Journal of the Hong Kong College of Cardiology

Volume 19
Issue 2 *October*

Article 1

Fifteenth Annual Scientific Meeting-Institute Of Cardiovascular Science And Medicine

Recommended Citation
Fifteenth Annual Scientific Meeting-Institute Of Cardiovascular Science And Medicine *Journal of the Hong Kong College of Cardiology* 2011;19(2) https://doi.org/10.55503/2790-6744.1062

Journal of the Hong Kong College of Cardiology

Journal of the Hong Kong College of Cardiology (ISSN 1027-7811) is published bi-yearly by **Medcom Limited**, Room 504-5, Cheung Tat Centre, 18 Cheung Lee Street, Chai Wan, Hong Kong, tel no. (852) 2578 3833, fax no. (852) 2578 3929.

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Journal of the Hong Kong College of Cardiology

October 2011
Volume 19, No. 2

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Prognostic Value of N-terminal Pro-brain Natriuretic Peptide in Risk Stratification of Non-ST Elevation Myocardial Infarction / Unstable Angina

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CHAN ET AL.: ***Prognostic Value of N-terminal Pro-brain Natriuretic Peptide in Risk Stratification of Non-ST Elevation Myocardial Infarction / Unstable Angina.*** *The study evaluated the prognostic value of N-terminal pro-Brain Natriuretic Peptide (NTproBNP), and its incremental predictive power over traditional prognostic indicators, in the risk stratification of Non-ST elevation myocardial infarction / unstable angina (NSTEMI/UA). One hundred and seventy-seven NSTEMI/UA patients were recruited. NTproBNP level was obtained within 72 hours after index admission. Patients were followed for up to 1 year. The primary composite endpoint was defined as all-cause mortality, myocardial infarction (MI) / unstable angina (UA), and congestive heart failure (CHF) requiring hospitalization. At 1-year follow-up, patients with NTproBNP >331 ng/L had higher rate of primary composite endpoint, total mortality, MI/UA and CHF (35.4%, 11.1%, 19.2%, and 15.2% respectively) than those with NTproBNP≤331 ng/L (10.3%, 1.3%, 10.3%, and 0% respectively). NTproBNP cutoff of >331 ng/L was the independent predictor of composite endpoint at 1 year (HR 3.4; CI 1.6-7.5; p=0.002). In patients who were classified as low risk by traditional prognostic markers, the NTproBNP>331 ng/L also predicted a trend towards more adverse clinical outcome. In conclusion, in patients with NSTEMI/UA, NTproBNP cutoff of >331 ng/L was an independent prognostic marker in prediction of all cause mortality, MI/UA, and CHF requiring hospitalization at 1 year. It provided incremental and independent prognostic value over traditional prognostic markers. Measurement of NTproBNP in NSTEMI/UA patients should be considered for early risk stratification. (J HK Coll Cardiol 2011;19:45-56)*

NSTEMI, NTproBNP, Risk stratification, UA

摘要

此項研究旨在評估氨基末端前腦利納肽（NTproBNP）在非ST段抬高型心肌梗死/非穩定性心絞痛（NSTEMI/UA）中的預後價值，及其相比傳統預測指標而言所擁有的更強的預測力。共招募177位NSTEMI/UA患者，篩選入組72小時內查得其NTproBNP值，隨訪1年。基礎複合終點定義爲所有原因導致的死亡，心肌梗死（MI）/非穩定性心絞痛（UA）及需住院治療的充血性心力衰竭（CHF）。到1年的隨訪期時，NTproBNP>331 ng/L的患者在基礎複合終點，總死亡率，MI/UA和CHF中所佔比例（分別爲35.4%，11.1%，19.2%和15.2%）均高於那些NTproBNP≤331 ng/L的患者（分別爲10.3%，1.3%，10.3%和0%）。在隨訪1年時計算複合終點，臨界值NTproBNP>331 ng/L可作爲獨立的預測指標（HR 3.4；CI 1.6-7.5；p=0.002）。在那些傳統預測指標分類爲低危險性的患者中，其NTproBNP>331 ng/L同樣

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Received June 9, 2011; revision accepted July 15, 2011

能提示其趨向於不利的臨床預後。總而言之，在NSTEMI/UA患者中，隨訪1年時臨界值NTproBNP>331 ng/L可作爲一項獨立預測因數判斷所有原因的死亡率，MI/UA及需住院治療的CHF的預後。相比傳統預測指標而言，NTproBNP能提供更有效更獨立的預測價值。在NSTEMI/UA患者中測量NTproBNP應被列入早期危險度分層。

關鍵詞：非ST段抬高型心肌梗死（NSTEMI），氨基末端前腦利鈉肽（NTproBNP），危險度分層，非穩定性心絞痛（UA）

Introduction

In Non-ST elevation myocardial infarction/unstable angina (NSTEMI/UA), early coronary intervention in high-risk patients can achieve mortality reduction of 25% (6.5% to 4.9%) and MI reduction of 17% (9.1% to 7.6%).[1] The current guideline[2] recommends an early invasive strategy for high-risk groups. Early and accurate risk stratification in NSTEMI/UA is of paramount importance.

However, traditional prognostic markers in NSTEMI/UA like clinical vascular risk factors, ECG changes, cardiac enzyme elevation, stress test, left ventricular ejection fraction (LVEF), and clinical scoring systems (e.g. TIMI Score) have their limitations in predicting adverse cardiac outcome.

N-terminal pro-Brain Natriuretic Peptide's prognostic power surpasses that of conventional prognostic indicators, in risk stratification of NSTEMI/UA patients.

The aim of the study was to evaluate the prognostic value of N-terminal pro-Brain Natriuretic Peptide (NTproBNP) and its incremental predictive power over traditional prognostic indicators, in the risk stratification of NSTEMI/UA in a local population.

Method

Patient Selection

Patients admitted to our acute medical unit presenting with NSTEMI/UA, who were aged ≥18 years were included. Enrollment period was between December 2006 and February 2008. Patients with ST-Elevation Myocardial Infarction (STEMI), septicemia, shock at the time of recruitment, severe valvular heart disease, renal impairment with creatinine level >220 micromole/litre (μmol/L), history of admission for congestive heart failure, myocardial infarction/unstable angina within 3 months prior to index admission, history of coronary intervention within 3 months prior to index admission and advanced organ failure or malignancies with life-expectancy ≤1 year were excluded. Patients receiving cardiotoxic chemotherapeutic agents were also excluded. Patients who refused to consent or who were unable to consent were excluded.

Study Endpoints Definition

The primary composite endpoints were all cause mortality, MI/UA, and Congestive heart failure (CHF) requiring hospitalization. Patients were followed for up to 1 year after recruitment. NSTEMI/UA was diagnosed when patients presented with angina at rest, or crescendo angina, which lasted for ≥5 minutes (occurring within 24 hours of admission), and fulfilled one of the following criteria: 1) ST-segment depression ≥0.1 mV or T-wave inversion ≥0.1 mV; 2) elevated cardiac troponin T (TnT) ≥0.1 microgram/Litre (μg/L). CHF was defined according to the Modified Framingham clinical criteria.[3]

Laboratory Analysis

After informed consent, venous serum samples were collected in heparinised bottles, within 72 hours after admission. Serum was frozen at -20°C after centrifugation. Serum NTproBNP was determined with a sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics). NTproBNP unit was expressed in nanogram per litre (ng/L). Serum creatinine kinase and cardiac troponin T were also obtained. If the first set of cardiac TnT was obtained within 6 hours of symptom onset and was negative, a second TnT would be repeated at ≥6 hours apart. Creatinine clearance was estimated by Cockcroft and Gault Equation.

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS 11.5) software (SPSS Inc., Chicago, Illinois). One-sample Kolmogorov-Smirnov Test was used to assess the normality of distribution of continuous variables. Differences between means for parametric variables were evaluated with independent sample T-test. Differences between means for nonparametric variables were evaluated with Mann-Whitney U test. Differences between categorical variables were evaluated with Chi-square test. The NTproBNP cutoff level was obtained from ROC analysis. Kaplan-Meier Survival analysis was used to assess the difference in event free survival. The relative risk and the 95% CI were calculated. The significance of differences in event rates between the two groups of patients with NTproBNP above and below cutoff level were assessed with log-rank test. Cox Regression analysis was used to assess the prognostic value of NTproBNP independent of other confounding variables. Hazard ratio comparing NTproBNP above and below the cutoff were calculated, with adjustment for statistically significant covariates (defined as $p<0.1$). Subgroup analysis was also performed in patients with LVEF≥55%, TIMI risk score ≤4 and negative TnT. All statistical tests were 2-tailed, and a P value of <0.05 was considered statistically significant.

Comparison with Other Traditional Prognostic Markers

The prognostic value of NTproBNP was assessed and compared with traditional prognostic markers including LVEF, Troponin T (TnT), TIMI Score, and the ten ACC/AHA Risk Factors (ACC Risk Factors) for NSTEMI/UA[2]: 1) Recurrent angina or ischemia at rest or with low-level activities despite intensive medical therapy, 2) Elevated cardiac biomarkers, 3) New or presumably new ST-segment depression, 4) Signs or symptoms of CHF or new or worsening mitral regurgitation, 5) High-risk findings from noninvasive testing, (6) Hemodynamic instability, 7) Sustained ventricular tachycardia. 8) PCI within 6 months, 9) Prior CABG, 10) High risk score (e.g. TIMI), 11) Reduced left ventricular function (LVEF less than 40%).

Results

A total of 329 patients were screened. One hundred and fifty-one patients were excluded during initial screening for various reasons including: renal impairment with creatinine >220 μmol/L (60), delayed recruitment (when NTproBNP sampling time could not be obtained within 72 hours after index admission) (31), inability/refusal to consent (26), history of MI, UA, CHF or coronary revascularisation within 3 months of index admission (22), coexisting advanced medical illness with life-expectancy ≤1 year (e.g. malignancies) (7), severe valvular heart disease (4), and expected loss of follow-up (non-local citizen) (1). One patient was excluded retrospectively due to septicemic shock at the time of NTproBNP sampling.

Baseline Characteristics

A total of 177 patients were included in the study. The baseline characteristics of patients were listed in Table 1. The median time between NTproBNP sampling and symptom onset was 36 hours. The mean LVEF of our study population was 52.2±12.4%. The median NTproBNP level in patients with unstable angina and NSTEMI was 262 ng/L and 874.6 ng/L respectively. The mean TIMI risk score was 3.7±1.4. Forty-four (24.9%) patients had TIMI risk score >4. The mean ACC risk factors was 2.1±1.4.

Assessment of NTproBNP Cutoff

The Receiver-Operator Curve (ROC) analysis yielded an area under curve of 0.746. (SE: 0.042; CI 0.663-0.83; $p<0.001$) (Figure 1). NTproBNP cutoff of 331 ng/L yielded a sensitivity of 81.4% and a specificity of 52.2% in predicting primary composite endpoints at 1 year. The positive and negative predictive values were 35.4% and 90% respectively.

Outcome Measures

At 1 year follow-up, a total of 43 (24.3%) patients reached primary composite endpoint of all cause mortality, MI/UA or CHF requiring hospitalization. Twelve patients (6.8%) reached mortality endpoint. Seven patients (4%) died of cardiac cause. Five patients (2.8%) died of noncardiac causes (namely intracranial

hemorrhage, ischemic stroke, chronic obstructive airway disease, pneumonia and unknown cause). Twenty-seven patients (15%) had MI/UA. Fifteen patients (8.5%) had CHF requiring hospitalization at 1 year (Table 2).

Among patients with NTproBNP ≤331 ng/L, the 1-year incidences of primary composite endpoint, total mortality, MI/UA and CHF rate were lower (10.3%, 1.3%, 10.3%, and 0% respectively, p all ≤0.01) than that in patients with NTproBNP >331 ng/L (35.4%, 11.1%, 19.2%, and 15.2% respectively, p all ≤0.01). In patients with NTproBNP >331 ng/L, there was a trend towards higher cardiac mortality (OR 4.97; CI 0.6-42.2; p=0.136) and MI/UA (OR 2.08; CI 0.9-5; p=0.1) at

Table 1. Baseline characteristics

Epidemiological factors (n=177)	
Age (years)	68.4±13.1
Gender (Male/Female)	112/65
Creatinine clearance (ml/min)	62±28
Creatinine (μmol/L)	90.7±24.9
Body weight (kg)	61.9±13.3
NTproBNP time from admission (hours)#*	22 (15-36)
NTproBNP time from symptom onset (hours)#*	36 (23-54)
Peak creatinine kinase (IU/L)#	150 (78.5-334)
Patients with angina	153 (86.4%)
Dynamic ECG changes	133 (75%)
Patients with Non-ST elevation myocardial infarction (NSTEMI)	72 (40.7%)
Patients with unstable angina	105 (59.3%)
Left ventricular ejection fraction (%)	52.2±12.4
Patients with left ventricular ejection fraction ≤40%	31 (17.5%)
NTproBNP (ng/L)#	591.8 (95.6-1757.5)
NTproBNP in unstable angina patients (ng/L)#	262 (76.3-1285.5)
NTproBNP in NSTEMI patients (ng/L)#	874.6 (219.9-3237.3)
TIMI score	3.7±1.4
Patients with TIMI risk score >4	44 (24.9%)
ACC risk factors	2.1±1.4
Patients with ACC risk factors≥1	159 (89.8%)
Patients received percutaneous coronary intervention at 1 year	72 (40.7%)
Patients received coronary artery bypass grafting at 1 year	2 (1.1%)

Continuous variables are expressed in mean ± standard deviation unless otherwise specified.

Variables expressed in median (25th/75th centile). All dichotomous variables are expressed in number (percentage); *NTproBNP time from admission=Time between NTproBNP sampling and admission; NTproBNP time from symptom onset=Time between NTproBNP sampling and symptom onset.

1 year, although it did not reach statistical significance. NTproBNP cutoff of >331 ng/L predicted 1-year composite endpoint with a sensitivity of 81.4% and specificity of 52.2%. It also provided a high negative predictive value of 90% in predicting composite endpoint at 1 year.

Major predictors of primary composite endpoint assessed in univariate and multivariate analysis were summarized in Tables 3a, 3b and 4. NTproBNP, Age, creatinine clearance, ST segment depression, ACC risk factors, TIMI score, CHF during index admission, hypertension, history of CHF, history of stroke, use of calcium channel blockers, nitrates, diuretics, and previous use of aspirin were found to be predictors of primary composite endpoint in univariate analysis. However, in multivariate analysis, NTproBNP>331 ng/L remained to be the independent predictor of primary composite endpoint.

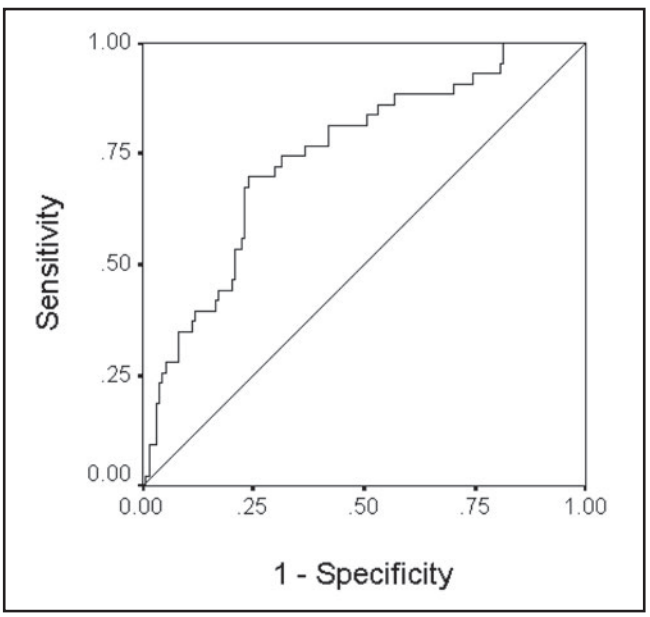

Figure 1. *Receiver-operator curve analysis. NTproBNP cutoff of >331 ng/L yielded a sensitivity of 81.4% and a specificity of 52.2% in prediciting primary composite endpoints at 1 year. The positive and negative predictive values were 35.4% and 90% respectively.*

Survival Analysis

Patients with NTproBNP≤331 ng/L demonstrated higher event free survival at 1 year in Kaplan Meier analysis (Log Rank 13.97; p=0.0002) (Figure 2). The composite endpoint event free survival at 1 year (HR 2.5; CI 1.1-5.8; p=0.028) (Figure 3) was higher in patients with NTproBNP >331 ng/L in Cox Regression analysis. NTproBNP was the independent prognostic indicator after adjusting for confounding variables including age, diabetes mellitus (DM), hypertension (HT), smoking status, creatinine clearance, CHF during index admission, history of MI/UA, history of CVA, ACC risk factors and the time between NTproBNP sampling and symptom onset. NTproBNP >331 ng/L predicted primary composite endpoint with a HR of 3.4 (CI 1.6-7.5; p=0.002). The prognostic value of NTproBNP was still retained after adjusting for revascularisation strategy.

Table 2. Outcome at 1 year

	NTproBNP		Odds	Confidence	p value
	≤331 ng/L (n=78)	>331 ng/L (n=99)	ratio	interval	
Composite endpoint	8 (10.3%)	35 (35.4%)	4.79	2.1-11.1	<0.001
Mortality	1 (1.3%)	11 (11.1%)	9.6	1.2-76.3	0.01
Cardiac mortality	1 (1.3%)	6 (6.1%)	4.97	0.6-42.2	0.136
Recurrent myocardial infarction/ unstable angina	8 (10.3%)	19 (19.2%)	2.08	0.9-5.0	0.1
Congestive heart failure 1 year	0 (0%)	15 (15.2%)	–	–	<0.001

Table 3a. Univariate predictors of primary composite endpoint (continuous variables)

	Composite endpoint		p value
	0 (n=134)	+ (n=43)	
Age (years)	66.8±13	73.4±12	0.004
Creatinine clearance (ml/min)	64.7±28.8	54.1±24.1	0.029
ST depression (mm)	1±1	2±2	0.012
ACC risk factors	1.9±1.3	2.6±1.6	0.021
TIMI score	3.5±1.4	4.4±1.4	<0.001
NTproBNP time* (hours)	35 (22-52)	44 (26-91)	0.087
NTproBNP (ng/L)#	314.6 (84.6-1081.3)	1543 (773.9-4564)	<0.001

0=negative; +=positive

*NTproBNP time=NTproBNP sampling time from symptom onset; #values expressed in median (25th/75th centiles)

Table 3b. Univariate predictors of primary composite endpoint (dichotamous variables)

	Composite endpoint		Odds ratio	Confidence interval (95%)	p value
	0 (n=134)	+ (n=43)			
CHF adm	19 (14.2%)	13 (30.2%)	2.6	1.2-5.9	0.017
Diabetes mellitus	45 (33.6%)	22 (51.2%)	2.1	1.0-4.2	0.039
Hypertension	77 (57.5%)	35 (81.4%)	3.2	1.4-7.5	0.005
History of congestive heart failure	7 (5.2%)	7 (16.3%)	3.5	1.2-10.7	0.044
History of stroke	11 (8.2%)	9 (20.9%)	3.0	1.1-7.7	0.029
Previous aspirin use	67 (50%)	29 (67.4%)	2.0	1-4.3	0.046
Enoxaparin	113 (84%)	40 (93%)	2.5	0.7-8.8	0.15
Aspirin	133 (99.3%)	42 (97.7%)	0.3	0.2-5.2	0.43
Clopidogrel	124 (92.5%)	37 (86%)	0.5	0.2-1.5	0.22
ACEI	112 (83.6%)	36 (83.7%)	1.0	0.4-2.6	0.98
Statin	120 (89.6%)	38 (88.4%)	0.9	0.3-2.6	0.78
Betablockers	112 (83.6%)	39 (90.7%)	1.9	0.6-5.9	0.25
Calcium channel blockers	20 (14.9%)	14 (32.6%)	2.8	1.2-6.1	0.011
Nitrates	57 (42.5%)	28(65.1%)	2.5	1.2-5.2	0.01
Diuretics	35 (26.1%)	18 (41.9%)	2.0	1.0-4.1	0.05
NTproBNP>331 ng/L	64 (47.8%)	35 (81.4%)	4.8	2.1-11.1	<0.001

0=negative; +=positive; CHF adm = congestive heart failure in index admission; ACEI=angiotensin-converting enzyme inhibitors

Table 4. Multivariate parameters in Cox Regression analysis: Predictors of primary composite endpoint

	HR	Multivariate analysis Confidence (95%)	p value
Age	1.0	0.98-1.04	0.463
Chronic smoker	1.1	0.4-2.8	0.915
CHF adm	1.1	0.4-2.0	0.851
NTproBNP time (hours)	1.0	0.998-1.003	0.714
History of MI/UA	1.2	0.6-2.3	0.551
Creatinine clearance	1.0	0.99-1.02	0.594
Diabetes mellitus	1.5	0.8-2.8	0.179
History of stroke	2.0	0.9-4.1	0.080
Hypertension	2.0	0.9-4.4	0.084
ACC risk factors	1.3	1.1-1.7	0.016
NTproBNP>331 ng/L	2.5	1.1-5.8	0.028

CHF adm=congestive heart failure in index admission; NTproBNP time=NTproBNP sampling time from symptom onset; ACC risk factors=The ten American College of Cardiology/ American Heart Association Risk Factors for Non-ST Elevation Myocardial Infarction/ Unstable Angina: 1) Recurrent angina or ischemia at rest or with low-level activities despite intensive medical therapy, 2) Elevated cardiac biomarkers, 3) New or presumably new ST-segment depression, 4) Signs or symptoms of CHF or new or worsening mitral regurgitation, 5) High-risk findings from noninvasive testing, 6) Hemodynamic instability, 7) Sustained ventricular tachycardia, 8) PCI within 6 months, 9) Prior CABG, 10) High risk score (e.g. TIMI), 11) Reduced left ventricular function (LVEF less than 40%).

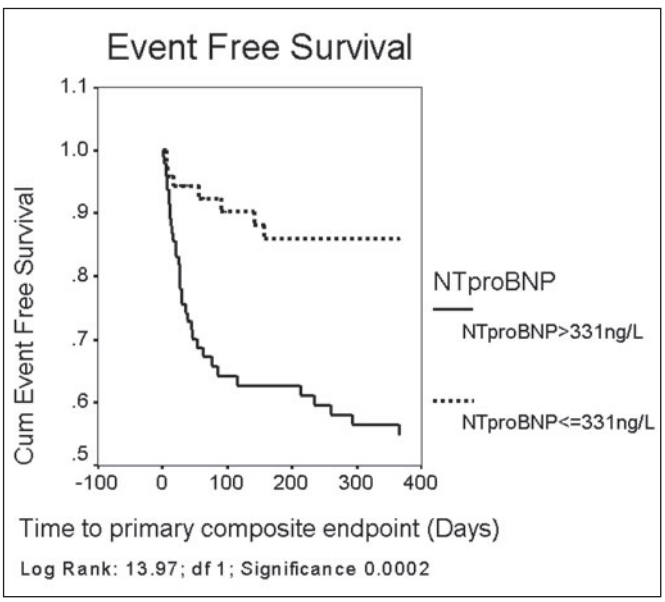

Figure 2. *Kaplan-Meier survival analysis.*

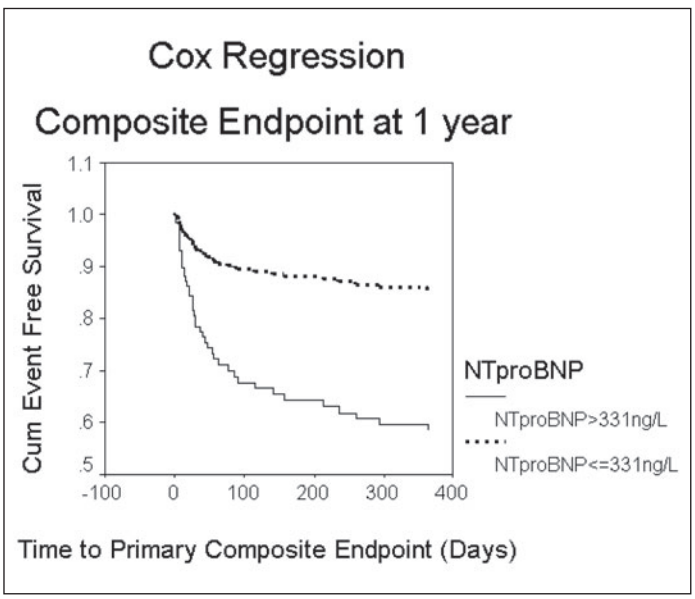

Figure 3. *Cox Regression – Primary composite endpoint at 1 year. NTproBNP>331 ng/L predicted primary composite endpoint with a HR of 3.4 (CI 1.6-7.5; p=0.002)*

Prognostic Power of NTproBNP Compared with Traditional Prognostic Indicators

Decompensated CHF alone could cause NTproBNP elevation.[4] In our study, 32 (18%) patients had decompensated CHF during index admission. CHF during index admission was a significant predictor of outcome in univariate analysis (OR 2.6; CI 1.2-5.9; p=0.017). However, the prognostic power of CHF during index admission was lost after addition of NTproBNP into Cox Regression analysis. Clinical diagnosis of decompensated CHF during index admission predicted primary composite endpoints with sensitivity of 30.2%, specificity of 85.8%, positive predictive value (PPV) of 40.6% and negative predictive value (NPV) of 79% (OR 2.623; CI: 1.165-5.91; p=0.017). TIMI risk score cutoff >4 predicted primary composite endpoint with sensitivity of 46.5%, specificity of 82.1% (OR 3.99; CI 1.89-8.4, p<0.001), PPV of 45.5% and NPV of 82.7%. LVEF<40% predicted primary composite endpoint with sensitivity of 72.1%, specificity of 14.2%, PPV of 21.2% and NPV of 61.3% (OR 0.427; CI 0.187-0.973; p=0.039). NTproBNP cutoff of >331 ng/L predicted primary composite endpoint with sensitivity of 81.4%, specificity of 52.2%; (OR of 4.79; CI2.07-11.1; p<0.001), PPV of 35.4% and NPV of 90%.

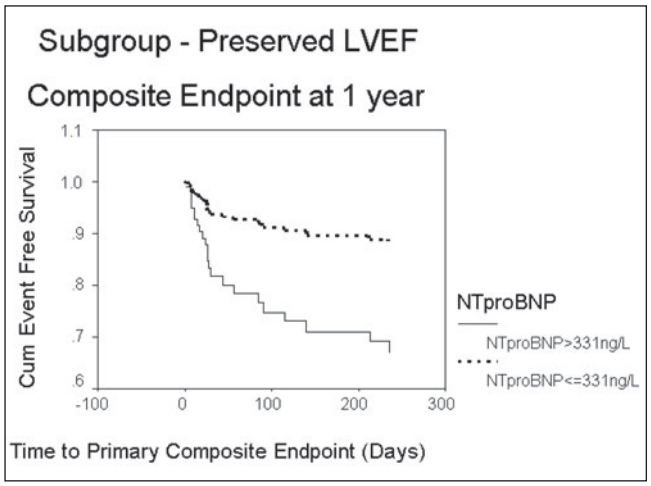

Figure 4. *Subgroup analysis (LVEF ≥55%). NTproBNP >331 ng/L predicted primary composite endpoint with a HR of 3.1 (CI 1.2-8.3; p=0.02).*

Subgroup Analysis

In subgroup analysis, NTproBNP>331 ng/L demonstrated its independent prognostic value in predicting primary endpoint at 1 year even in patients with preserved left ventricular systolic function (LVEF ≥55%) (HR 3.1, CI 1.2-8.3; p=0.02), normal troponin (HR 5.6, CI 1.9-16.7; p=0.02) and TIMI score ≤4 (HR 2.6, CI 1.1-6.3; p=0.036) (Figures 4-6).

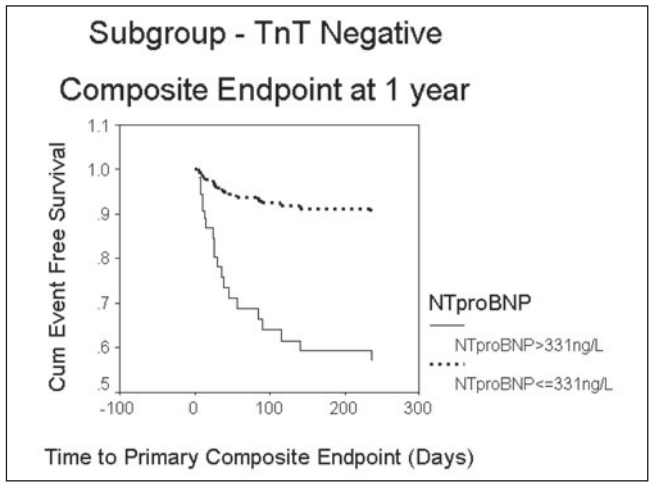

Figure 5. *Subgroup analysis (Troponin negative). NTproBNP >331 ng/L predicted primary composite endpoint at 1 year with HR 5.6 (CI 1.9-16.7; p=0.02).*

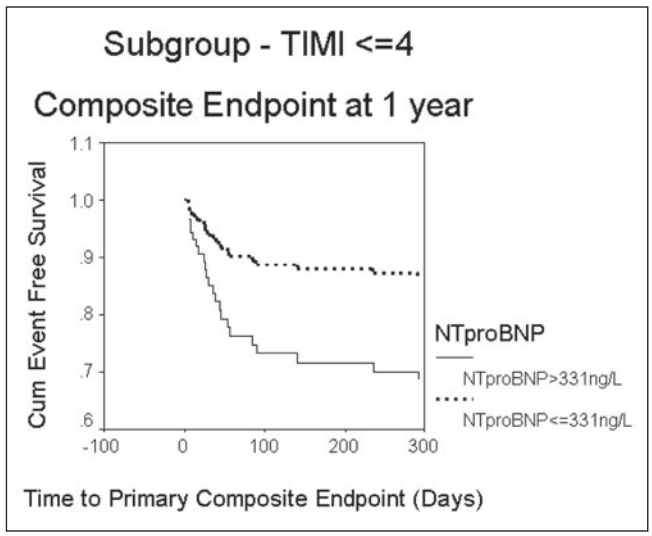

Figure 6. *Subgroup analysis (TIMI risk score ≤4). NTproBNP >331 ng/L predicted 1 year composite endpoint with HR of 2.6 (CI 1.1-6.3; p=0.036).*

Discussion

An ideal prognostic biomarker should have high sensitivity, negative predictive value and cost-effectiveness, compared with traditional prognostic markers and clinical scoring systems. Traditional prognostic markers in NSTEMI/UA include clinical vascular risk factors e.g. DM, HT, smoking status, family history of coronary artery disease (CAD), angina symptom, ECG changes, cardiac enzyme elevation, stress test, LVEF, and clinical scoring systems (e.g. TIMI Score). However, these risk stratification systems have their own limitations in terms of sensitivity, negative predictive value and cost-effectiveness.

Cardiac enzyme has limited role in further risk stratifying patients with UA without enzyme leak. In our study, the 1 year rate of primary composite endpoint was up to 21% (19 out of 92) among the subgroup of patients with unstable angina. This means 1 out of 5 patients classified as low risk by negative cardiac enzyme alone could potentially develop adverse cardiac events at 1 year.

TIMI score is limited by its low negative predictive value. In our study, the 1 year rate of primary composite endpoint was up to 17% in the unstable angina subgroup. In the subanalysis study of TIMI 11B and ESSENCE, NSTEMI/UA patients with TIMI score =3 still had a 13.2% risk of all-cause mortality, MI, or recurrent ischemia requiring urgent revascularization at 14 days.[5] In the CURE substudy, the risk of cardiovascular death, myocardial infarction and stroke at 9 months for NSTEMI/UA patients with TIMI score ≤4 was still ~14% in the clopidogrel arm.[6]

Similarly, echocardiogram is limited by its lack of sensitivity in further risk stratifying patients with preserved LVEF (left ventricular ejection fraction >55%). In our study, patients with preserved LVEF still had 1 year rate of primary composite endpoint of 21%.

NTproBNP surpasses traditional prognostic factors by its distinctive characteristics. In the setting of acute coronary syndrome (ACS), it is cardiac specific, has more rapid release (as early as 3 hours) and higher sensitivity than cardiac enzymes.[7] It could be elevated even before the occurrence or in the absence of myocardial necrosis.[8] In ACS patients with normal LVEF, myocardial wall stress and neurohormonal activation can cause NTproBNP elevation, even in the absence of myocardial necrosis.[9,10] This could account for the high sensitivity of NTproBNP in predicting adverse outcome in ACS patients without cardiac enzyme elevation.

As a result, measurement of BNP or NTproBNP is recommended for assessment of global risk in patients with suspected ACS.[2] Major studies yielded conflicting results on the role of natriuretic peptides in risk stratification intervention strategy.[11,12] According to current guideline,[2] measurement of BNP or NT-proBNP is a class II b recommendation, in risk stratification of ACS patients. At the moment, there is no international consensus on the cutoff value of NTproBNP in risk stratification of NSTEMI/UA patients and guidance on intervention strategy. NTproBNP level was found to be lower among healthy Chinese population compared with their Western counterparts.[13] The prognostic value and cutoff level of NTproBNP among Chinese ACS population has remained undefined.

In acute myocardial infarction, plasma NTproBNP level rises rapidly, starting from 3 hours,[7] to a maximum at 20-30 hours from onset of symptoms.[7,9,14,15] The profile of plasma NTproBNP release after MI depends on the extent of infarct. In small or moderate myocardial infarction (MI), NTproBNP peaks at 24-48 hours.[16] In major MI, it demonstrates biphasic peaks - with the first peak on first 1-2 days, and the second peak on days 5-7 (indicative of adverse ventricular remodeling).[9] Therefore, the NTproBNP sampling timeframe is of paramount importance in prognostication of ACS patients. In our study, the mean and median time between symptom onset and NTproBNP sampling was 62.9±138.1 hours and 36 hours (interquartile range 23-54 hours) respectively, which coincided with the initial peak of NTproBNP after myocardial infarction.

The cutoff level of NTproBNP in our study population was 331 ng/L (interquartile range 95.6-1758 ng/L). NTproBNP cutoff of >331 ng/L predicted 1 year all cause mortality (11.1% vs 1.3% for those above and below the cutoff respectively, p=0.01) and composite endpoint of all-cause mortality, MI/UA and CHF requiring hospitalization (35.4% vs 10.3%

for those above and below the cutoff respectively, p<0.001).

NTproBNP also predicted a trend towards higher future risk of myocardial infarction (MI) and cardiovascular (CVS) death at 1 year (6.1% vs 1.3% for those above and below the cutoff, p=0.136), although it did not reach statistical significance. The use of single cutoff level of NTproBNP might limit its sensitivity in predicting future MI and CVS death, as it is not a specific marker in reflecting myocardial necrosis. Adoption of interquartile range might overcome this potential limitation, but a larger sample size would be required.

Compared with traditional risk stratification strategy such as TIMI score, clinical diagnosis of CHF during index admission, and LVEF<40%, NTproBNP cutoff value of 331 ng/L provided superior sensitivity (81.4%) and NPV (90%) in prediction of composite endpoint. Its low PPV (35.4%) could be accounted by the use of single cutoff value at low level. The PPV could be improved by using inter-quartile ranges instead of a single cutoff value. However, a larger sample size would be needed to generate adequate prognostic power.

Brain-natriuretic peptide has been demonstrated to be independently associated with mortality in left ventricular dysfunction secondary to myocardial ischemia.[17] Level of brain natriuretic peptide also reflects the size of ischemia.[18] The ability to reflect myocardial stress even in the absence of myocardial necrosis and left ventricular systolic function impairment[9,10,19] explains its high sensitivity. In our study, we have demonstrated that NTproBNP was able to predict adverse outcome even in ACS patients with normal left ventricular systolic function and normal cardiac enzymes. These patients would be otherwise classified as low risk by conventional prognostic markers. NTproBNP predicted MACE (OR 4.8), CHF, mortality (OR 9.6) at 1 year independent of other confounding variables and conventional prognostic markers.

Impact of Revascularization Strategies

There was no major difference in the incidence of 3 vessels disease (23.3% vs 27.6%; p=0.729) and left main disease (7% vs 6%; p=0.73) between the two groups of patients with and without composite endpoint.

In our study, 84 (47.5%) patients received coronary angiogram, 72 (40.7%) patients received percutaneous coronary intervention (PCI), 2 (1.1%) patients received coronary artery bypass grafting (CABG). The mean time to coronary intervention was 12.4±33 days. The revascularization rate in our study population was 42% (74 out of 177). The early revascularization rate (within 7 days of admission) was 11.3% (20 out of 177). There was no statistically significant difference in the revascularisation rate among the two groups of patients categorized by NTproBNP cutoff of 331 ng/L or categorized by composite endpoint.

The mean time to PCI in patients who reached and who did not reach composite endpoints was 8±16 days and 14±37 days respectively (p=0.28) The mean time to PCI in patients with NTproBNP below and above cutoff level of 331 ng/L was 10±17 days and 14±41.5 days respectively (p=0.623)

Revascularisation strategy could potentially confound the prognostic value of NTproBNP. However, in our study population, there was no statistically significant difference in intervention strategy in the two groups of patients categorized by NTproBNP cutoff and composite endpoint at 1 year. The coronary intervention timing among the two groups of patients was also comparable.

Study Limitations

There are a number of limitations in our study. Firstly, we have not performed serial NTproBNP sampling. Studies have shown that serial monitoring of NTproBNP post MI revealed a biphasic profile of plasma concentration.[9] Serial NTproBNP sampling at baseline and 72 hours had been shown to predict adverse outcomes.[20] In our study, the median NTproBNP sampling time was 36 hours. A single sampling timeframe might limit its comprehensiveness in prognostication as compared with serial sampling. The effect of early medical therapy/coronary intervention for NSTEMI/UA might not be fully manifested at the time of NTproBNP sampling.

Secondly, the early revascularisation rate (within index admission) in our study was lower than that in

major clinical trials. The low early revascularisation rate (22.7%) in high risk patients (defined as TIMI score >4) would lead to heterogeneity of clinical outcomes due to the diversity of intervention timing. This reflected the limitation of resources and capacity of catheterization service. Revascularisation strategy could potentially confound the prognostic value of NTproBNP. However, in our study population, there was no statistically significant difference in intervention strategy in the two groups of patients categorized by NTproBNP cutoff and composite endpoint at 1 year. The coronary intervention timing, percentage of left main disease among the two groups of patients were also comparable.

Conclusion

In clinically high risk patients with NSTEMI/UA, NTproBNP provided incremental prognostic information over traditional prognostic markers. Among patients classified as low risk by conventional prognostic markers (patients with LVEF >55%, negative TnT, TIMI risk score ≤4), NTproBNP >331 ng/L also predicted a trend towards more adverse clinical outcomes. Whether NTproBNP could be used to guide intervention strategy in ACS patient should await future randomized trials. In patients with NSTEMI/UA, NTproBNP cutoff of 331 ng/L is an independent prognostic marker in prediction of all cause mortality, MI/UA, and CHF requiring hospitalization at 1 year. Measurement of NTproBNP in NSTEMI/UA patients should be considered for early risk stratification.

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Mitral Valve Prolapse Associated with Atrial Level Communication

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YUAN and LAVEE: ***Mitral Valve Prolapse Associated with Atrial Level Communication*****. Objectives:** *The association of mitral valve disorder and atrial communication, either atrial septal defect or patent foramen ovale, remains a topic of debate over time. The aim of the present article is to describe the clinical features of this entity.* **Patients and Methods:** *Eighteen patients with an atrial communication that required closure were selected into this study, and were divided into two groups: Group A patients were associated with mitral valve prolapse, and Group B patients were not.* **Results:** *Pulmonary hypertension was noted in eight patients of Group A, and in one patient of Group B. Three patients in Group A and none of Group B had infective endocarditis. Group A patients had larger left ventricular diastolic dimension, left atrial dimension, and tricuspid valve peak systolic pressure gradient than Group B patients. Regression analysis revealed an inverse relationship between left ventricular diastolic dimension and peak systolic pressure gradient across the tricuspid valve in Group A (p=0.033), but no significant correlation was noted in Group B (p=0.183).* **Conclusions:** *The presence of mitral valve prolapse with various degrees of mitral regurgitation in the patients with atrial level communication may implicate an impaired diastolic function of the left ventricle, and increased pulmonary artery pressure. Surgical intervention to the atrial level communication and mitral regurgitation may lead to a better prognosis in such patients. (J HK Coll Cardiol 2011;19:57-62)*

Heart septal defects, atrial; Hypertension, pulmonary; Mitral valve prolapse; Ventricular function, left

摘要

目的：二尖瓣病變合併心房水平分流（心房中隔缺損或未閉卵圓孔）是長期以來一個存在爭議的問題。本文著重討論該症的臨床特點。**方法：**18 例需要閉合心房水平分流的患者被列入本研究，並分成兩組：A 組患者合併二尖瓣脫垂，B 組不合併二尖瓣脫垂。**結果：**A 組中8 例患者有肺動脈高壓，B 組中1 例患者有肺動脈高壓。A 、B 兩組中各有3 例患者有感染性心內膜炎。A 組的左室舒張期內徑、左房內徑及經三尖瓣的峰值壓力階差顯著大於B 組。回歸分析結果顯示A組左室舒張期內徑與經三尖瓣的峰值壓力階差之間具有顯著的負相關（p＝0.033），而B組該兩參數間則無顯著關聯性（p＝0.183）。**結論：**在心房水平分流的患者，二尖瓣脫垂伴不同程度的二尖瓣返流可能會使左室的舒張功能受損、肺動脈壓力增加。對心房水平分流及二尖瓣返流的外科治療會使此類患者獲得較好的預後。

關鍵詞：心房中隔缺損，肺動脈高壓，二尖瓣脫垂，左心室功能

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Received June 19, 2011; revision accepted September 29, 2011

Introduction

The association of mitral valve disorder and atrial septal defect (ASD) or patent foramen ovale remains a topic of debate over time. Mitral valve prolapse that was associated with secundum ASD was regarded as compatible to congenital lesion in some young children,[1] but could be a rheumatic disorder in young adults.[2] Due to the fact that right ventricular volume loading and dilation, as well as paradoxical septal movement, mitral valve prolapse in patients with an ASD was surmised to be functional.[3]

Patients and Methods

From January 2004 to June 2008, 41 adult patients with an atrial communication (secundum ASD or patent foramen ovale) received an ASD/patent foramen ovale closure solely or combined with other cardiac operations in this department. Eighteen patients with or without mitral valve prolapse were sorted out and were included into this study. The exclusion criteria were,

1. mitral stenosis, or predominant mitral stenosis mixed with mitral regurgitation;
2. flail mitral valve caused by acute mitral chordae rupture;
3. a redo cardiac operation and the atrial level communication had been closed in the primary operation;
4. primum atrial septal defect with mitral cleft;
5. associated with other complex congenital heart defects.

Group A included 11 patients with mitral valve prolapse associated with coronary artery disease, atrial fibrillation, infective endocarditis and anemia. Seven patients without mitral valve prolapse were involved into Group B as control. Informed consent was obtained from the study participants, and the study was approved by institutional ethical committees.

The degree of severity of mitral regurgitation can be quantified by the regurgitant fraction, which is the percentage of the left ventricular stroke volume that regurgitates into the left atrium. Methods that have been used to assess the regurgitant fraction in mitral regurgitation included echocardiography, cardiac catheterization, computed tomography, and cardiac magnetic resonance imaging. Regurgitant fraction <20%, 20-40%, 40-60% and >60% was defined as mild, moderate, moderate to severe, and severe mitral regurgitation, respectively.[4]

Pulmonary hypertension was defined as a mean pulmonary arterial pressure of greater than 25 mmHg at rest or greater than 30 mmHg during exercise. Based on the normal values of right ventricular systolic pressure (<35 mmHg) and tricuspid regurgitant velocity (<2.7 m/s), mild pulmonary arterial hypertension was defined when right ventricular systolic pressure was 36-50 mmHg, or tricuspid regurgitant velocity was 2.8-3.4 m/s, and moderate-severe pulmonary arterial hypertension was defined when right ventricular systolic pressure >50 mmHg, or tricuspid regurgitant velocity >3.4 m/s.[5]

Comparative study was conducted between the two groups in terms of age, ASD size, and echocardiographic measurements, including left ventricular function, left atrial diameter, tricuspid valve peak systolic pressure gradient, and right atrial pressure. Data were expressed as mean±SD. Unpaired *t*-test, Fisher's exact test, and linear regression were applied. $p<0.05$ was considered of statistical significance.

Results

The demographics of the patients were listed in Table 1. In systole, the thickened scallop of the posterior mitral valve leaflet prolapsed beyond the annular plane into the left atrium was seen by echocardiography in each patient of Group A. The severity of mitral regurgitation of Group A patients was mild, moderate, moderate-to-severe, and severe secondary to mitral valve prolapse in 4 (36.4%), 2 (18.2%), 2 (18.2%), and 3 (27.3%) patients, respectively. There were no mitral valve disorders in Group B patients. No difference was noted in patients' age and ASD size between the two groups. Pulmonary hypertension was noted in eight patients of Group A, and in one patient of Group B. Three patients in Group A and none of Group B had

Table 1. Demographic data

Parameters	Group A	Group B	*p* value
Case, n	11	7	–
Female gender, n (%)	4 (36.4)	2 (28.6)	1.0000
Age, year	56.00±13.25	55.00±12.83	0.8765
Prolapse valve mitral, n (%)	11 (100)	0 (0)	<0.0001
Mild MR, n (%)	4 (36.4)	–	–
Moderate MR, n (%)	2 (18.2)	–	–
Moderate to severe MR, n (%)	2 (18.2)	–	–
Severe MR, n (%)	3 (27.3)	–	–
ASD, n (%)	4 (36.4)	4 (57.1)	0.6305
Patent foramen ovale, n (%)	7 (63.6)	3 (42.9)	0.6305
ASD/patent foramen ovale size, cm	1.4636±1.5042	1.6929±1.1798	0.7377
Patch repair of ASD, n (%)	3 (27.3)	3 (42.9)	0.6267
Mitral valve repair, n (%)	5 (45.5)	–	–
Mitral valve replacement, n (%)	6 (54.5)	–	–
Pulmonary hypertension, n (%)	8 (72.7)	1 (14.3)	0.0498
Infective endocarditis, n (%)	3 (27.3)	0 (0)	0.2451
Ischemic heart disease, n (%)	0 (0)	0 (0)	1.0000
Mellitus diabetes, n (%)	2 (18.2)	1 (14.3)	1.0000
Hypertension, n (%)	3 (27.3)	2 (28.6)	1.0000

ASD: atrial septal defect; MR: mitral regurgitstion.

infective endocarditis.

Of the echocardiographic measurements, statistical significance was found in left ventricular diastolic dimension, left atrial dimension, and tricuspid valve gradient between the two groups. No intergroup difference was noted in left ventricular systolic dimension, interventricular septum in diastole, left ventricular posterior wall thickness in diastole, estimated left ventricular mass index, and estimated right atrial pressure (Table 2). Regression analysis revealed a close inverse relationship between left ventricular diastolic dimension and peak pressure gradient across the tricuspid valve in Group A (p=0.033) (Figure 1), but no significant correlation in Group B (p=0.183) (Figure 2). All the patients survived and were well in both groups.

Discussion

Mitral valve prolapse is the most common congenital valvular heart disease in adults. The prevalence of mitral valve prolapse in the United States has been estimated at four to five percent, but varies by age and gender. The prolapse of the mitral valve involves superior and posterior displacement of one or both mitral valve leaflets across the plane of the mitral valve annulus into the left atrium during systole.[6] Mitral valve prolapse is classified as primary, secondary, or functional based upon the anatomic or physiologic defects. Although the prognosis is usually benign, mitral valve prolapse can be associated with serious complications, including mitral regurgitation, infective endocarditis and arrhythmias.[6]

Table 2. Hymodynamics of the patients with versus without mitral valve prolapse

Parameters	Group A	Group B	*p* value
Left ventricular ejection fraction (%)	56.11±9.28 (35-65)	58.57±5.56 (50-65)	0.5467
Left ventricular diastolic dimension (cm)	5.0900±0.8543 (3.9-6.1)	4.1871±0.5849 (3.5-5.41)	0.0317
Left ventricular systolic dimension (cm)	3.1044±0.5242 (2.5-3.85)	2.6657±0.5349 (2.2-3.58)	0.1220
Interventricular septal thickness in diastole (cm)	1.0533±0.1539 (0.8-1.3)	1.0971±0.1468 (0.9-1.3)	0.5738
Left ventricular posterior wall thickness in diastole (cm)	1.0211±0.0867 (0.9-1.15)	1.0229±0.1643 (0.7-1.16)	0.9784
Estimated left ventricular mass index (g/m^2)	106.238±30.027 (62.2-139)	87.367±28.906 (56.5-137.2)	0.2601
Left atrial diameter (cm)	4.8778±1.0008 (3.4-5.74)	3.9271±0.6581 (2.9-4.7)	0.0480
Peak gradient across the tricuspid valve (mmHg)	46.1625±8.6560 (42.3-61)	27.8900±11.4061 (8-39)	0.0051
Estimated right atrial pressure (mmHg)	10.00±4.63 (5-15)	8.33±6.06 (5-20)	0.5690

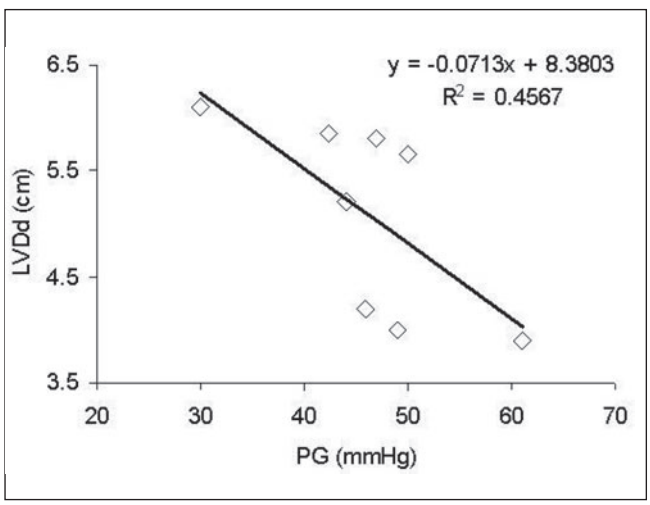

Figure 1. *Regression analysis revealed an inverse relationship between the left ventricular diastolic dimension and peak systolic pressure gradient across the tricuspid valve in Group A (p=0.033). LVDd=left ventricular diastolic dimension; PG =peak systolic pressure gradient across the tricuspid valve.*

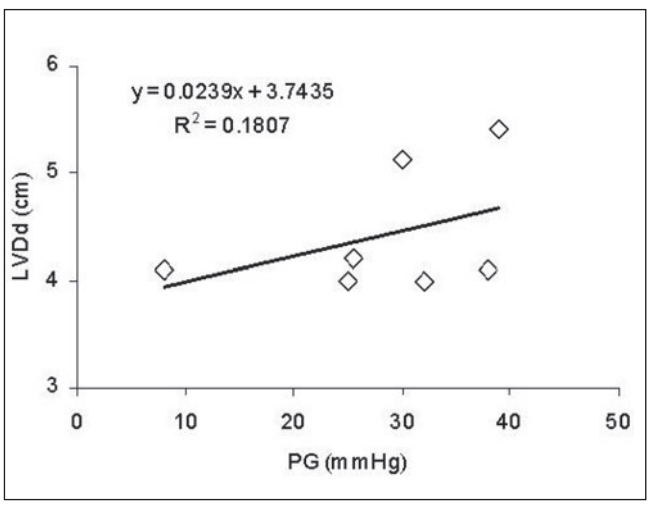

Figure 2. *No significant correlation was noted between left ventricular diastolic dimension and peak systolic pressure gradient across the tricuspid valve in Group B (p=0.183). LVDd=left ventricular diastolic dimension; PG=peak systolic pressure gradient across the tricuspid valve.*

The incidence of the association of mitral valve prolapse and secundum atrial septal defect is increasing, and has led to much deliberation over the years.[3] This entity was regarded as benign, but prognosis of mitral valve malfunction exists, and infective endocarditis might occur as a complication of secundum ASD.[7] The risk of infective endocarditis in patients with isolated ASD of the fossa ovalis type is exceedingly small, but it may develop with the associated mitral valve disorder, especially prolapse of posterior mitral leaflet.[8] The development of mitral valve prolapse would alter the prognosis of ASD.[9] And mitral valve prolapse could, in turn, regress after ASD closure.[3]

Our results based on echocardiographic hemodynamics showed a similar trend with those of Burleson et al,[10] who noted patients with mitral valve prolapse had a greater pulmonary artery pressure preoperatively than those without (102±19 vs. 84±21 mmHg). No significant differences were noted in mitral annulus dimensions or left ventricular chamber areas. Kestlli[3] obtained an extensive significance in a comparative study on mitral valve prolapse. He found that patients with an ASD had decreased values of diastolic ventricular septum thickness and diastolic left ventricular posterior wall thickness, but ejection fraction was high, when comparing with those patients without mitral valve prolapse. The development of mitral valve prolapse was explained by a theory of imbalanced stability of a triangle formed by mitral leaflet, papillary muscle-chord, and left ventricular wall. Patients with an ASD yielded mitral valve prolapse due to a better left ventricular filling and a higher left ventricular ejection fraction.[3] His novel explanation has furnished considerable evidence for the understanding of this entity.

We noted a significant correlation between left ventricular diastolic dimension and peak pressure gradient across the tricuspid valve in patients with mitral valve prolapse (Group A) but not in patients with no mitral valve disease (Group B). However, Group B may not reach statistical significance due to small number of patients. Animal studies showed that an end-diastolic deformity in the right ventricular outflow tract may predispose the production of low early diastolic pressure in the local region.[11] The hemodynamic status of Group B patients in this study may thus represent an underlying cause of the impaired early diastolic function in the right ventricular outflow tract region.

Severe pulmonary hypertension secondary to mitral valve prolapse was very rare. Tago et al[12] reported such a rare association in a 65-year-old female patient, who had her condition improved by surgical operation with mitral valve replacement and tricuspid annuloplasty, and postoperative prostagrandin E1 proved effective for residual pulmonary hypertension. The pulmonary hypertension was considered to be related to the presence of latent left ventricular dysfunction.[13] In fact, right ventricular systolic pressure may correlate with diastolic function in other cardiovascular disease group of patients. The peak systolic pressure gradient over the tricuspid valve of 27 mmHg indicated mildly increased pulmonary pressures.[14] Morrison et al[15] observed that most of their elevated pulmonary artery pressure patients were passive elevations secondary to global or regional left ventricular dysfunction. Elevated left ventricular end-diastolic pressure was often associated with left ventricular systolic dysfunction, left ventricular hypertrophy, or pulmonary hypertension, which may predict increased perioperative mortality and morbidity.[16] These may account for why the majority of Group A patients showed pulmonary hypertension comparing to Group B patients in this study.

In conclusion, the presence of mitral valve prolapse with various degrees of mitral regurgitation in the patients with atrial level communication may implicate an impaired diastolic function of the left ventricle, and increased pulmonary artery pressure. Surgical intervention to the atrial level communication and mitral regurgitation may lead to a better prognosis in such patients.

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Institute of Cardiovascular Science and Medicine
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The University of Hong Kong

The Fifteenth Annual Scientific Meeting

17 September 2011
Hong Kong Convention and Exhibition Centre
Hong Kong

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SCIENTIFIC PROGRAMME

17 SEPTEMBER 2011 (SATURDAY)

08:30-09:00	**Registration**
09:00-10:40	**Oral presentations for Young Investigator Award** **Sponsored by Sun Chieh Yeh Heart Foundation** *Chairmen: Prof. O. Binah, Technion-Israel Institute of Technology* *Prof. P.M. Vanhoutte, The University of Hong Kong*
10:40-11:10	**Coffee break, poster viewing and booth visit**
11:10-12:00	**Poster presentations for Young Investigator Award** **Sponsored by Sun Chieh Yeh Heart Foundation** *Chairmen: Prof. Y.W. Kwan, Chinese University of Hong Kong* *Dr. M.L. Fung, The University of Hong Kong*
12:00-13:20	**Lunch**
13:20-13:30	**Opening ceremony** *Prof. P.M. Vanhoutte, The University of Hong Kong*
13:30-14:30	**Symposium I** **Sponsored by Boehringer Ingelheim Hong Kong Limited** *Chairmen: Prof. X.Q. Yao, Chinese University of Hong Kong* *Dr. L.F. Lee, Hong Kong College of Cardiology* **A new approach to anticoagulation to prevent thromboembolic events** *Dr. C.W. Siu, The University of Hong Kong, HKSAR* **The new NICE guideline on the treatment of hypertension** *Prof. B.M.Y. Cheung, The University of Hong Kong, HKSAR*
14:30-16:00	**Symposium II** **Sponsored by Stem Cell & Regenerative Medicine Consortium** *Chairman: Prof. R. Li, The University of Hong Kong* **Mitochondrial superoxide flashes in progenitor cells** *Prof. H. Cheng, Peking University* **Gene therapy for congestive heart failure** **– Inhibition of protein phosphatase 1 as a promising therapeutic target** *Dr. D. Sigg, NanoCor Therapeutics, Inc.*
16:00-16:20	**Coffee break, poster viewing and booth visit**
16:20-17:50	**Symposium III** *Chairmen: Prof. H.F. Tse, The University of Hong Kong, HKSAR* *Prof. Y. Huang, Chinese University of Hong Kong, HKSAR* **Molecular characterization and functional properties of induced pluripotent stem cells-derived cardiomyocytes from healthy and diseased individuals** *Prof. O. Binah, Technion-Israel Institute of Technology* **Therapeutic potential of pluripotent stem cell-derived mesenchymal stem cells in cardiovascular regeneration** *Dr. Q. Lian, The University of Hong Kong*
17:50-18:00	**Closing remarks and Young Investigator Award ceremony** *Prof. P.M. Vanhoutte, The University of Hong Kong, HKSAR*
18:00	**Annual General Meeting**

ABSTRACTS

Abstracts for Plenary Lectures:

PL1.

THE NEW NICE GUIDELINES ON THE TREATMENT OF HYPERTENSION

BMY Cheung
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In 2011, American and British guidelines on the management of hypertension are scheduled to be updated. The British guidelines, developed by the National Institute for Clinical Excellence, have just been published in August 2011. There are several significant changes in these guidelines. Ambulatory blood pressure is named the new gold standard in the diagnosis of hypertension. It is valuable not only in people with white coat hypertension, but also in people whose ambulatory blood pressures are higher than blood pressure readings in the clinic. Home blood pressure monitoring is now recognised as being useful and informative. Largely as a result of the ASCOT study, calcium channel blockers (CCB) and blockers of the renin-angiotensin system have surpassed diuretics and beta-blockers as first line drugs. Patients 55 or younger should receive an ACE inhibitor, or an angiotensin receptor blocker if the former is not tolerated. Older patients are started on a CCB. A thiazide diuretic is used as the third drug, but interestingly, chorthalidone and indapamide are preferred as they showed favourable outcomes in large clinical trials. Treatment with these three drug classes should be sufficient in the majority of patients, but if triple therapy is insufficient, referral to a hypertension specialist is recommended. Alpha and beta blockers do not even make fourth place, which is taken up by spironolactone.

While the new UK guidelines are controversial, the changes are based on the best available current evidence. In Hong Kong, the prevalence of hypertension has been increasing among young and middle-aged men, and is approaching 50% among the elderly. Current efforts are channelled towards the detection and treatment of hypertension in middle and old age. The linear rise in the prevalence of hypertension with age means that measures to prevent hypertension, such as a healthy diet and regular physical activity, should start early in life.

PL2.

MITOCHONDRIAL SUPEROXIDE FLASHES IN PROGENITOR CELLS

H Cheng
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The mitochondrion is a prominent cellular organelle pivotal to bioenergetics, cell proliferation, cell survival, and cell death. As the primary source of reactive oxygen species (ROS), mitochondria constantly generate low level of ROS due to the slippage of the electron transport chain (ETC). By employing a newly developed mitochondrial matrix-targeted superoxide indicator, mt-cpYFP, we have recently shown that individual mitochondria undergo spontaneous bursts of superoxide generation, termed "superoxide flashes", in many types of cells under physiological conditions, in intact beating hearts, or even in skeletal muscles and sciatic nerves of living animals. Individual flashes are triggered by transient openings of the mitochondrial permeability transition pore (mPTP), and the bursting superoxide production depends also on the integrity of the ETC, suggesting that they are a highly regulated phenomenon. Evidence is further provided that such superoxide flash activity offers a measure of oxidative stress under pathophysiological conditions (e.g., during ischemia-reperfusion injury). In cancer cells (e.g., HeLa cells), it serves as an early mitochondrial signal for oxidative stress-related apoptosis. In transgenic mice, the superoxide flash activity in skeletal muscles surges in response to systemic glucose challenge or insulin stimulation, in an apparently frequency modulatory (FM) manner. This result uncovers a tight metabolism-mPTP-superoxide flash coupling as a housekeeping mechanism that connects distinct functions of the mitochondria. Moreover, most recent results suggest a novel role for mitochondrial superoxide flashes in regulating neural progenitor cell fate, and as a mediator of the negative impact of amyloid β-peptide (Aβ) on neurogenesis. Taken together, our studies suggest that (i) superoxide flashes represent a novel, mPTP-regulated mode of ROS production and signaling by the mitochondria; (ii) activities of superoxide flashes could serve as a valuable biomarker for oxidative stress-related diseases, and (iii) the discovery of superoxide flashes may shed new light on mPTP and ETC functions as well as their heretofore unappreciated coupling in health and disease.

ABSTRACTS

Abstracts for Plenary Lectures:

PL3.

GENE THERAPY FOR CONGESTIVE HEART FAILURE – INHIBITION OF PROTEIN PHOSPHATASE 1 AS A PROMISING THERAPEUTIC TARGET

DC Sigg
CEO NanoCor Therapeutics, Inc.

A defect in calcium cycling via reduced SERC2a activity is a primary pathogenic mechanism in heart failure (HF). Approaches to increasing SERCA2a involve modulating upstream proteins that impact its activity. A central protein in the regulation of SERCA2a is protein phosphatase 1 (PP1). The association between PP1 and calcium cycling involves several upstream and downstream events. In HF, PP1 activity is increased leading to the dephosphorylation of phospholamban (PLN) which in turn decreases SERCA2a activity. Inhibition of PP1 results in inhibition of phospholamban (PLN) which in turn increases SERCA and Ca^{2+} uptake and contractility. In addition, inhibition of PP1 has other effects relevant to HF including inhibition of apoptosis and inhibition of the activity of MAP kinases. Apoptosis represents a common pathway in ischemic and non-ischemic HF in which cells progress from injury to cell death. The MAP kinases play a major role in the remodeling processes (i.e. fibrosis and hypertrophy) that occurs in the more chronic stages of HF (either ischemic or non-ischemic). Thus, inhibition of PP1 in the failing heart results in increases in phospholamban phosphorylation, leading to improvement of SR Ca^{2+}-handling, contractility and reversal of adverse remodeling by directly decreasing fibrosis and cardiac hypertrophy. An upstream modulator of PP1's activity is protein phosphatase inhibitor-1, designated I-1, which is activated via phosphorylation by protein kinase A (PKA). As further evidence of I-1's role in HF, I-1 has been reported to be inactivated (via reduced PKA-phosphorylation) in human failing hearts (Carr et al. 2002). While I-1 protein levels were found to be similar in non-failing and failing hearts, the inactive (dephosphorylated) form of I-1 was the predominant form (~60%) in the failing hearts, consistent with increased PP1 activity and PLN inhibition of SERCA2a. Since I-1 is inactive in human failing hearts, interventions to increase its activity may be beneficial. Rodent studies have shown that constitutively activating inhibitor 1 (I-1c) of PP1 (AA 1-65 with T35D) within the failing rat heart improves not only contractility but also reverses adverse remodeling by directly decreasing fibrosis and cardiac hypertrophy (Pathak et al., 2005). New translational research data will be presented demonstrating that overexpression of I-1c by intracoronary in vivo gene transfer of adeno-associated viral vector type 9 encoding for I-1c preserves cardiac function and reduces scar size in a preclinical model of heart failure.

PL4.

MOLECULAR CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF INDUCED PLURIPOTENT STEM CELLS-DERIVED CARDIOMYOCYTES FROM HEALTHY AND DISEASED INDIVIDUALS

O Binah
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In view of the therapeutic potential of cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CM), our overall goal is to investigate their molecular characteristics, functional properties related to the excitation-contraction coupling (e.g., $[Ca^{2+}]_i$ handling), pacemaker function and underlying ion currents, the effects of β-adrenergic stimulation, and responsiveness to common modifiers of cardiac function (e.g., I_f blocker). The iPSC clones we investigated were derived from human foreskin fibroblasts, dermal fibroblasts and hair keratinocytes. Reprogramming was accomplished by infecting the cells with retroviruses containing the four human genes: OCT4, Sox2, Klf4 and C-Myc. Our major findings show that iPSC-CM: (1) express cardiac specific RNA and proteins; (2) exhibit regular pacemaker activity; (3) exhibit key features of the excitation contraction coupling machinery; (3) respond to ryanodine and caffeine (albeit less than adult cardiomyocytes), and express the SR-Ca^{2+} handling proteins ryanodine receptor and calsequestrin. Hence, our work demonstrated that iPSC-CM exhibit features resembling the adult myocardium, and thus constitute a potential source for cardiac regeneration. Additionally, we investigated iPSC-CM generated from skin biopsies obtained from patients with the inherited arrhythmia Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). Our study focused on the autosomal recessive form of the disease caused by the missense mutation D307H in the cardiac calsequestrin gene, CASQ2. The major findings were that the CPVT cardiomyocytes demonstrated catecholamine-induced arrhythmogenesis, indicating that the CASQ2-mutated cardiomyocytes can be used to study the electrophysiological mechanisms underlying CPVT and for tailoring patient-specific anti-arrhythmic therapy.

ABSTRACTS

Abstracts for Plenary Lectures:

PL5.

THERAPEUTIC POTENTIAL OF PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS IN CARDIOVASCULAR REGENERATION

Q Lian

Cardiology Division, Department of Medicine; Research Centre of Heart, Brain, Hormone, and Healthy; Eye Institute, Faculty of Medicine, the University of Hong Kong, Hong Kong

Recent experimental and clinical studies suggest that transplantation of bone marrow derived mesenchymal stem cells (BM-MSCs) is an attractive therapeutic option for ischemic cardiovascular diseases. However, there are several potential limitations of using BM derived-MSCs (BM-MSCs). Firstly, BM-MSCs display a limited proliferative capacity and a large variability of cell quality derived from different donors. Secondly, during ex-vivo expansion before possible therapeutic use, these cells quickly loose differentiation potential and decrease ability to produce protective factors. Importantly, survival capacity and stem cell functions of BM-MSCs derived from aged or diseased donors are profoundly impaired, and thus limit their therapeutic efficacy. Human pluripotent stem cells (i.e.hESCs/iPSCs) may provide an alternative source of functional MSCs for tissue repairs. Here we will introduce the generation, characterization and application of human ESC and iPSC-derived MSCs for the treatment of cardiovascular diseases.

ABSTRACTS

Abstracts for Oral Communications:

OC1.

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

J Liu, WT Wong, XY Tian, LM Liu, CW Lau, X Yao, Y Huang
Institute of Vascular Medicine and School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

Objectives: Heme oxygenase-1 (HO-1), an inducible isoform of heme oxygenase, can exert vaso-protective effects. However, such vascular benefit in diabetic vasculopathy remains largely unclear. The present study aims at investigating the impact of HO-1 induction on endothelial function in diabetic *db/db* mice.

Methods: Diabetic *db/db* and non-diabetic *db/m+* mice were treated intraperitoneally with hemin (a potent inducer of HO-1) or vehicle for two weeks. The aortae were isolated and suspended in myograph for isometric force measurement; levels of marker proteins were determined by the Western blotting method; nitric oxide (NO) production in cultured human umbilical vein endothelial cells (HUVEC) was estimated using NO-sensitive DAF-DA fluorescence dye.

Results: Two-week hemin administration augmented endothelium-dependent relaxations in response to acetylcholine in *db/db* mouse aortae; this beneficial effect was antagonized by the co-treatment with the HO-1 inhibitor Sn(IV) mesoporphyrin IX dichloride (SnMP) and largely inhibited by the PI3K inhibitor wortmannin. Overnight treatment with hemin and bilirubin (a metabolic product of HO-1 activity) in organ culture reversed the impaired endothelial function in *db/db* mouse aortae and SnMP inhibited the effect of hemin but not that of bilirubin. Furthermore, hemin treatment increased the phosphorylation level of eNOS and Akt in diabetic mouse aortae. Hemin and bilirubin can restore the lost NO production in HUVECs exposed to high glucose and this effect was inhibited by the Akt inhibitor V and dominant-negative Akt transfection which suppresses the Akt expression. In addition, hemin treatment also improved the acetylcholine-induced relaxation in renal arteries from diabetic patients.

Conclusion: The present study demonstrates that the PI3K/Akt signaling pathway is likely to mediate the vascular benefit of HO-1 induction in restoring the impaired endothelial function in diabetic mice. The novel findings suggest that HO-1 induction could be a useful strategy for alleviation of diabetes-associated vascular dysfunction.

OC2.

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

SYW Kwan
Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong

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

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

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

ABSTRACTS

Abstracts for Oral Communications:

OC3.

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

NR Samaranayake,[1] KL Ong,[2] RYH Leung,[1] BMY Cheung,[1]
[1]Department of Medicine, The University of Hong Kong, Hong Kong, and [2]Lipid Research Group, Heart Research Institute, Sydney, New South Wales, Australia

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

OC4.

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

YY Zhang, GR Li
Department of Medicine, The University of Hong Kong, Hong Kong

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

ABSTRACTS

Abstracts for Oral Communications:

OC5.

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

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

OC6.

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

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

ABSTRACTS

Abstracts for Posters:

CP1.

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

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

CP2.

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

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

ABSTRACTS

Abstracts for Posters:

CP3.

INHIBITION OF NUCLEOSIDE TRANSPORTERS IN ENDOTHELIAL CELLS BY EMODIN

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

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

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

CP4.

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

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

ABSTRACTS

Abstracts for Posters:

CP5.

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

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

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

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

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

CP6.

CELLTHERAPY BEHIND THE ESSAY TUBES AND THE LAB COAT

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

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

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

Results:

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

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

ABSTRACTS

Abstracts for Posters:

CP7.

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

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

ABSTRACTS

Abstracts for Posters:

P1.

FUNCTIONAL TRANSIENT RECEPTOR POTENTIAL CHANNELS AND ADIPOGENIC REGULATION IN HUMAN PREADIPOCYTES

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Background: Preadipocytes are extensively used as a type of proliferative cell culture model to investigate proliferation and differentiation of adipocytes and lipodystrophy (e.g. obesity)-related metabolic dysfunctions and disorders. However, cell biology is not well understood in human preadipocytes. The present study was to investigate the expression of transient receptor potential (TRP) channels in human preadipocytes, and their role in regulating adipogenesis.
Methods: Whole-cell patch voltage-clamp, RT-PCR, Western blot, and confocal microscopic approaches were used to determine functional expression of TRP channels in cultured human preadipocytes. ShRNA targeting TRP channels were constructed to silence the related TRP channels. Adipogenesis and oil red O staining were applied to observe the effect of the TRP channels on cell differentiation.
Results: A small background current was inhibited by the TRPC channel blocker La^{3+}. Removal of Mg^{2+} of pipette solution or bath solution induced a Mg^{2+}-sensitive current, and the current was suppressed by the TRP channel blocker 2-aminoethoxydiphenyl borate. In addition, an intracellular Ca^{2+}-activated current was inhibited by the TRPV channel blocker capsazepine. RT-PCR revealed significant mRNA expression of TRPC1, TRPC4, TRPV2, TRPV4, and TRPM7 channels in human preadipocytes. Western blot analysis confirmed the protein expression of these TRP channels. Interestingly, shRNAs targeting TRPV2, TRPV4 and TRPM7 suppressed the corresponding gene and protein expression and significantly reduced adipogenesis of human preadipocytes, which was revealed by the reduced oil red O staining and the decreased expression of peroxisome proliferator-activated receptor gamma (PPARγ, a marker of adipogenesis).
Conclusion: Our results demonstrate for the first time that multiple TRP channels, TPC1/4, TRPV1/2/4, and TRPM7, are present in human preadipocytes. TRPV2, TRPV4 and TRPM7 channels participate in regulating adipogenesis.

P2.

AGING, HYPERTENSION AND HEART ENLARGEMENT INCREASED THE RISK OF VENTRICULAR ARRTHYMIA IN GENERAL POPULATION

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Objectives: To investigate the risk factors of arrthymia in general population.
Methods: A total of 25788 subjects were enrolled from an annual health examination. Their average age was 45±11.1 years, each of them received ECG examination. Heart enlargement was determined by cardio-thoracic proportion >= 0.5 on chest X-ray.
Results: Prevalence of hypertension was 30.6%, and that of diabetes was 6.0%. The rate of all cause of ECG abnormalities was 20.8%, and that of ventricular premature was 0.6%. Logistic regression showed that age>=65 years (OR 2.65, 95% CI 1.68-4.17, P<0.001), hypertension (OR 1.56, 95% CI 1.11-2.20, P=0.01) and heart enlargement (OR 4.74, 95% CI 1.11- 20.3, P=0.36) were independent predictors for presence of ventricular premature, but other variables such as hypercholesterolemia and hyperglycerides were not.
Conclusions: This study showed that aging, hypertension and heart enlargement increased risk of ventricular arrthymia in general population.

Table. Logistic Regression Analysis for Predicting

	Ventricular Premature	
Variables	**OR (95% CI)**	**P-value**
Age >=65 years	2.65 (1.68-4.17)	<0.001**
Female gender	1.09 (0.78-1.51)	0.63
Hypertension	1.56 (1.11-2.20)	0.01*
Diabetes	1.26 (0.72-2.21)	0.42
Left ventricular hypertrophy	2.13 (0.66-6.89)	0.21
Heart enlargement	4.74 (1.11-20.3)	0.036*

ABSTRACTS

Abstracts for Posters:

P3.

DRUG-INDUCED QT PROLONGATION IN GUINEA PIG IS AUTOMATICALLY ANALYZED BY MICRO-MAGNETOCARDIOGRAPHY SYSTEM WITH SUPERCONDUCTING QUANTUM INTERFERENCE DEVICE: COMPARISON WITH ECG

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Objectives: Drug-induced QT prolongation is a critical problem for drug development process. The guideline of International Conference on Harmonization requires to evaluate pre-clinical potential hazards of drug-induced QT prolongation. We have developed a micro-magnetocardiography (MCG) system for small animals comprised of ultrafine magnetometer array consisting of a 3×3 matrix of superconducting quantum interference device (SQUID) on a single silicon chip of 10 mm square.
Methods: Ten male Hartley guinea pigs (250 g) were anesthetized with pentobarbital. Platinum needle electrodes were attached to animals. Excessive quinidine (60 mg/kg) was administered intraperitoneally. MCG and ECG were recorded simultaneously at baseline, 3, 5, 7, 10, 15 min after injection. QT interval was automatically analyzed (PowerLab, ADInstruments Pty Ltd) and QTc was calculated by Bazett formula.
Results: QTc was prolonged from 284±10 to 299±11 msec in ECG (QTc-ECG). QTc was prolonged from 280±16 msec to 295±10 msec in MCG (QTc-MCG). QTc-MCG correlated well with QTc-ECG at baseline (r^2=0.944, $p<0.0001$) and at 15 min after injection (r^2=0.780, $p=0.0007$).
Conclusion: Micro-MCG successfully measured drug-induced QT prolongation with good correlation with ECG in guinea pig. Non-contact characteristics of MCG may enable high throughput screening test of drug-induced QT prolongation in the small animal.

P4.

PROTECTION AGAINST OXIDATIVE STRESS-INDUCED DAMAGE IN ENDOTHELIAL CELLS BY ERGOTHIONEINE

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Ergothioneine is abundantly found in mushroom. It possesses strong antioxidant effects by removing radical species or chelating metal ions. However, these antioxidant effects are mainly studied in simple cell-free systems but not in *in intro* or *in vivo* study. It is also not known whether ergothionine can enter the cells to exert antioxidant effect since ergothioneine is not permeable to cell membrane and a specific carrier noval organic cation transporter (OCTN)-1 is required for its cellular internalization. The objective of this study was to investigate whether or not ergothioneine can be taken up by endothelial cells, thereby protecting endothelial cells against oxidative stress-induced cellular damage.

Experimental results show that [^{3}H]ergothioneine could be taken up by human umbilical vein endothelial cells (HUVECs) through a sodium-dependent and transporter-dependent system. This ergothionine transport system was probably OCTN-1. In line with this notion, the results of RT-PCR demonstrated that OCTN-1 was expressed in HUVECs. Reactive oxygen species (ROS) generation of HUVECs was detected by the fluorescence intensity of DCF. The pyrogallol and xanthine/xanthine oxidase-induced production of ROS could be decreased by ergothionine. In addition. MTT assay was used to study the viability of HUVECs. The cytotoxic effects of hyperglycemia and hydrogen peroxide on HUVECs could be reduced by ergothionine.

In conclusion, our recent findings suggest that ergothioneine can be taken up by endothelial cells, probably through OCTN-1. Besides, ergothioneine is a potential agent that can protect endothelial cells against oxidative stress-induced cellular damage.

Acknowledgement: This study was financially supported by the RGC general research fund (project number: 771410).

ABSTRACTS

Abstracts for Posters:

P5.

OVARIECTOMY PROMOTES THE ENHANCEMENT OF ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR-MEDIATED RELAXATION IN MESENTERIC ARTERIES OF RATS WITH CHRONIC NITRIC OXIDE SYNTHASE INHIBITION

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Background and objectives: Gender differences exist in the incidence and manifestation of vascular diseases, of which endothelial dysfunction is the underlying cause. Endothelial dysfunction is associated with a reduced bioavailability of nitric oxide. Therefore, the effects of ovariectomy with and without 17β-estradiol supplement on endothelial function were examined in rats following chronic inhibition of nitric oxide synthases with L-NAME.
Methods: Female Sprague Dawley rats were ovariectomized or sham-operated at 12 weeks old. Half of ovariectomized rats were supplemented with 17β-estradiol (25 $\mu gkg^{-1}day^{-1}$, intramuscularly) or its vehicle (olive oil) until they were sacrificed. At 18 weeks old, all rats were administered daily with L-NAME (60 $mgkg^{-1}$, by gavage) or its vehicle (drinking water) for 6 weeks. Rats were then anaesthetized for blood pressure measurement and for isolation of mesenteric arteries for isometric tension measurement in organ baths.
Results: Chronic L-NAME treatment did not increase blood pressure in all rats, but impaired endothelium-dependent relaxation in ovariectomized rats without 17β-estradiol supplement. This impairment was reversed in the presence of indomethacin (a cyclooxygenase inhibitor). Chronic L-NAME treatment improved endothelium-derived hyperpolarizing factor (EDHF)-mediated relaxation only in mesenteric arteries of ovariectomized rats without 17β-estradiol supplement, although ovariectomy alone reduced EDHF-mediated relaxation.
Conclusions: Inhibition of cyclooxygenase alone, but not 17β-estradiol supplement, improved endothelial function in ovariectomized rats with chronic nitric oxide synthase inhibition. This greater relaxation appears to be mediated by EDHF, the function of which is enhanced when there is the occurrence of cyclooxygenase-dependent contraction.

This study was supported by a General Research Fund of the Research Grant Council of HKSAR, and a Committee on Research and Conference Grant, The University of Hong Kong.

P6.

PURINERGIC RECEPTORS MEDIATE PROLIFERATION AND MIGRATION IN CULTURED HUMAN CARDIAC FIBROBLASTS

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Objectives: Physiological role of ATP in human cardiac fibroblasts is unknown, and the present study was to investigate whether ATP regulates proliferation and migration and purinergic receptors involvement in cultured human cardiac fibroblasts.
Methods and results: Cell proliferation and migration assay, RT-PCR, Western blot, siRNA gene silence, and flow cytometric analysis were used in cultured human cardiac fibroblasts. We found that ATP (1-100 μM) increased cell proliferation in a concentration-dependent manner. The P2X receptor agonist AMP-CPP and P2Y receptor agonist ATP-γS displayed a similar role in regulating cell proliferation. The P2 receptor antagonists suramin and reactive blue-2 countered the ATP-induced increase of proliferation. Silence of P2X4, P2X7 and P2Y2 with the corresponding siRNAs significantly inhibited proliferation of fibroblasts. In addition, ATP significantly promoted migration of human cardiac fibroblasts. Flowcytometry and Western blot analysis revealed that ATP promoting G0/G1 cells to S phase via increasing cyclin D1 and cyclin E protein expression, and phosphorylated Akt and ERK1/2 levels. Suramin, reactive blue-2, the PI3K inhibitor wortmannin, the Akt inhibitor API-2, and the MAPK inhibitors PD98059 reduced phosphorylated Akt and ERK1/2 levels antagonized ATP-induced increase of proliferation and migration in cultured human cardiac fibroblasts.
Conclusions: These results demonstrate the novel information that ATP stimulates proliferation and migration of human cardiac fibroblasts mediated by activating P2X4, P2X7 and P2Y2 receptors, increasing phsophorylated PI3K/Akt, MAPK/ERK1/2, and modulating cyclin D1 and cyclin E expression, which likely plays a role in cardiac remodeling of the injured heart.

ABSTRACTS

Abstracts for Posters:

P7.

ELECTROPHYSIOLOGICAL PROPERTIES OF ENDOTHELIAL PROGENITOR CELLS FROM RAT BONE MARROW MONONUCLEAR CELLS

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Objectives: Bone marrow endothelial progenitor cells (BMEPCs) are believed to be a promising cell source for regenerative medicine; however, their electrophysiology properties have not been fully clarified, which is important to the clinical application of BMEPCs. The current study was designed to determine the transmembrane ion currents and mRNA expression levels of related ion channel subunits in rat BMEPCs.
Methods: Bone marrow mononuclear cells were isolated by density gradient separation and cultured in EPC medium. The transmembrane ion currents were determined using whole cell patch-voltage clamp technique, the levels of mRNA expression of functional ionic channels were measured using reverse transcription polymerase chain reaction (RT-PCR).
Results: We observed two types of ionic currents in undifferentiated rat BMEPCs. One was Ca^{2+}-activated potassium current (I_{kca}), which was seen in approximate 90% of cells when 1 μM Ca^{2+} was employed in pipette solution, and it was predominantly inhibited by intermediate-conductance inhibitor clotrimazole. The other one was volume-sensitive chloride current (I_{cl}), which was detected in 85.7% of cells when BMEPCs were subjected to K^+-free hypotonic extracellular solution, whose currents could be inhibited by 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB). The corresponding ion channel genes and proteins, KCNN4 for I_{kca} and Clcn3 for I_{cl}, were confirmed by RT-PCR and western immunoblotting analysis in BMEPCs.

P8.

ROLES OF TRPV1 AND TRPV2 CHANNELS IN THE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS TO CARDIOMYOCYTES

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The transient receptor potential vanilloid (TRPV) channels are Ca^{2+}-permeable non-selective cation channels, which can be activated by a variety of mechanisms, including thermo, low pH, osmolarity and stretch stress. However their roles in the differentiation of mouse embryonic stem cells (mESC) to ventricular cardiomyocytes are unknown. In this study, we tested the effects of capsaicin (an activator of TRPV1 channel, 1 μM), capsazepine (a TRPV1 antagonist, 10 μM), SB366791 (a potent and selective TRPV1 inhibitor, 10 μM), and probenecid (a TRPV2 agonist, 100 μM), on mESC differentiation. In mESC differentiation stage, the capsazepine and SB366791 suppressed the embryonic body formation. qPCR results also demonstrated capsazepine and SB366791 significantly inhibited the differentiation of mESC to cardiomyocytes. Treatment of capsazepine and SB366791 suppressed the expression level of cardiomyocyte marker genes (cardiac actin, c-TnT, c-TnI, -MHC and MLC2a) to only about one tenth of that in DMSO control group. However, capsaicin did not have any significant effect on mESC differentiation. Interestingly, as an activator of TRPV2, probenecid did not up-regulate the cardiomyocyte marker genes expression, on the contrary, it inhibited this process. The marker genes expression is less than half of the control group. Together these data suggest that TRPV1 and TRPV2 channels present important roles in the differentiation of mESC to cardiomyocytes. Further study may offer a new strategy to regulate the stem cells differentiation for therapeutic transplantation.

P9.

WY14643, THE DUAL PPAR ALPHA/GAMMA AGONIST, IMPROVES ENDOTHELIAL FUNCTION IN THE AORTA OF THE SPONTANEOUSLY HYPERTENSIVE RAT

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Wy14643 is an agonist at peroxisome proliferator-activated receptors (PPAR) α and γ. PPAR α and ψ agonists are currently used in the treatment of dyslipidemia and insulin-resistance, respectively. The present study aimed to determine whether or not Wy14643 improves endothelial dysfunction in hypertension, and if so, to determine the mechanism involved. Isometric tension was measured in isolated thoracic aortic rings of spontaneously hypertensive rats (SHR). Wy14643 caused more pronounced relaxations than fenofibrate (PPARα agonist) or rosiglitazone (PPARγ agonist). MK886 (PPARα antagonist) nearly abolished the Wy14643-induced relaxation while GW9662 (PPARγ antagonist) caused a modest inhibition of the response. L-NAME (nitric oxide synthase inhibitor) and ODQ (soluble guanylyl cyclase inhibitor), given alone or in combination, inhibited these relaxations to the same extent. Compound C [adenosine monophosphate-activated protein kinase (AMPK) inhibitor] reduced Wy14643-induced relaxations to the same extent as L-NAME. Wy14643 and fenofibrate, but not rosiglitazone, significantly increased the protein expression (determined by Western blotting) of phosphorylated endothelial nitric oxide synthase and AMPK and their ratio to the total forms of the enzymes in aortae of SHR but not in those of WKY. Compound C but not L-NAME abolished the above effect. Endothelium-dependent contractions evoked by acetylcholine in the presence of L-NAME were reduced by Wy14643 and fenofibrate but not by rosiglitazone. MK886, but not GW9662, prevented the Wy14643-induced inhibition. Wy14643 and fenofibrate inhibited acetylcholine-induced prostanoid release to the same extent. Cyclooxygenase-1 expression and activity were reduced after administration of Wy14643 and fenofibrate in the aorta of SHR. The present data suggest that the PPARα agonists induce nitric oxide-mediated relaxation through activation of AMPK. This relaxing effect is more prominent with Wy14643. Together with the ability to reduce the release of endothelium-dependent contracting factors, it appears that dual PPAR agonists such as Wy14643 provide better protection against the endothelial dysfunction of spontaneous (essential) hypertension.

ABSTRACTS

Abstracts for Posters:

P10.

UPREGULATED TRPM2 AND ITS POTENTIAL ROLE IN NEOINTIMAL HYPERPLASIA

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A hallmark in atherosclerosis is progressive intimal thickening, which leads to occlusive vascular disease such as myocardial infarction and stroke. A causation of neointimal hyperplasia is the migration and proliferation of smooth muscle cells, in which reactive oxygen species (ROS) play an important role. This study was designed to investigate the involvement of TRPM2, a member of the transient receptor potential superfamily, in neointimal hyperplasia. Neointimal hyperplasia of rat femoral artery was induced by cuff placement. The TRPM2 expression and ROS generation were detected. The effect of TRPM2 inhibitors on hydrogen peroxide-induced proliferation of primary rat aortic smooth muscle cells was further examined. It was found that arteries with cuff placement for 14 days showed distinct intimal thickening. The neointima has enhanced cell cycle activity and upregulated TRPM2 expression. ROS generation was dramatically increased in the neointima and media layer of arteries after cuff placement. *In vitro*, exposure to hydrogen peroxide at low concentration promoted the proliferation of smooth muscle cells. This effect was abolished by both TM2E3, a specific blocking antibody to TRPM2, and 2-aminoethoxydiphenyl borate, a chemical blocker. These results suggest that TRPM2 is involved in neointimal hyperplasia and is a potential therapeutic target of ROS-induced hyperplasia of vascular smooth muscle cells.

This work was supported by Hong Kong RGC Grant CUHK477408, CUHK479109 and CUHK478710, Focused Investment Scheme and Group Research Grant of CUHK.

P11.

THE FUNCTIONAL ROLE OF TRPM2 CHANNELS IN H_2O_2-INDUCED CA^{2+} RISES AND CELL APOPTOSIS IN ENDOTHELIAL CELLS

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Objectives: Melastatin-like transient receptor potential channel 2 (TRPM2) is an oxidant-sensitive, non-selective cation channel which has been shown to be widely expressed in mammalian tissues, including the vascular endothelium. Here we investigated the functional role of TRPM2 channels in hydrogen peroxide (H_2O_2)-induced endothelial Ca^{2+} responses and cell apoptosis in microvessel endothelial cells (H5V).

Methods: With the use of Cytosolic Ca^{2+} measurement, DNA ladder formation assay, DAPI Staining, western blotting, MTT assay, TRPM2 shRNA and transfection methods, it was found that TRPM2 play a key role in H_2O_2-induced endothelial Ca^{2+} responses and cell apoptosis. Application of H_2O_2 caused a significant increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$), which was inhibited by TM2E3, a TRPM2 specific blocking antibody, or by TRPM2 shRNA. In addition, the data of DNA ladder formation assay showed that pretreatment of TM2E3 successfully protected the cells from H_2O_2-induced apoptosis, which was also confirmed by TRPM2 shRNA. Moreover, over-expression of TRPM2 in H5V cells significantly increased cell sensitivity to Ca^{2+} overload and cell apoptosis under H_2O_2 treatment.

Results: These data strongly suggest that TRPM2 channels are involved in important intrinsic mechanisms, which mediate H_2O_2-induced Ca^{2+} overload and cell apoptosis in H5V cells. Inhibition of endogenous ROS-sensitive TRPM2 channels with TM2E3 or their specific shRNA significantly protects the vascular cells from apoptotic cell death.

ABSTRACTS

Abstracts for Posters:

P12.

ACTIVE TRANSPORT FOR CARDIOVASCULAR HEALTH

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Background: Active transport is the travel by walking, cycling, and other non-motorized modes from a place to destinations. Research suggested that walking or cycling in about 30 minutes daily is a kind of moderate physical activity to prevent ill health. Nevertheless, it is recognized that active transport offers health gain for cardiovascular health. The objective of this study is to identify the benefits of active transport to cardiovascular health from current literature.
Methods: A review of literature published between 2005 and 2011 was conducted. Results focusing on the benefits of active transport to cardiovascular health were summarized.
Results: The review of literature showed beneficial results of active transport to cardiovascular health. Active transport could modulate heart rate and maximize blood flow to contracting muscle during exercise, at the same time, minimize the energy cost of contraction of cardiac muscle. People who walk more could have a improved systolic and diastolic blood pressure and body mass index, thus, improve the heart workload. Also, people who did less active transport reported higher risk of cardiovascular death. Nevertheless, it could improve cardiorespiratory fitness for ill-health patients.
Conclusions: It is concluded that active transport benefits cardiovascular health in a number of ways. Thus, it is recommended that participating in active transport should be encouraged and be integrated into individual's daily life in order to promote cardiovascular health.

P13.

BONE MORPHOGENIC PROTEIN-4 IMPAIRS THE ENDOTHELIAL FUNCTION THROUGH INCREASING OF OXIDATIVE STRESS IN TYPE 2 DIABETIC MICE

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Objectives: Bone morphogenic protein (BMP4) stimulates superoxide production and exerts proinflammatory effects in the endothelium. BMP4 also mediates endothelial dysfunction in hypertension. However, the role of BMP4 in endothelial dysfunction of type 2 diabetes remains unknown. The present study aims to investigate whether inhibition of BMP4 can improve the endothelial function of type 2 diabetic mice, and to study the mechanism underlying BMP4-induced oxidative stress in diabetes.
Methods: *db/db* mice were infused with BMP4 inhibitor noggin (0.4 mg/kg/day) or vehicle through osmotic pump for two weeks, and vasoreactivities of mouse aortae and mesenteric arteries were measured. Isolated aortae of *db/db* mice were treated with BMP4 inhibitors including noggin, chordin, and follistatin for 24 hours. Reactive oxidative species (ROS) in both aortic endothelium and cultured endothelial cells were measured by DHE and CM-H_2DCFDA fluorescence.
Results: Noggin infusion in *db/db* mice reduced systolic blood pressure without affecting the insulin and glucose tolerance. Endothelium-dependent relaxations (EDRs) in both aortae and resistance mesenteric arteries improved after noggin infusion in *db/db* mice. Treatment with noggin, chordin, or follistatin for 24 hours improved EDRs in *db/db* mouse aortae. ROS production was reduced by BMP4 inhibitors in *en face* endothelium of *db/db* mouse aorta. BMP4 treatment also increased ROS production in both *en face* endothelium of C57BL/6J mice and HUVECs, which was inhibited by BMP4 inhibitors, diphenyleneiodonium, and tempol. In addition, p38 inhibitor SB202190 or JNK inhibitor SP600125 also improved EDRs in *db/db* mouse aortae, and EDRs in C57BL/6J mice aortae impaired by BMP4.
Conclusions: The present study showed that inhibition of BMP4 *in vivo* reduces blood pressure and improves endothelial function of *db/db* mice. Oxidative stress, p38 and JNK activation contribute to BMP4-induced endothelial dysfunction in diabetes. BMP4 could be a potential therapeutic target in the treatment of diabetic vascular dysfunction.

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