K^+ current), I_{KCa} (big conductance Ca^{2+} -activated K^+ current), $I_{Cl.vol}$ (volume-activated chloride current), and I_{Na} were present in cultured human cardiac fibroblasts. Little is known about the functional involvement of these ion channels in cardiac fibroblasts, and the present study was therefore designed to examine the possible involvement of I_{KDR}, I_{KCa}, $I_{Cl.vol}$, and I_{Na} in proliferation of human cardiac fibrioblasts.
Methods and results: Using MTT assay, we found that cell proliferation of human cardiac fibroblasts was remarkably suppressed the I_{KDR} blocker 4-aminopyridine (3.0 mM, by 27.2%, $P<0.05$ vs vehicle control), the specific big conductance I_{KCa} blocker paxilline at 1 and 3 μM (by 12.0% and 58.4%, $P<0.05$ vs control), and the volume-regulated chloride channel blocker NPPB (200 μM, by 12.1%, $P<0.01$) or DIDS (200 μM, by 25.9%, $P<0.01$ vs control) with 48 h incubation. However, sodium channel blocker, TTX (1 and 10 μM), had no significant effect on proliferation of human cardiac fibroblasts.
Conclusion: These results demonstrate that IKDR, big conductance I_{KCa} and $I_{Cl.vol}$, but not I_{Na}, regulate the proliferation of human cardiac fibroblasts. The further study will be performed using specific siRNAs targeting to specific ion channel genes to investigate how these channels modulate cell cycle progression and whether proliferation-related kinases are affected by these interventions.

P17.

ROLE OF MONOAMINE OXIDASES IN THE EXAGGERATED 5-HT-INDUCED TENSION DEVELOPMENT IN VITRO OF HUMAN PRE-ECLAMPTIC UMBILICAL ARTERY

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We investigated the role(s) of monoamine oxidases (MAOs) on the altered 5-hydroxytryptamine (5-HT)-induced tension development of the isolated umbilical artery of preeclamptic (PE) pregnancy of Chinese women. An enhanced 5-HT-induced tension development of the umbilical artery of PE pregnancy was observed when compared with that of normal pregnancy. The enhanced component of 5-HT-induced tension development was eradicated by clorgyline (a MAO_A inhibitor). Blockade of eNOS (N^{ω}-nitro-L-arginine methyl ester), 5-HT transporter (citalopram) and 5-HT receptor subtypes ($5HT_{2B}$, SB 204741; $5\text{-}HT_{2C}$, RS 102221; $5\text{-}HT_7$, SB 269970) of the umbilical artery of normal pregnancy mimicked the enhanced 5-HT-induced tension development as observed in the PE tissues. In contrast, no apparent changes in 5-HT-induced tension development of the umbilical artery of PE pregnancy were observed with the same pharmacological manipulations. A decreased protein expression levels of MAO_A and eNOS (no iNOS expression was detected), and an increased expression of PTEN were demonstrated in the umbilical artery (endothelium intact) lysate of PE pregnancy, compared to that of the umbilical artery of normal pregnancy. Thus, in the umbilical artery of PE pregnancy, a decrease of MAO_A and eNOS protein expression levels is probably associated with, or responsible for, the exaggerated 5-HT-induced tension development.
Acknowledgements: This project was financially supported by Direct Grants for Research (The Chinese University of Hong Kong) (Reference no.: 2007. 1.079).

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P18.

EFFECTS OF HIGH GLUCOSE (25 MM) ON HMG COA REDUCTASE AND CAVEOLIN-1 PROTEIN EXPRESSION IN PORCINE ISOLATED PANCREATIC ISLETS

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Recent studies have demonstrated that inhibition of HMG CoA reductase by statins inhibited the glucose-induced insulin release in various animal models, and uses of statins provide beneficial outcomes in diabetic patients. However, the biochemical existence of HMG CoA reductase in the pancreas, and the effects of hyperglycaemia on HMG CoA reductase are unknown. In this study, we hypothesised that glucose levels modulate the expression of HMG CoA reductase and caveolin-1 in porcine isolated pancreatic islets. Fresh pancreatic islets were harvested and incubated in medium supplemented with normal (5 mM) or high (25 mM) glucose for 24 and 48 hr before subjecting to Western blot analysis. Our results demonstrate, for the first time, the expression of HMG CoA reductase in porcine isolated pancreatic islets. Under high glucose (25 mM) conditions (48 hr), a significant increase and decrease of the expression of HMG CoA reductase and caveolin-1, respectively, were observed. In contrast, there was apparent change in the expression of HMG CoA reductase and caveolin-1 of the pancreatic islets incubated under the normal glucose (5 mM) conditions (24 and 48 hr). Thus, our results suggest that hyperglycaemia has significant modulatory effects on HMG CoA reductase expression/functions of porcine pancreatic islets. The physiological significance of HMG CoA reductase in diabetes mellitus remains to be determined.

Acknowledgements: This project is financially supported by RGC Earmarked Grant of Hong Kong (Project code: 2140565).

P19.

SIMVASTATIN SUPPRESSES CYTOKINES-INDUCE INOS EXPRESSION IN PORCINE ISOLATED PANCREATIC ISLETS

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Inflammation is important in the pathophysiology of diabetes mellitus. Inflammatory cytokines such as IL-1β, TNF-α and INF-γ induced iNOS expression and inhibited insulin secretion from the pancreatic islets of various animal models. Statins (HMG CoA reductase inhibitors) possess anti-inflammatory effects but the effects of statins on the inflammatory cytokines-induced iNOS expression of the pancreatic islets are unknown. In this study, we hypothesised that simvastatin (a HMG CoA reductase inhibitor) modulates the cytokines-induced iNOS expression of freshly harvested porcine isolated pancreatic islets. The harvested pancreatic islets were incubated with or without IL-1β (10 ng/ml) and TNF-α (10 ng/ml), alone or in combination, for 24 hr. In some preparations, the islets were pre-incubated with simvastatin (10 μM; 60 min) before the addition of cytokines. The protein expression of iNOS of the pancreatic islets was evaluated and compared. IL-1β and TNF-α, applied alone, failed to induce iNOS protein expression. In contrast, a combination of IL-1β and TNF-α markedly induced iNOS expression which was significantly suppressed by simvastatin. Thus, we have demonstrated that a combination of IL-1β and TNF-α regulated iNOS expression of porcine isolated pancreatic islets which was sensitive to the presence of simvastatin. Acknowledgements: This project is financially supported by RGC Earmarked Grant of Hong Kong (Project code: 2140565).

ABSTRACTS

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P20.

PROTECTIVE EFFECTS OF RALOXIFENE AND ESTROGEN ON RAT PROSTATE ENDOTHELIAL CELL LINE YPEN-1 DAMAGED BY HYPOXANTHINE-XANTHINE OXIDASE (HXXO) OXYGEN RADICAL DONOR SYSTEM

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Reactive Oxygen Species (ROS) have been traditionally regarded as toxic by-products of aerobic metabolism. ROS, however, also act as intracellular signaling molecules and can mediate phenotypes in vascular endothelial cells, which may be physiological or pathological in nature. Estrogen has been demonstrated to protect different types of cells from apoptosis induced by various substances. Selective estrogen receptor modulators (SERMs) such as raloxifene are compounds that have both estrogen agonistic and estrogen antagonistic properties. They can function in the same way as estrogen in some tissues (e.g. bone) and more like antiestrogen in some other tissues (e.g. breast) and have important clinical applications. This study is to examine the effects of estrogen and raloxifene on the HX-XO (hypoxanthine-xanthine)-induced death of rat prostate endothelial cell line YPEN-1 and the mechanism of its protective effects. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay showed that in the control group, cell survival rate decreased significantly. After DAPI staining, in the estrogen and raloxifene-pretreated group the number of cells with apoptotic morphology decreased significantly. The mechanism of estrogen and raloxifene protection was studied using two-dimensional gel electrophoresis coupled with mass spectrometry. In comparison with controls cells, the differential proteomic analysis of YPEN-1 treated by HX-XO revealed the variation of some proteins such as rab GDP dissociation inhibitor β-2, histone H2B, alcohol dehydrogenase, peroxiredoxin, adenylate kinase isoenzyme, dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, protein disulfide-isomerase A6 and peptidyl-prolyl cis-trans isomerase A. Raloxifene and estrogen were shown to restore the altered proteins to the control level. These proteins induced by estrogen or raloxifene might play an important role in protecting endothelial cells from oxidative stress-induced cellular damage. (Supported by GRF grant, CUHK Li Ka Shing Institute of Health Sciences and CUHK Focused Investment Scheme).

P21.

INHIBITORY EFFECTS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) ON NUCLEOSIDE TRANSPORTERS IN HUMAN AORTIC SMOOTH MUSCLE CELLS

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Objective: It is known that the equilibrative nucleoside transporters (ENTs) play an important role in adenosine functions because they fine-tune the extracellular concentrations of adenosine. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of pain and inflammation. NSAIDs affect vascular functions due to their inhibitory effect on cyclooxygenase, which attenuates the synthesis of vasoactive prostanoids. In the present study, we sought to investigate whether or not NSAIDs could act on ENTs in vascular smooth muscle cells.

Methods: The ENT activity in human aortic smooth muscle cells (HASMCs) was determined by measuring the initial rate of [^{3}H]adenosine uptake. Since ENTs can be subdivided into ENT-1 and ENT-2, the effects of NSAIDs on these specific isoforms will also be studied using PK15NTD/ENT-1 and PK15NTD/ENT-2 cells.

Results: The effects of different NSAIDs (100 μM) on [^{3}H]adenosine uptake were screened. Aspirin and naproxen had no effect on [^{3}H]adenosine uptake. Etodolic, ibuprofen and ketoprofen inhibited [^{3}H]adenosine update by 15% while indomethacin, mefenamic acid and piroxicam inhibited adenosine uptake by 30%. Sulindac inhibited [^{3}H]adenosine uptake by 20% but its active metabolites sulindac sulfide and sulindac sulfonate inhibited [^{3}H]adenosine uptake by 80% and 30%, respectively. Sulindac sulfide inhibited [^{3}H]adenosine uptake in PK15NTD/ENT-1 cells with IC_{50} values of 22.6 ± 3.35 μM and kinetic study revealed that sulindac sulfide was a competitive inhibitor of ENT-1. In contrast, sulindac sulfide did not affect the [3H]adenosine uptake in PK15NTD/ENT-2 cells.

Conclusions: Among the NSAIDs studied, sulindac sulfide appears to be the most potent in inhibiting the ENT-1-mediated adenosine uptake in HASMCs. It suggests that sulindac sulfide may affect the vascular functions through its potential effect on regulating the availability of adenosine in the vicinity of adenosine receptors.

ABSTRACTS

Abstracts for Posters:

P22.

EFFECT OF 2',3'-DIDEOXYADENOSINE ON THE RELAXATION OF RAT BASILAR ARTERIES

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Objective: 2',3'-Dideoxyadenosine (ddA) is a nucleoside analogue, which is used as an antiviral drug for HIV-infected patients. At the same time, ddA is an inhibitor of adenylyl cyclase. It has been suggested that ddA reduces vasodilation in rabbit iliac arteries because ddA decreases cAMP level in endothelial cells, which in turn inhibits gap junction and reduces the endothelium-dependent hyperpolarization factor (EDHF) response (Griffith et al., 2004). However, evidence has demonstrated that EDHF responses in other vascular beds are not necessarily linked to gap junction. Therefore, it is hypothesized that ddA may show different effects on different types of blood vessels.
Methods: The effect of ddA on rat basilar arteries was investigated using wire myograph.
Results: Pre-incubation of ddA did not affect the acetylcholine-induced endothelium-dependent relaxation of basilar arteries. In contrast, ddA itself caused relaxation of basilar arteries in a dose-dependent manner (2 x 10-6-2 x 10-4 M). The vasorelaxing effect of ddA was unaffected even the endothelium was removed. In addition, although ddA is an adenosine analogue, the vasorelaxing effect of ddA was not inhibited by ZM 241385 (1 x 10-6 M, an adenosine receptor blocker).
Conclusion: The present findings have demonstrated that the effect of ddA on rat basilar arteries is distinct from that on rabbit iliac arteries. ddA reduces vasodilation in rabbit iliac arteries but exerts vasorelaxing effect on rat basilar artery through an endothelium-independent and adenosine receptor-independent mechanism.
Reference: Griffith TM, Chaytor AT, Edwards DH, Daverio F, McGuigan C. Enhanced inhibition of the EDHF phenomenon by a phenyl methoxyalaninyl phosphoramidate derivative of dideoxyadenosine. Br J Pharmacol 142: 27-30; 2004.

P23.

MOLECULAR MECHANISM AND CHARACTERIZATION OF MATERNALLY INHERITED ESSENTIAL HYPERTENSION

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Objective: We aimed to observe the relationship between the mitochondrial tRNA mutation and the essential hypertension by examining the mutation of four tRNA ($tRNA^{Lys}$, $tRNA^{Ile}$, $tRNA^{Gln}$ and $tRNA^{Met}$). We also wanted to explore the inherited signs and clinical characters of maternally inherited essential hypertension.
Methods: We collected the data of general information, blood routine test, blood biochemical examination and color Doppler echocardiography examination of the subjects. We extracted DNA from subject's white blood cell, and amplificated the target fragment using the special primers. We then purified the PCR products, and then we directly sequenced them. At last, we analysised the sequencing results and blasted it on net. We also made a comparative analysis of the collected data of the essential hypertension subjects who carried tRNA mutation and those who did not carry mutation using the methods of 1:1 case-control study.
Results: (1) From the mutation analysis of mitochondrial DNA of 2,000 essential hypertensive subjects, we totally found 26 mutation sites in 57 subjects, and 22 mutation sites were new. The most frequently occurrence of the mutation site was A4386G in tRNAGln gene, next to this was G4394A in the same tRNA gene. (2) The onset ages of the individuals carrying the mutation were earlier than those who did not bear them, which was not associated with the change of body mass index. (3) tRNA mutations significantly affected serum lipids, blood electrolyte, blood creatinine, blood urea nitrogen and heart structure and function, and different tRNA mutations produced different effections. (4) Most essential hypertensive patients had maternally inherited history, which fulfilled the feature of mitochondrial hereditary.
Conclusion: 1. Mitochondrial tRNA mutations might result in the change of their structure and function, and then damaged the blood metabolism, the balance of the blood electrolyte, the steady-state of the blood cells and the heart structure and function, which were involved in the progress of the essential hypertension. (2) Part of the essential hypertension patients clinically presented the characters of maternal inheritance, which might be associated with the tRNA mutation.

ABSTRACTS

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P24.

UP-REGULATION OF ENDOGENOUS NITRIC OXIDE PRODUCTION IN RAT ADRENAL GLAND IN CHRONIC HYPOXIA

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Background: Adrenal gland is an effector of the sympathetic nervous system, which plays an important role in the cardiovascular response to hypoxia. We have shown the endogenous nitric oxide (NO) produced by NO synthases (NOS) is increased by hypoxia in oxygen-sensitive tissues. Yet, there is a paucity of information on the adrenal NO production under chronically hypoxic (CH) conditions simulating in subjects sojourning to high altitude or patients with chronic cardiopulmonary diseases.
Hypothesis: CH increases the endogenous NO production and the NOS expression in adrenal gland.
Methods: Normoxic (N) and CH rats were exposed to air and 10% O_2 for 7 days, respectively. The level of endogenous NO was measured by electrochemical microsensor placed on the surface of superfused adrenal gland slices. The expression of NOS in adrenal gland was determined by RT-PCR and Western Blot. RESULTS: L-arginine (Arg, 1 mM) increased the adrenal NO level. The Arg-induced NO elevation was significantly more in the CH group than that of the N group. By contrast, the endogenous NO level was decreased by NOS inhibitor L-NMMA (100 μM). The effect of L-NMMA on the endogenous NO production in CH slices was more significant than that of the N group. Moreover, the mRNA and protein expression of NOS in adrenal gland was also increased in the CH group. Comparable results were observed in PC12 cells.
Summary: Chronic hypoxia upregulates the NOS expression and endogenous NO production in rat adrenal glands. The elevated NO production in the adrenal gland may play patho- or physiological roles in the activation of sympathetic-adrenal axis in responding to chronic hypoxia.

P25.

VOLTAGE-DEPENDENT ANION CHANNEL IS INVOLVED IN THE APOPTOSIS OF CELL LINES CARRYING MITOCHONDRIAL DNA A4263G MUTATION

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In this report, we studied the effect of voltage-dependent anion channel (VDAC) contributed to the apoptosis of the cell lines carrying mitochondrial DNA A4263G mutation. We established lymphoblastoid cell lines derived from 3 symptomatic and 1 asymptomatic hypertension individuals in the family carrying A4263G mutation compared with 3 control cell lines. The mitochondrial potential ($\Delta\Psi m$) was detected by flow cytometry and the co-localization of VDAC and Bax was evaluated by confocal laser scanning microscopy. The results showed that the expression of VDAC and Bax of the lymphoblastoid cell lines in individuals carrying mtDNA A4263G mutation increased compared with control group, while the expression of small conductance calcium dependant potassium (sK_{Ca}) had no change. The confocal imaging showed co-localization of VDAC/Bax on the outer membrane of mitochondrial of the cell lines from individuals carrying mtDNA A4263G mutation, while the interaction was not seen on control group. Flow cytometry showed mitochondrial potential of cell lines from individuals carrying mtDNA A4263G mutation decreased 32% compared with control group ($P<0.05$) and this difference was attenuated by Cyclosporin A (CsA, 2μM), a blocker of VDAC. In conclusion, the change of expression of mitochondrial VDAC and subcellular co-localization of VDAC/Bax leads to the significant increase of mitochondrial permeability and apoptosis of cell lines carrying mtDNA A4263G mutations.

ABSTRACTS

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P26.

RELATIONSHIP BETWEEN QRS TIME AND LONG-TERM EFFECT OF VENTRICULAR RESYNCHRONIZATION IN ELDERLY

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Objective: The purpose of the study is to explore the relationship between QRS time and long-tern effect of ventricular resynchronization in the treatment of congestive heart failure in elderly.
Methods: Consecutive elderly patients with congestive heart failure were selected with the criteria of: (1) Sinus rhythm. (2) NYHA 3-4 class. (3) LVEF≤40%. (4) LVED ≥55mm. (5) delta ventricular ejection time≥30ms. (6) moderate mitral insufficiency. (7) refractory to conservative medication. Patients were divided into wide (>120 ms) and narrow (≤120 ms) QRS group according to the QRS time on surface electrocardiogram. The parameters of procedure, pacing and follow-up between two groups were comparable (P<0.05).
Results: Twenty-seven elderly patients (male 21 cases, mean age 67.3± 5.6years) were enrolled. Sixteen and eleven patients were divided into wide and narrow QRS groups respectively (176.8±13.2ms vs116.5±8.6ms). Twenty-seven tri-chamber pacemakers were successfully implanted without mortality and complications. After procedure, QRS time was shortened by 31.2±5.7ms in wide QRS group and prolonged by 11.4±3.2ms in narrow QRS group. Two groups were followed by 15.4±3.8 months with event free. Compared with pre-procedure, both groups had significant improvement in NYHA, LVEF, LVED and delta ventricular ejection time (P<0.05). There were no significant different between two groups in above parameters during follow-up (P>0.05).

	NYHA(class)		LVEF(%)		LVED(mm)		Delta ejection time (ms)	
	Control	F/U	Control	F/U	Control	F/U	Control	F/U
Wide QRS	3.3±1.6	2.5±1.2	31.9±7.2	47.3±8.6	62.8±5.7	47.1±4.9	46.6±8.9	33.6±5.1
Narrow QRS	3.1±1.5	2.6±1.7	34.2±6.5	45.8±7.7	63.2±5.3	46.3±5.2	45.7±6.1	31.4±3.9
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Conclusions: Ventricular resynchronization has long-term improvement effect in elderly patients with congestive heart failure and selected by delta ventricular ejection time. QRS time in surface electrocardiogram has no influence on this long-term effect.

P27.

ANTIARRHYTHMIC EFFECT OF ETHYL ACETATE EXTRACT FROM FLOS CHRYSANTHEMI ON RATS

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Objective: To investigate the effect of ethyl acetate extract from Flos Chrysanthemi (EFC) on experimental arrhythmia induced by ischemia/ reperfusion or aconitine in rats and its underlying mechanisms.
Methods: Arrhythmia model in intact rat was induced by aconitine (30 μg/kg body weight, *i.v.*). In isolated Langendorff-perfused rat hearts, regional ischemia and reperfusion was induced by ligation (for 30 min) and release (for 15 min) of left anterior descending artery. The effect of EFC on ventricular fibrillation threshold (VFT), effective refractory period (ERP), and diastolic excitation threshold (DET) in rat heart was measured. The action potentials of papillary muscle in rat right ventricle were recorded by conventional glass microelectrode technique and four parameters including resting potential (RP), amplitude of action potential (APA), maximal velocity of phase 0 depolarization (V_{max}), and action potential duration at 90% repolarization (APD_{90}) were measured.
Results: We found that (1) EFC significantly decreased the number and duration of ventricular tachycardia (VT), delayed the occurrence of ventricular premature beats (VPB) and VT induced by aconitine. Arrhythmia score of the EFC group was lower than that in aconitine-treated group. (2) EFC markedly prolonged the ERP and increased the VFT in the isolated perfused rat hearts during ischemia and reperfusion, but did not affect the DET. (3) EFC significantly decreased V_{max}, prolonged APD_{90}, but had no effect on RP and APA in papillary muscle from the right ventricle compared with ischemia/ reperfusion group.
Conclusion: EFC antagonizes the arrhythmia induced by aconitine, and decreases the vulnerability of I/R myocardium, which may be mediated by the inhibition of Na^+ influx and Na^+ channel inactivation kinetics and the decrease of K^+ efflux during repolarization, thus increasing myocardial electrophysiological stability.

ABSTRACTS

Abstracts for Posters:

P28.

FUNCTIONAL EXPRESSION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID-RELATED CHANNELS IN CHRONICALLY HYPOXIA HUMAN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS

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Transient receptor potential vanilloid (TRPV)-related channels are nonselective cation channels pertinent to diverse physiological functions. Multiple TRPV channel subtypes have been identified in different tissues and cloned. The aim of this study was to investigate the role of TRPV channels in hypoxia-induced proliferation of human pulmonary artery smooth muscle cells (PASMCs) and its possible signal pathway. RT-PCR, real-time PCR and Western blot were used to detect the expression of TRPV in human PASMCs. Cell number was determined with a hemocytometer. Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) was measured using a dynamic digital Ca^{2+} imaging system. The mRNA of TRPV1-4 was detected in human PASMCs and chronic hypoxia up-regulated expression levels of the TRPV1 gene and protein. The ability to proliferate, the resting $[Ca^{2+}]_{cyt}$, and CPA-induced capacitative Ca^{2+} entry in human PASMCs were enhanced significantly by chronic hypoxia compared with the control, and these effects were inhibited in a dose-dependent manner by capsazepine, a TRPV1 channel inhibitor. These results suggested that TRPV1 may be a critical pathway or mediator in chronic hypoxia-induced proliferation of human PASMCs.

P29.

A STUDY ON MATERNALLY INHERITED HYPERTENSION AND MITOCHONDRIAL DNA POINT MUTATION A4263G IN A LARGE CHINESE FAMILY

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Objective: To find a novel mitochondrial DNA point mutation A4263G, we characterized clinically and evaluated hereditarily a large Chinese family with the characteristics of maternally inherited hypertension.
Methods: The mitochondrial DNA point mutation A4263G 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 of this family.
Results: All the members with mitochondrial DNA point mutation A4263G were maternal members, a finding consistent with the maternal inherited characteristics. The morbidity of hypertension in the maternal members is up to 53.8%, while that in the nonmaternal members is only 11.8% ($P<0.01$); the onset age of hypertension is tend to be advanced (from 64.3±5.0 y to 23.3± 2.9 y); the levels of blood glucose, total cholesterol and sodium of maternal members were different with those of nonmaternal members ($P<0.05$), while the results of echocardiogram has no difference between two groups. Finally, the blood pressure of maternal members was relevant with age, smoking, height and high salt diet.
Conclusions: By far all findings, including the same mitochondrial DNA point mutation in all maternal individuals and clear pattern of maternal inheritance, suggested mitochondrial DNA point mutation may be associated with hypertension and play an important role in onset of hypertension.

ABSTRACTS

Abstracts for Posters:

P30.

ANTIARRHYTHMIC EFFECT OF ATORVASTATIN ON ISCHEMIA/REPERFUSION RATS

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Objective: To investigate the effect of Atorvastatin (Ator), a traditional blood-lipid lowering drugs, on arrhythmia induced by ischemia/reperfusion (I/R) in SD rats and to explore its underlying mechanisms.

Methods: In isolated Langendorff-perfused rat hearts, arrhythmia was produced by regional ischemia and reperfusion via ligation (for 30 min) and release (for 15 min) of left anterior descending artery. The effect of Ator on ventricular fibrillation threshold (VFT), effective refractory period (ERP), and diastolic excitation threshold (DET) in rat heart were measured.

Results: We found that 10 μmol/L Ator significantly decreased the number and duration of ventricular tachycardia (VT), delayed the occurrence of ventricular premature beats (VPB) and VT induced by ischemia/reperfusion. Ator also prolonged the ERP and increased the VFT (P<0.05, 0.01 vs. I/R group) in the isolated perfused rat hearts during ischemia and reperfusion, but did not affect the DET. However, L-NAME cancelled these antiarrhythmic effects induced by Ator.

Discussion: The results suggest that Ator exerts its antiarrhythmic effects against ischemia/reperfusion. This antiarrhythmic mechanism may be related to that Ator could prolong the ERP and increase VFT to enhance the cardiac electrophysiological stability through NO pathway. However, the precise mechanism of NO involving in the antiarrhythmic effect of Ator against ischemia/reperfusion needs to be further explored.

P31.

W-7, A CALMODULIN ANTAGONIST, DIRECTLY BLOCKS HUMAN ETHER A-GO-GO RELATED GENE POTASSIUM CHANNELS STABLY EXPRESSED IN HEK 293 CELLS

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W-7 is a well-known calmodulin antagonist and believed to be a potential anti-arrhythmic agent. The purpose of the present study was to determine whether W-7 would block human ether a-go-go-related gene (hERG or Kv11.1) potassium channel and human Kv1.5 and Kir2.1 channels. Whole-cell patch voltage-clamp technique was used to record membrane currents in HEK 293 cells expressing hERG, hKv1.5, or hKir2.1 gene. We found that W-7 blocked hERG current (I_{hERG}) in a concentration-dependent manner (IC_{50}: 3.5 μM). Blockade of hERG channels showed a closed channel blocking property. Steady-state activation $V_{0.5}$ of hERG channels was negatively shifted by 9.3 mV (from -5.1±1.3 mV of control to -14.4±1.9 mV, n=11, P<0.01), while inactivation V0.5 was negatively shifted by 9.9 mV (from -57.5±2.4 mV of control to -67.4±2.7 mV, n=7, P<0.01) with application of 3 μM W-7. The S6 mutant Y652A, F656V and the pore helix mutant S631A had a reduced channel block by W-7, and IC_{50} was 5.5 μM, 12.0 μM, and 24.6 μM, respectively. In addition, W-7 inhibited human Kv1.5 channel (IC_{50}: 6.5 μM) and human Kir2.1 channel (IC_{50}: 15.1 μM). The block also showed a closed channel blocking property. These results indicate that W-7 is a multiple-channel blocker by binding to the closed channels of hERG, hKv1.5 and Kir 2.1. The concentrations of ion channel blockage are lower than that of calmodulin inhibition; thus, caution should be taken when it is used as a calmodulin inhibitor or a potential anti-arrhythmic agent.

ABSTRACTS

Abstracts for Posters:

P32.

EGFR KINASE REGULATES HUMAN CARDIAC NA(V)1.5 CURRENTS

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Nav1.5 is the pore-forming a-subunit protein of the cardiac sodium channels which plays a pivotal role in the initiation and propagation of the cardiac action potential. It is generally believed that cardiac sodium current (I_{Na}) is regulated by protein phosphorylation. The present study was designed to determine whether protein tyrosine kinases (PTKs) regulate human cardiac Nav1.5 channels stably expressed in HEK 293 cells using a whole-cell patch clamp technique.

It was found that human cardiac INa was enhanced by epidermal growth factor (EGF, 100 ng/ml) or the protein tyrosine phosphatases (PTPs) inhibitor orthovanadate (1 mM). The selective EGFR kinase inhibitor AG556 (5 μM) remarkably inhibited INa amplitude, shifted the inactivation voltage toward negative potentials, and slowed the recovery of INa from inactivation These effects were countered by orthovanadate. However, insulin and the Src-family tyrosine kinase inhibitor PP2 had no significant effect on I_{Na}.

These results suggest that EGFR kinase (but not Src-family kinase) and PTPs regulate human cardiac Nav1.5 channels stably expressed in HEK-293 cells. EGFR kinase positively, while PTPs negatively modulates the channels. Additional experiments are required to confirm tyrosine phosphorylation level of Nav1.5 using immunoprecipitation and Western blot analysis and to find out the tyrosine phosphorylation site(s) of Nav1.5 using site-directed mutagenesis.

P33.

CARDIOPROTECTIVE OF INTERMITTENT HYPOBARIC HYPOXIA AGAINST ISCHEMIA/REPERFUSION INJURY IN RAT HEART

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Abstract: The aim of this study was to investigate the mechanisms of cardioprotection against ischemia/reperfusion-induced injury in rat hearts adapted under intermittent hypobaric (IHH) hypoxia. Adult male SD rats were put into the hypobaric chamber (simulated 5000m) for 6h daily, lasting 42 days. Isolated hearts were subjected to 30 min global ischemia followed by 30 or 120 min reperfusion. Glibenclamide, 5-hydroxydecunoate or pinacidil were administrated before ischemia. Cardiomyocytes $[Ca^{2+}]_i$ were measured using a digital CCD. The activation and translocation of PKC-α, ε, and δ isozymes, Bax and Bcl-2 were examined by Western Blotting; Incidence of apoptosis in cardiomyocytes was determined by TUNEL.

Results: Post-ischemic functional recovery of LVDP and ±dp/dtmax were better in IHH hearts. [Ca2+]i in cardiomyocytes from normoxic hearts gradually increased during ischemia and kept at higher level during reperfusion. However, in cardiomyocytes isolated from IHH hearts, $[Ca^{2+}]_i$ kept at lower level during ischemia and reperfusion. Glibenclamide and 5-hydroxydecunoate respectively abolished this effect. However, they had no effects on nomoxic myocytes. Pinacidil attenuated calcium overloading during ischemia and reperfusion in normoxic myocytes, but had no effect on IHH myocytes. IHH up-regulated the baseline protein expression of particulate fraction of PKC-α, ε, and δ. Ischemia and reperfusion induced the particulate/cytosolic ratios of PKC-α, ε, in IH hearts was higher than those of normoxic hearts, and the particulate/cytosolic ratio of PKC-δ, in IH hearts was higher than that of normoxic hearts during ischemia period. Ischemia/reperfusion-induced apoptosis was significantly reduced in IH group. After ischemia/reperfusion, the expression of Bax in both cytosolic and membrane fractions were decreased, and the expression of Bcl-2 in membrane fraction was upregulated in IHH hearts.

Conclusions: The results indicated that KATP channels and PKC contributed to the cardiaoprotection afforded by IHH against ischemia/reperfusion injury. The elimination of calcium overload might underlie the mechanism of cardioprotection. IHH attenuated ischemia/reperfusion-induced apoptosis via increasing the ratio of Bcl-2/Bax in membrane fraction. (The study was supported by grants: No.30393130; 2006CB504100)