



Hong Kong College of Cardiology

Fourteenth Annual Scientific Meeting - Institute of Cardiovascular Science and Medicine

Follow this and additional works at: <https://www.jhkcc.com.hk/journal>

Recommended Citation

Fourteenth Annual Scientific Meeting - Institute of Cardiovascular Science and Medicine *Journal of the Hong Kong College of Cardiology* 2010;18(2) <https://doi.org/10.55503/2790-6744.1067>

This Full Issue is brought to you for free and open access by Journal of the Hong Kong College of Cardiology. It has been accepted for inclusion in Journal of the Hong Kong College of Cardiology by an authorized editor of Journal of the Hong Kong College of Cardiology.

Journal of the Hong Kong College of Cardiology



Editor-in-Chief

Chu-Pak Lau

Editorial Board

Raymond Hon-Wah Chan

Wai-Kwong Chan

Wai-Hong Chen

Chun-Ho Cheng

Bernard Cheung

Chung-Seung Chiang

Moses S S Chow

Wing-Hing Chow

Katherine Fan

Chi-Lai Ho

Kau-Chung Ho

David Sai-Wah Ho

Cyrus R Kumana

Suet-Ting Lau

Yuk-Kong Lau

Tin-Chu Law

Kathy Lai-Fun Lee

Stephen Wai-Luen Lee

Maurice P Leung

Sum-Kin Leung

Wai-Suen Leung

Wing-Hung Leung

Shu-Kin Li

Archie Ying-Sui Lo

Che-Keung Mok

Ngai-Shing Mok

Chiu-On Pun

John E Sanderson

Brian Tomlinson

Hung-Fat Tse

Kai-Fat Tse

Tak-Ming Tse

Siu-Hong Wan

Kwok-Yiu Wong

Alexander Shou-Pang Wong

Kam-Sang Woo

Cheuk-Man Yu

International Editorial Consultants

A John Camm

Shih-Ann Chen

Victor Dzau

Barry A Franklin

Dayi Hu

Hamid Ikram

David T Kelly

Bertram Pitt

William C Roberts

Delon Wu

Section Editors

John E Sanderson, Editor of Clinical Cardiology

Suet-Ting Lau, Editor of Preventive Cardiology

Kau-Chung Ho, Editor of Invasive Cardiology

Yuk-Kong Lau, Editor of Non-invasive Cardiology

Chu-Pak Lau, Editor of Pacing and Electrophysiology

Cyrus R Kumana, Editor of Basic Cardiology: Pharmacology

Wai-Kwong Chan, Editor of Images in Cardiology: ECG

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.

INSTRUCTION FOR AUTHORS

The Journal of the Hong Kong College of Cardiology publishes peer-reviewed articles on all aspects of cardiovascular disease, including original clinical studies, review articles and experimental investigations. As official journal of the Hong Kong College of Cardiology, the journal publishes abstracts of reports to be presented at the Scientific Sessions of the College as well as reports of the College-sponsored conferences.

Manuscripts submitted to this journal must not be under simultaneous consideration by any other publication and should not have been published elsewhere in substantially similar form. The letter of submission must so affirm. A transfer of copyright form to be signed by all authors to the Hong Kong College of Cardiology should accompany all submitted articles. All manuscripts should be submitted to the **Editor-in-Chief, Journal of the Hong Kong College of Cardiology, c/o Medcom Limited, Room 504-5, Cheung Tat Centre, 18 Cheung Lee Street, Chai Wan, Hong Kong.**

Manuscript Preparation

Manuscripts must be submitted in English in triplicate (one original and two copies) and typed double-spaced on A4 size white bond paper. This applies to all parts of the manuscript, i.e. references, legends, etc. Liberal margins should be left at the top and bottom, as well as the sides. Except for editorials, images/ECG and letters, all manuscript should be submitted in the following order: Title Page, Abstract, Text, References, Tables, Legends, and Figures. Each page, beginning with the summary, should also include the senior author's surname typed on the upper, left-hand corner. The author should not make any changes in the proofs except for corrections of editorial errors, if any, and/or correction of typesetter's errors. Employees of industry may not evaluate or comment about the products of a competitor. A commercial name should not be part of a manuscript title. Finally, authors should make no claims of priority in their manuscripts.

Title Page

- Include full name(s), degree(s) and affiliation(s) of author(s); list under file.
- Give a running title of 3 to 6 words.
- At the bottom of the page, include information about grants, if applicable.
- Add: "Address for reprint:...", followed by full name, address, telephone and fax numbers.

Abstract

- Abstract should be after title page and numbered page 1.
- It should not exceed 250 words for major articles; case reports should have abstracts of no more than 100 words.
- At the end of the abstract, provide a maximum of 6 key words suitable for indexing.
- Abbreviations should be kept to a minimum and must be explained when they first appear; after first use, abbreviations alone may be used.
- Standard abbreviations should be used for all measurements (SI units).

Text

- The text should follow the abstract and begin on a new page, as should References, Tables, and Legends.
- Abbreviations not defined in the abstract should be explained when they first appear in the text.
- References should be cited in numerical order, as should tables and figures.

References

- Number in the order in which they appear in the text.
- Abbreviate titles of periodicals according to the style of the Index Medicus.
- Follow the format (arrangement, punctuation) shown below:

Periodicals

1. Lewis T. Paroxysmal tachycardia. *Heart* 1909;1:43-72.
(if more than three authors, please use "et al." after the third).

Books (edited by other authors of article)

2. Furman S. Pacemaker follow-up. In Barold SS, (eds): *Modern Cardiac Pacing*. Mount Kisco, New York, Futura Publishing Company, 1985, pp. 889-958.

Books (identical author and editor)

3. Chung EK. *Principles of Cardiac Arrhythmias*. Baltimore, MD, Williams & Wilkins, 1977, pp. 97-188.

Abstracts

4. Same as periodicals and followed by "(abstract)".

Tables

- Tables should supplement, but not duplicate, the text.
- Tables should be numbered consecutively in order of appearance in the text.
- Each table must be given an Arabic numeral and a title, placed at the top of the page.
- Abbreviations used in the table should be foot-noted and explained in the order in which they appear in the table, if they have not been previously used.
- Any material which is not self-explanatory should be foot-noted as well.

Legends

- Be sure that legends and figures correspond.
- Identify all abbreviations used in a figure at the end of each legend, if the abbreviation has not been used in the text.
- Be sure abbreviations used for measurements are standard SI unit.

Figures

- Submit either 3 black and white glossy prints or 2 prints and one photocopy, preferably of 13 cm x 18 cm (5" x 7") size.
- On the back of each figure, indicate number, senior author's surname, top of illustration; all of this should be written lightly with soft, black pencil.
- Submit written permission from publisher(s) for any figure which has been published previously.
- Do not use clips on illustrations; submit them in an envelope backed by cardboard.
- Any lettering or scale of measurement used in an illustration must be large enough to be legible in the event of half-size reduction.
- Do not send original art-work, X-rays, or ECGs.
- Photographs in which a patient or other person is identifiable must have written permission from that person. The consent must state specifically what the person is consenting to and what restrictions, if any, the person has placed upon the publication of the photograph. All restrictions must be strictly observed.
- Colour illustrations are costly and will be charged to the author.
- Authors should inquire about cost from the publisher before submitting a colour illustration.

Ethics

Published studies on human subjects should indicate the nature of consent and the approval of the institutional ethics committee if deemed appropriate. In case of animal experiments, ethical approval must be enclosed.

The author is responsible for all material presented in a paper. The journal disclaims all responsibility for such material. No product or service advertised in this publication is guaranteed or warranted either by the Editors or publisher. Neither the Editors nor publisher guarantee any claims made by a manufacturer or an author in regard to a product or service. If a trademark item is named, the name(s) and address(es) of the manufacturer(s) or supplier(s), in addition to the generic name, should be foot-noted.

Reprints are available. Ordering information can be obtained from the above address.

Subscription Rates

Local Subscription: HK\$200/year (including postage)
Overseas Subscription: US\$120/year (including airmail postage)



Table of Contents

- **REVIEW ARTICLES**

- Evaluation of Pulmonary Hypertension Using the Right to Left Ventricular Diameter Ratio on Contrast-Enhanced Computed Tomography**

- Tetsuo Ichinose, Yuji Nakazato, Hiroyuki Daida, Youichi Katoh, Tetsuya Oigawa, Hakuoh Konishi, Kosei Tanimoto, Yasunobu Kawano, Miki Yamase, Shuta Tsuboi, Yuki Yokomatsu, Hiroshige Yuu44

- In the Wireless Era: Leadless Pacing**

- Kathy LF Lee and Chu-Pak Lau53

- **FOURTEENTH ANNUAL SCIENTIFIC MEETING**

- Institute of Cardiovascular Science and Medicine*

- Organizing Committee.....60**

- Scientific Programme.....61**

- Abstracts.....62**

The Hong Kong College of Cardiology



The Council

President
President-Elect
Honorary Secretary
Honorary Treasurer
Immediate Past President
Accreditation and Education Committee Chairman
Scientific Committee Chairman
Chief Editor
General Affairs and Public Relations Committee Chairman
Council Members

Chung-Seung Chiang
Chris Kwok-Yiu Wong
Kam-Tim Chan
Shu-Kin Li
Chiu-On Pun
Tak-Fu Tse
Chiu-On Pun
Chu-Pak Lau
Shu-Kin Li
Raymond Hon-Wah Chan
Wai-Kwong Chan
Chun-Ho Cheng
Wing-Hong Fung
Charles Kau-Chung Ho
Patrick Tak-Him Ko
Chu-Pak Lau
Suet-Ting Lau
Yuk-Kong Lau
Stephen Wai-Luen Lee
Kai-Fat Tse
Cheuk-Man Yu
Peggy Cheung
Walter Ma

Honorary Legal Adviser
Honorary Auditor

Correspondence for Hong Kong College of Cardiology

Secretariat, Room 1116, Bank of America Tower, 12 Harcourt Road, Hong Kong.

Tel: (852) 2899 2035, Fax: (852) 2899 2045

E-mail: enquiry@hkccchk.com

Evaluation of Pulmonary Hypertension Using the Right to Left Ventricular Diameter Ratio on Contrast-Enhanced Computed Tomography

TETSUO ICHINOSE,¹ YUJI NAKAZATO,¹ HIROYUKI DAIDA,² YOUICHI KATOH,¹ TETSUYA OIGAWA,¹ HAKUOH KONISHI,² KOSEI TANIMOTO,¹ YASUNOBU KAWANO,¹ MIKI YAMASE,¹ SHUTA TSUBOI,¹ YUKI YOKOMATSU,¹ HIROSHIGE YUU¹

From The Department of Cardiology, ¹Juntendo University Urayasu Hospital, Chiba; ²Juntendo University Hospital, Tokyo, Japan

ICHINOSE ET AL.: *Evaluation of Pulmonary Hypertension Using the Right to Left Ventricular Diameter Ratio on Contrast-Enhanced Computed Tomography.* **Objective:** To assess the severity of a right heart overload due to pulmonary hypertension (PH), the estimated right ventricular systolic pressure (RVSP) determined by echocardiography has generally been measured. The purpose of the present study was to evaluate the correlation between the right to left ventricular diameter (RV/LV) ratio determined with contrast-enhanced computed tomography (CE-CT) and the RVSP by echocardiography. **Methods and Materials:** 63 patients were enrolled and placed in two groups consisting of those with or without a right heart overload that was defined as an RV pressure of more than 40 mmHg on echocardiography. PH was observed in 31 patients (PH group) and the remaining 32 patients exhibited no PH (control group). In both groups, the RV and LV diameters were measured as the maximum inner diameter of each heart chamber in the axial image obtained from the CE-CT. **Results:** The RVSP of the PH and control groups was 58.5 ± 20.7 mmHg and 26.1 ± 0.4 mmHg, respectively. The RV/LV ratio for the PH group was significantly greater than that of the control group (1.1 ± 0.4 and 0.8 ± 0.1 , $p < 0.05$). In both groups, the RV/LV ratio was correlated ($r^2 = 0.21$, $p < 0.05$) with the RVSP. An optimal cut off value of the diagnostic value of the RV/LV ratio of above 1.0 in the PH group had a sensitivity of 64.5%, specificity of 100%, positive predictive value of 100% and negative predictive value of 74.4%. **Conclusion:** The RV/LV ratio significantly correlated with the RVSP and that ratio was a useful parameter for diagnosing a right heart overload. (*J HK Coll Cardiol* 2010;18:44-52)

Computed tomography, Echocardiography, Estimated right ventricular systolic pressure, Pulmonary hypertension, Right and left ventricular diameter

摘要

目的：超聲心動圖的右室收縮壓（RVSP）廣泛用於評估肺動脈高壓引起的右室超負荷嚴重程度。本研究的目的是評估增強-電腦掃描（CE-CT）測量的右/左室直徑比（RV/LV）和超聲心動圖的右室壓之間的關係。**方法與材料：**本研究共納入63例患者。以超聲心動圖的右室壓超過40 mmHg為標準，分為右室超負荷組及非右室超負荷組。其中31例為肺動脈高壓組，32例為非肺動脈高壓組（對照組）。以增強CT測量心臟搏動週期內軸向最大內徑為右室和左室直徑。**結果：**肺動脈高壓組和對照組的右室收縮壓分別為 58.5 ± 20.7 mmHg 和 26.1 ± 0.4 mmHg。肺動脈高壓組RV/LV比

Address for reprints: Dr. Tetsuo Ichinose
The Department Cardiology, Juntendo University Urayasu
Hospital, 2-1-1 Tomioka, Urayasu, Chiba, 279-0021, Japan

Email: tichinose@juntendo-urayasu.jp

Received April 28, 2010; revision accepted August 13, 2010

顯著高於對照組 (1.1 ± 0.4 vs 0.8 ± 0.1 , $p < 0.05$)。兩組內RV/LV比與RVSP有顯著相關性 ($r^2 = 0.21$, $p < 0.05$)。肺動脈高壓組內RV/LV > 1.0 為最優診斷意義值，敏感度64.5%，特異性100%，陽性預測率100%，陰性預測率74.4%。
結論：RV/LV值與RVSP顯著相關，可作為診斷右室超負荷的有效指標。

關鍵詞：電腦掃描 超聲心動圖 估計右室測量壓 肺動脈高壓 右室左室直徑

Introduction

Echocardiography has been recognized as a rapid and non-invasive method for evaluating the right heart overload. Using Doppler echocardiography, the right ventricular systolic pressure (RVSP) has been shown to be highly correlated with the pulmonary artery pressure during right heart catheterization.^{1,2} The assessment of pulmonary hypertension which suggests a right heart overload has been performed by measuring the estimated RVSP using echocardiography. However, some studies³⁻⁶ have reported that the right to left ventricular diameter (RV/LV) ratio determined with contrast-enhanced computed tomography (CE-CT) is useful for assessing the severity of pulmonary thromboembolisms (PTEs). The purpose of the present study was to evaluate the correlation between the RV/LV ratio determined by CE-CT and the RVSP by echocardiography.

Materials and Methods

1) Patient Characteristics

In this retrospective study, from December 2008 to March 2009, consecutive 63 patients were divided into two groups consisting of those with or without a right heart overload. In standard echocardiographical definition of pulmonary hypertension (PH), maximum flow velocity and pressure gradient of a tricuspid regurgitation considered as more than 2.8 m/second and 30 mmHg at a rest.⁷ If a right atrial pressure estimate of more than 10 mmHg is used for PH, estimated RVSP of more than 40 mmHg is reasonable to consider PH. So the pulmonary hypertension group consisted of those diagnosed with an estimated RVSP of more than 40 mmHg on the echocardiography, and the control

group as those diagnosed with an estimated RVSP of less than 39 mmHg. Those with an acute or active stage of PH were excluded from this study. Other exclusion criteria included (i) dilatation of the thickness or lumen such as with a myocardial infarction, pacemaker implantations, left bundle branch block, left ventricular hypertrophy, or dilated and hypertrophic cardiomyopathy, and (ii) pericardial involvement such as a pleural effusion or pericarditis.

The baseline patient characteristics and comorbidities are presented in Table 1. Sixty-three patients (29 males and 35 females) were included in this study. Thirty-one patients (mean age, 60.5 ± 20.8 , 13 males and 18 females) were enrolled in the PH group and the remaining 32 (mean age, 59.8 ± 13.6 , 16 males and 17 females) in the control group. The clinical pathogenesis of the PH was as follows; 9 patients with congestive heart failure (6 with valvular disease, 1 with hypertensive heart disease, 1 with atrial septal defect and 1 with ischemic heart disease), 6 with malignant disease, 5 with chronic obstructive pulmonary disease, 4 with pulmonary PTEs, 3 with primary pulmonary hypertension and 4 with collagen disease. Maximum pressure gradient of a tricuspid regurgitation in the PH group and the control group were 3.5 ± 0.6 mmHg and 2.2 ± 0.3 mmHg ($p < 0.001$), respectively. And its severity were as follows, 49 with mild tricuspid regurgitation (PH group, $n=17$, control group, $n=32$), 5 with moderate tricuspid regurgitation (PH group, $n=5$) and 4 with severe tricuspid regurgitation (PH group, $n=4$). The patients received warfarin potassium (PH group, $n=11$; control group, $n=3$), Beraprost sodium (PH group, $n=5$), Bosentan hydrate (PH group, $n=2$), Sildenafil citrate (PH group, $n=3$), diuretics (PH group, $n=9$; control group, $n=2$) and depressors (calcium channel antagonists, $n=8$, angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin II receptor blockers

Table 1. Clinical basis of the disease in the 63 patients

	PH group (n=31)		Control group (n=32)		P value
Age (years)	60.5±20.8		59.8±13.6		NS
Male/Female	13/18		16/17		NS
BMI (kg/m ²)	21±5.4		23±3.7		NS
Comorbidities					NS
Diabetes mellitus	7		2		NS
Hypertension	14		15		NS
Hyperlipidemia	7		5		NS
Hyperuricemia	5		1		NS
	CHF	9	Malignancy	15	
	Malignancy	6	Vascular disease	5	
	COPD	5	Collagen disease	6	
	PTEs	4	IHD	2	
	IPAH	3	COPD	2	
	Collagen disease	4	PTE	1	
			Infectious disease	1	

All continuous variables are presented as mean±S.D.

BMI, body mass index; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; IHD, ischemic heart disease; IPAH, idiopathic pulmonary arterial hypertension; PH, pulmonary hypertension; PTE, pulmonary thrombo-embolism.

(ARBs), n=14) and home oxygen therapy (PH group, n=9). Four patients died from cancer (3) or a PH crisis (1) after 7 months.

2) CE-CT Acquisition and Image Analysis

CE-CT was performed using a multi-detector row helical CT scanner (Aquilion TSX-101A, Toshiba Medical Systems Co. Ltd., Japan). The scanning parameters were 120 kV, and 150~300A with 0.5 mm thick sections. A nonionic iodinated contrast medium (Iopamidol 612 mg/ml) was intravenously administered at a total dose of 2 ml/kg and injection rate of 1.5 ml/second, and the CT data were available after 70 seconds from the start of the contrast medium injection. The scanned images were retrospectively reviewed with a DICOM viewer (ExaVision LITE, Ziosoft Inc., Japan). The RV/LV ratio was evaluated by measuring the right and left ventricular diameters of the heart in the axial view of the transverse plane at the maximum point between the inner surfaces of the lumen (Figure 1A). The pulmonary artery trunk diameter (PA) and ascending aorta diameter (Ao) were measured at the maximum inner diameter of the transverse plane (Figure 1B).

3) Echocardiographic Data

Standard two-dimensional and Doppler echocardiography were performed with a SONOS 5500 (Phillips Medical Systems Inc., USA) standard ultrasound system with a 1 to 3 MHz wideband transducer. The left ventricular diastolic dimension and systolic dimension, left atrium dimension, ascending aorta dimension, pulmonary artery dimension and inferior vena cava dimension (IVC) were obtained in a left parasternal, apical, and subxiphoid approach. The estimated RVSP was calculated using the following formula:

$$\text{Estimated RVSP} = 4 \times v^2 + \text{Estimated RAP}$$

[#], Bernoulli's formula; v, maximum flow velocity of the tricuspid regurgitation; RAP, right atrium pressure.

The RAP was calculated as in Table 2.

A echocardiographic examination was performed within 4 weeks of the CE-CT examination. Two-experienced sonographers without any knowledge of the CE-CT examination reviewed the patients undergoing the echocardiography.

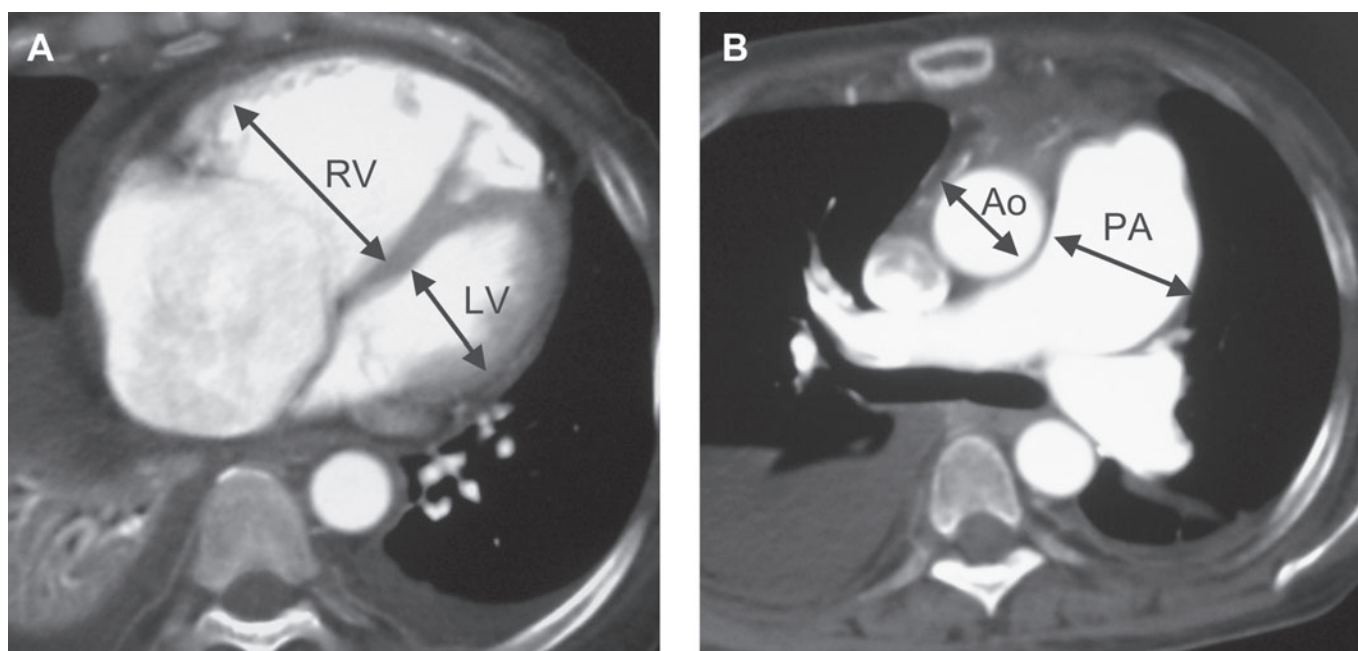


Figure 1. Axial image of the contrast-enhanced computed tomography. (A) Measurement of the RV/LV ratio. LV, left ventricle inner maximum lumen diameter; RV, right ventricle inner maximum lumen diameter. The scan shows the measurements of the maximum inner lumen diameter of the left and right ventricles. (B) Measurement of the PA/Ao ratio. PA, pulmonary artery inner lumen diameter; Ao, ascending aorta inner lumen diameter. This scan shows the measurement of the ascending aortic inner diameter and maximum pulmonary artery trunk inner diameter.

Table 2. Estimation of the right atrial pressure

IVC diameter (mm)	Respiratory variation*	Estimated RAP (mmHg)
12~15	+	0~5
12~15	-	5~10
15<	+	10~20
15<	-	15~25

IVC, inferior vena cava; RAP, right atrial pressure.

*respiratory variations were positive when there was a greater than 50% variation of the minimum to maximum diameters of the IVC and negative when there was less than a 50% variation in those diameters.

4) Statistical Analysis

The results are expressed as the mean ± the standard deviation (S.D.). The qualitative parameters were compared by means of a t-test or Fisher's exact test. A *p* value <0.05 was considered to indicate statistically significant differences. A statistical analysis was performed with a statistical software system (Stat Mate III, ATMS Tokyo, Japan)

Results

1) RV/LV Ratio

The estimated RVSP in the PH and control groups were 58.5±20.7 mmHg and 26.1±0.4 mmHg, respectively. The mean RV/LV ratio in the PH group was 1.1±0.3, and differed statistically significantly (*p*<0.05) from that in the control group (0.8±0.3)

(Figure 2A).

In both groups, the RV/LV ratio correlated ($r=0.46$, $r^2=0.21$, $p<0.05$) with the estimated RVSP (Figure 2B). In a calculation from the receiver operating characteristics (ROC)

curve, an optimal RV/LV ratio of more than 1.0 diagnosed PH with a sensitivity of 65%, specificity of 100%, positive predictive value (PPV) of 100% and negative predictive value (NPV) of 74% (Figure 2C).

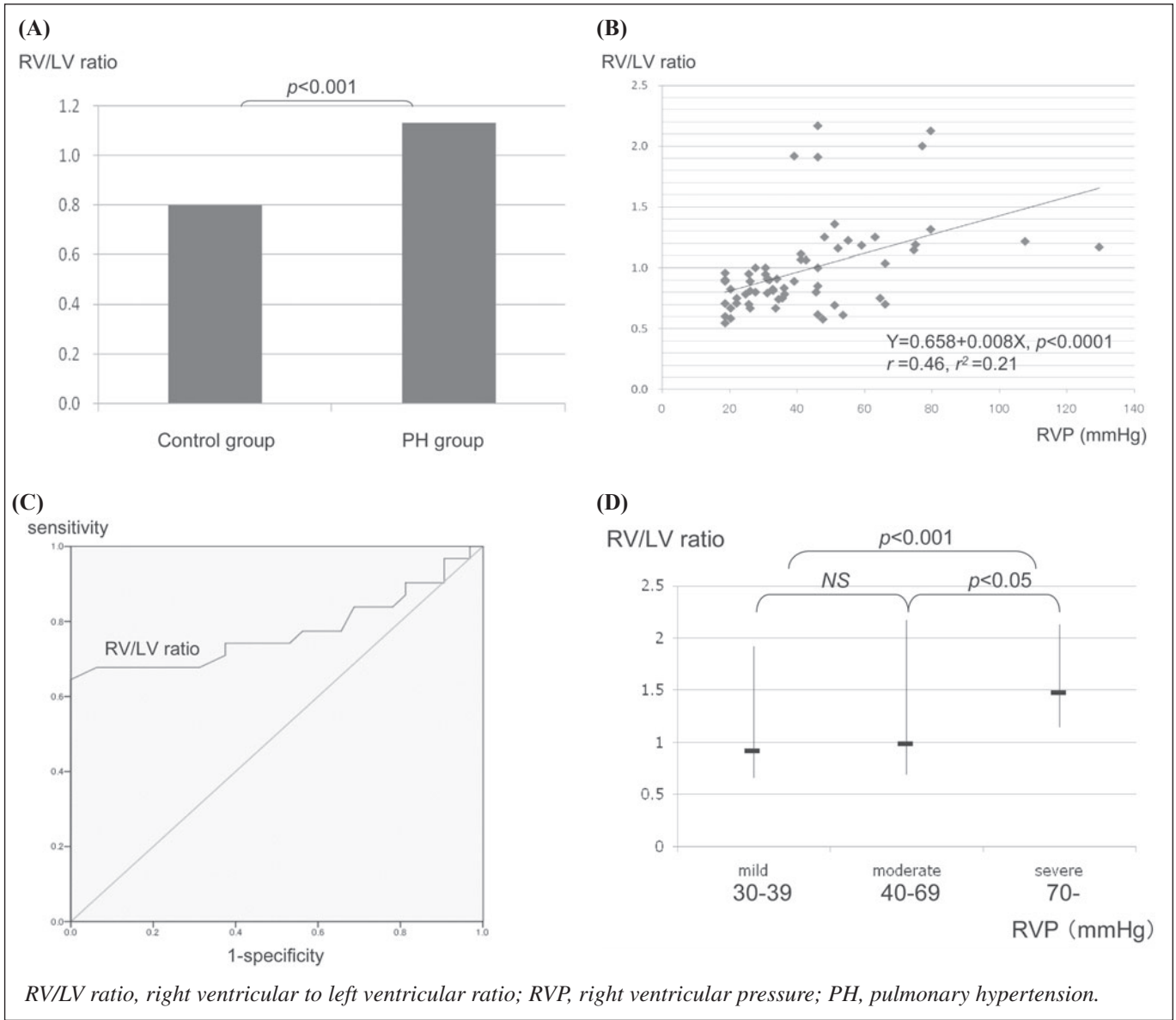


Figure 2. (A) The RV/LV ratios between the PH and control groups. The estimated RVP in the PH and control groups from the echocardiogram were 58.5 ± 20.7 mmHg and 26.1 ± 0.4 mmHg, respectively. The mean RV/LV ratio was significantly higher in the PH group than in the control group ($p<0.0001$, t-test). (B) Scattergram illustrating the correlation between the right ventricular to RV/LV ratio and estimated RVP in the PH and control groups. There was a significant correlation between the RV/LV ratio and estimated RVP. The regression equation was determined as follows: $RV/LV\ ratio=0.0077\times\ estimated\ RVP+0.66$ ($r=0.46$, $r^2=0.21$, $p<0.0001$ Fisher's test). (C) Receiver operating characteristic curve for the RV/LV ratio which diagnosed PH ($RVP\geq 40$ mmHg). (D) Relationship between the RV/LV ratio and severity of the RVP. Moderate and severe RVP had a statistically higher RV/LV ratio than that of mild RVP.

2) Other Parameters

The mean value of the PA/Ao ratios other than for thoracic aortic aneurysms was 1.04 ± 0.36 in the PH group and 0.86 ± 0.24 in the control group. The PA/Ao ratio in the PH group was significantly higher ($p < 0.05$) than that in the control group (Table 3) and that ratio statistically correlated with the RVSP ($r^2 = 0.23$, $p < 0.001$). An optimal PA/Ao ratio threshold of more than 0.9 calculated from the ROC curve diagnosed PH with a sensitivity of 74%, specificity of 62%, PPV of 68%, and NPV of 69% (Figures 3A & 3B).

The RV and PA diameters were significantly increased in the PH group ($p < 0.01$ and $p < 0.001$, respectively). The LV and Ao diameters did not statistically differ in the two groups (Table 3).

Discussion

Echocardiography is a non-invasive and rapid imaging technique for demonstrating the signs of PH, such as RV dilatation, leftward bowing of the intraventricular septum (IVS), collapse of the LV and the estimated RVSP. However, this examination has an operator-dependent bias and the need for special echocardiographic techniques. On the other hand, CT is an objective examination. The purpose of this study was to determine whether the RV/LV ratio of the CT parameters could predict PH when compared with the RVSP of the echocardiographic parameters.

1) RV/LV Ratio

For PTEs, some literature³⁻⁶ has been available for assessing the severity of the PH using CE-CT. Those studies estimated that an RV/LV ratio of >1.5 indicated the severity and the potential of dying from the PTE within 30 days. Collomb et al.³ reported the severity assessment of acute PTEs using CT. In their study, the clinically severe PTE group had a statistically larger RV/LV ratio than the non-severe PTE group (1.63 vs. 1.09, $p < 0.0001$). Further, the diagnostic accuracy of an RV/LV ratio of greater than 1.5 as a sign of a severe PTEs occurrence had a sensitivity of 60%, specificity of 83%, PPV of 83% and NPV of 76%. In our study, clinically severe PH was defined as a patient death due to PH, state of shock (systolic blood pressure of greater than 80 mmHg) or New York Heart Association (NYHA) class III-IV. In the ROC curve, if using an optimal RV/LV ratio of more than 1.1, severe PH could be accurately diagnosed with a sensitivity, specificity, PPV and NPV of 79%, 88%, 65% and 94%, respectively, which was comparable to the prior study. However, the optimal cut off value of the RV/LV ratio differed between our study and that of the previous study done by Collombo et al. The reason was considered as follows; in the acute phase of a severe PTE, the RV chamber is enlarged and the LV collapses. However, our study included all causes or stages of PH such as right sided heart failure, and a chronic state of PH. Therefore, the LV chamber size became compensated to some degree and it was not necessary complicated

Table 3. Results of the contrast-enhanced computed tomography parameters in the pulmonary hypertension and control groups

Parameters	PH group	Control group	P value*
Maximum diameter			
RV (mm)	38.7 ± 9.6	32.3 ± 6.5	< 0.05
LV (mm)	38.2 ± 12.0	40.2 ± 5.1	NS
PA (mm)	31.6 ± 5.9	26.5 ± 5.6	< 0.05
Ao (mm)	32.7 ± 9.3	32.6 ± 9.0	NS
RV/LV ratio	1.16 ± 0.44	0.80 ± 0.12	< 0.05
PA/Ao ratio	1.04 ± 0.36	0.86 ± 0.24	< 0.05

All continuous variables are presented as mean \pm S.D.

PA, pulmonary artery diameter; PA/Ao ratio, pulmonary artery diameter to aortic diameter ratio; PH, pulmonary hypertension; LV/RV ratio, left ventricle to right ventricle diameter ratio. RV, right ventricular.

*The LV/RV ratio, PA/Ao ratio, PA, and RV significantly differed between the two groups ($p < 0.05$).

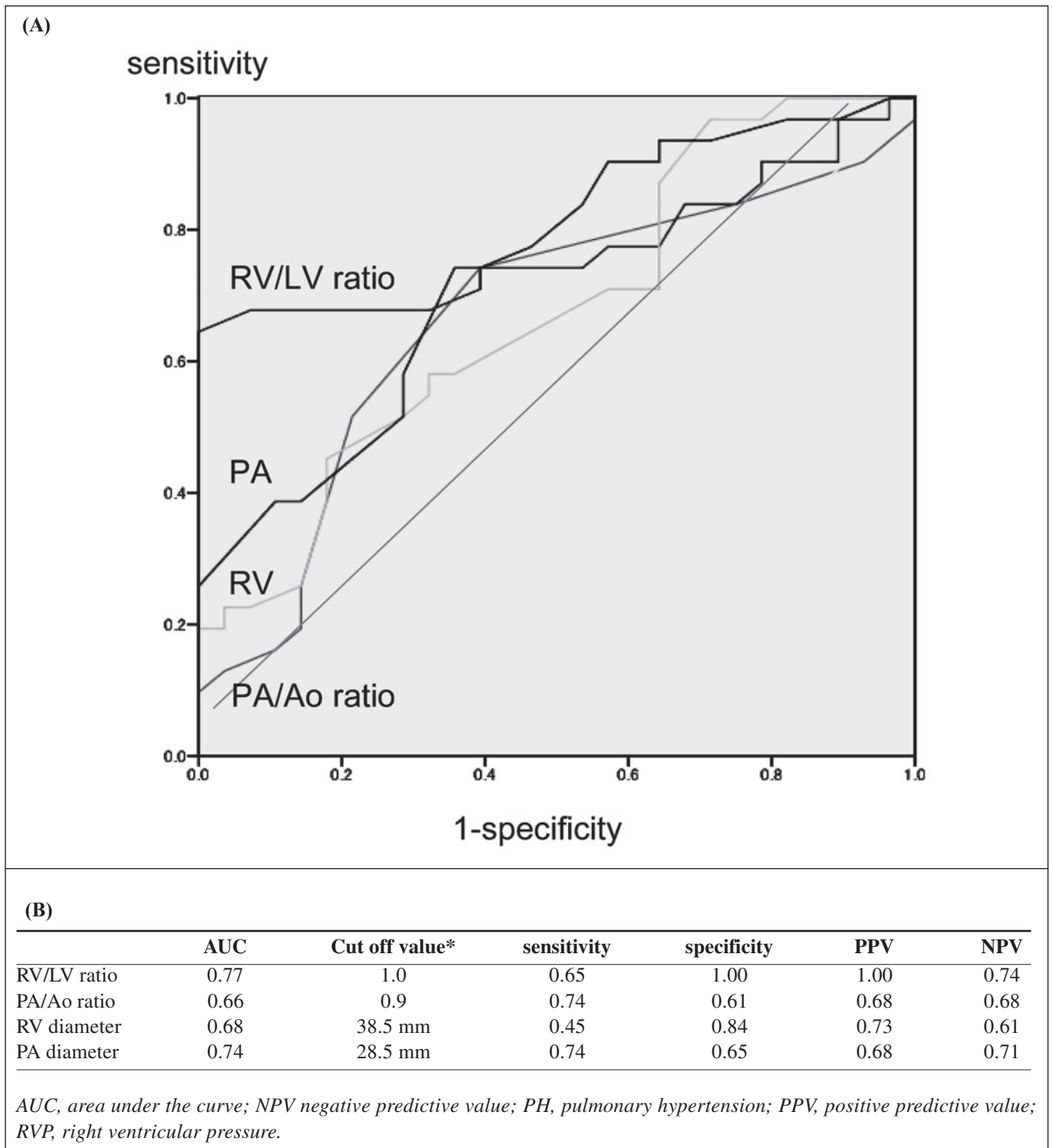


Figure 3. (A) Receiver operating characteristic curve for all the parameters for diagnosing PH ($RVP \geq 40$ mmHg). (B) The accuracy of the parameters for predicting an echocardiographic diagnosis of PH ($RVP \geq 40$ mmHg).

*the optimal cutoff levels were made available with a Youden index.

by an LV collapse. In addition, the RV dimension was enlarged with an increasing RVSP, however, the LV dimension was not. That might demonstrate that the optimal cut off value in our study was low compared with the previous data. Nevertheless, the RV/LV ratio was correlated with the estimated RVSP. If the patients were divided into three groups based on the severity of the RVSP with mild PH (30-39 mmHg), moderate PH (40-69 mmHg) and severe PH (above 70 mmHg), the RV/LV ratio for severe PH was significantly higher than that for the moderate ($p < 0.05$) and mild PH ($p < 0.001$). To the best of our knowledge there have been no previous studies which have demonstrated a correlation between the RV/LV ratio of the CE-CT and the severity of the RVSP.

In two studies on massive PTEs, Contractor et al.⁸ and Lim et al.⁹ reported that CT signs of RV dysfunction (such as an RV/LV ratio > 1.0 , and leftward bowing of the IVS) had a sensitivity of 78-92%, specificity of 100% and PPV of 100%, as compared to the echocardiographic findings of RV dysfunction (RV dilatation, leftward bowing or paradoxical motion of the IVS). In our study, using similar criteria, CT demonstrated a sensitivity, specificity, PPV and NPV of 50%, 100%, 100% and 79%, respectively for the diagnosis of RV dysfunction. Only the sensitivity was lower than that of those two studies despite the similar specificity and PPV.

The CT images obtained in the axial view were not similar to those in the four-chamber view. Quiroz et al.¹⁰ reported that the CT measurements obtained from the four chamber view images differed from those in the axial view images. In acute PTEs, the RV/LV ratio determined by the four-chamber view is more correlative than that of the axial view with the clinical adverse events including the mortality, necessity for cardiopulmonary resuscitation, hypotension and rescue thrombolysis. In our study, the RV/LV ratio was analyzed by the axial view because of the advantage of rapidly obtainable results without any reconstruction-imaging process. However, if we had performed a reconstruction from the four-chamber view, more accurate data might have been obtained.

2) Other Parameters

The RV diameter and RV/LV ratio determined by CT have demonstrated a significant positive correlation with the severity of PTEs or fatal outcomes, whereas the LV diameter has shown a negative correlation.³⁻⁶ In our study, the RV, RV/LV, PA, and PA/Ao ratios exhibited a positive correlation to the RVSP (the individual r indexes were 0.44, 0.46, 0.45 and 0.48 and r^2 indexes 0.19, 0.21, 0.20 and 0.23, respectively. All p values were < 0.001). On the other hand, there was no correlation between the LV and RVSP. The ROC curve for the RV/LV ratio, PA/Ao ratio, RV and PA are demonstrated in Figure 3A and the optimal cut off value is demonstrated Figure 3B.

In Ng¹¹ and colleagues reported the correlation between the PA/Ao ratio determined by CT and the right atrial pressure (RAP) measured by right heart catheterization in chronic PH patients with cardiovascular and pulmonary disease. The PA diameter and PA/Ao ratio were also correlated with the systolic pulmonary artery pressure (PAP) (the individual r indexes were 0.75 and 0.72, respectively, $p < 0.0005$). In the patients with the mean PAP measured by catheterization, the accuracy values (sensitivity, specificity, PPV and NPV) of a PA/Ao ratio of > 1.0 in predicting a diagnosis of PH, were 68%, 100%, 100% and 52%, respectively. Those values for a PA diameter of > 30 mm were 70%, 92%, 96% and 52%, respectively. Our data of the optimal cut off value for the PA/Ao ratio and PA diameter are shown in Figure 3A. Although the definition of PH and cut off value for the indices differed, the PA/Ao ratio and PA diameter from our study were less accurate in diagnosing PH than that in Ng's study. The RV/LV ratio had the highest accuracy among the four parameters constructing the area under the curve (AUC) (Figures 3A & 3B). To the best of our knowledge, no previous studies have demonstrated the relationship between the RV/LV ratio and estimated RVSP in PH due to various diseases. Our study was the first to demonstrate that the RV/LV ratio had a good correlation to the RVSP, and that an RV/LV ratio of > 1.0 was the most powerful index for diagnosing PH.

Study Limitations

The patient background in this study consisted of the chronic stage of PH, because the CE-CT examination was hard to perform during the active stage of PH, particularly with congestive heart failure. This evaluation may be useful for only patients in the chronic stage of PH. Second, the end-diastolic ventricular diameter may be obtained if an electrocardiogram-synchronized gating system is used together with CE-CT. However that technique increases the radiation exposure and requires a specific gating system. Third, in our study, right heart catheterization was not performed, however the RVSP has been recognized to have a good correlation to the PAP in previous studies^{1,2} More accurate results might have been obtained if the PAP was measured by a right heart catheterization.

References

1. Chan KL, Currie PJ, Seward JB, et al. Comparison of three Doppler ultrasound methods in the prediction of pulmonary artery pressure. *J Am Coll Cardiol* 1987;9:549-54.
2. Schiller NB. Pulmonary artery pressure estimation by Doppler and two-dimensional echography. *Cardiol Clin* 1990;8:277-87.
3. Collomb D, Paramelle PJ, Calaque O, et al. Severity assessment of acute pulmonary embolism: evaluation using helical CT. *Eur Radiol* 2003;13:1508-14.
4. van der Meer RW, Pattinama PM, van Strijen MJ, et al. Right ventricular dysfunction and pulmonary obstruction index at helical CT: prediction of clinical outcome during 3-month follow-up in patients with acute pulmonary embolism. *Radiology* 2005;235:798-803.
5. Ghaye B, Ghuysen A, Willems V, et al. Severe pulmonary embolism: Pulmonary artery clot load scores and cardiovascular parameters as predictors of mortality. *Radiology* 2006;239:884-91.
6. Reid JH, Murchison JT. Acute right ventricular dilatation: A new helical CT sign of massive pulmonary embolism. *Clin Radiol* 1998;53:694-8.
7. Badesch DB, Champion HC, Sanchez MA, et al. Diagnosis and assessment of pulmonary arterial hypertension. *J Am Coll Cardiol*. 2009 Jun 30;54 (1 Suppl):S55-66.
8. Contractor S, Maldjian PD, Sharma VK, et al. Role of helical CT in detecting right ventricular dysfunction secondary to acute pulmonary embolism. *J Comput Assist Tomogr* 2002;26:587-91.
9. Lim KE, Chan CY, Chu PH, et al. Right ventricular secondary to acute massive pulmonary embolism detected by helical computed tomography pulmonary angiography. *Clin Imaging* 2005;29:16-21.
10. Quiroz R, Kucher N, Schoepf UJ, et al. Right ventricular enlargement on chest computed tomography. Prognostic role in acute pulmonary embolism. *Circulation* 2004;109:2401-4.
11. Ng CS, Wells AU, Padley SP. A CT signs of chronic pulmonary arterial hypertension: The ratio of main pulmonary artery to aortic diameter. *J Thorac Imaging* 1999;14:270-8.

In the Wireless Era: Leadless Pacing

KATHY LF LEE AND CHU-PAK LAU

From Department of Medicine, Queen Mary Hospital, Hong Kong

LEE AND LAU: *In the Wireless Era: Leadless Pacing.* Cardiac pacemakers have been the standard therapy for patients with bradyarrhythmias for several decades. The pacing lead is an integral part of the system that serves as a conduit for delivery of energy pulses to stimulate the myocardium. However, it is also an Achilles tendon that directly causes most device complications both acutely during implant and chronically years afterwards. Both durability and optimization of stimulation site are important areas of improvement for manufacturers and implanters. Elimination of the pacing lead and utilization of other means for energy transfer is the only way to avoid lead complications and allow better choice of stimulation site. Leadless pacing with ultrasound-mediated energy has been demonstrated in animals and humans in acute studies. This has aroused intense interest in the field of cardiac pacing. With concerted effort from the profession and the industry, leadless pacing may indeed become a sound idea. (*J HK Coll Cardiol* 2010;18:53-58)

Cardiac resynchronization therapy, Heart failure, Pacemaker, Ultrasound

摘要

過去的幾十年間，心臟起搏器已經成為治療有症狀的心動過緩患者一種標準的方法。而整體系統一部分的起搏器導聯，有著傳輸能量脈衝以刺激心臟的作用。然而無論是在起搏器植入後初期抑或是若干年後，有時仍會導致裝置相關的急慢性併發症。續航能力和刺激部位的適宜強度，無論對於製造商還是植入者而言，都是重要的關注點。去除起搏器的導聯，而運用其他方式進行能力傳輸是避免導聯相關併發症的唯一途徑，並且它為刺激部位提供更好的選擇。通過超聲介導能量的無導聯起搏器已經見諸與動物和人體的急性期研究中。這在心臟起搏器領域引起了強烈的關注。在醫學專業和製造專業的共同籌畫下，無導聯起搏器將會成為一個健全的構想。

關鍵詞：心臟再同步治療 心衰竭 起搏器 超聲波

Introduction

All pacing leads are associated with complications like infection, fracture, failure and dislodgment. Lead extraction is a high risk procedure. With new device systems that often require implantation of multiple leads, and with patients living longer, the

incidence of lead complications becomes compounded over time.¹ Therefore, there is a strong demand to develop a pacing system that eliminates the pacing lead as a conduit for energy transfer. Randomized clinical trials on cardiac resynchronization therapy have demonstrated its clinical benefits. Access to the left ventricle is achieved with the use of a pacing lead to be advanced into the coronary sinus and positioned in a coronary vein branch. Implantation of this lead is technically demanding and associated with a significant incidence of failure to implant, implantation in a suboptimal location, and complications.^{2,3} Comparing to epicardial pacing, endocardial left ventricular stimulation that minimizes the conduction delay from the epicardium to the endocardium may be more physiological and therefore give rise to greater

Address for reprints: Dr. Kathy LF Lee
Department of Medicine, Queen Mary Hospital, 102 Pokfulam
Road, Hong Kong SAR, China

Email: klflee@hku.hk

Received November 22, 2010; revision accepted November 23,
2010

hemodynamic benefits.^{4,5} Leadless pacing will enable endocardial left ventricular stimulation without the risks of systemic thromboembolism and mitral regurgitation. It will also avoid diaphragmatic stimulation, avoid right ventricular apical pacing, enable multi-site pacing, and allow almost free choice of stimulation location within the left or right ventricle. It may also address challenges in pediatric pacing, and development of a truly MRI-compatible pacing system.

Acoustic Energy for Leadless Pacing

Many concepts have been patented for the development of a leadless pacing system. Having a long history and wide application in medical technology, ultrasound energy is considered safe and was chosen to prove the concept of energy transfer. The use of ultrasound-mediated energy to drive a remotely positioned electrode for direct myocardial stimulation is the first to be reported in the medical literature. This new technology uses the mechanical-to-electrical properties of piezoelectric materials for transformation of energy. The experimental set-up include: an ultrasound transmitting transducer connected to an ultrasound generator, a steerable bipolar electrophysiology catheter incorporating a receiver-electrode at the distal tip. Ultrasound energy was amplitude-adjusted and transmitted at around 330 kHz. The lower frequency used in this application achieves better tissue penetration. Ultrasound pulses generated from the transmitting transducer traveled through the chest wall to reach the receiver-electrode with circuitry to transform the pulses into electrical energy for myocardial stimulation. The feasibility and safety of this novel technology was first demonstrated acutely in animals.⁶ In the acute safety study, histological examination was performed in pigs that were exposed to continuous ultrasound transmission from the investigational system for two hours. No mechanical or thermal bio-effect was identified in the sacrificed animals. In the acute feasibility study, the receiver-electrode incorporated into a transvascular catheter was selective positioned and in contact with various heart chambers of a pig. An external transmitter was then

placed on the chest wall of the animal, with acoustic gel used for coupling. Ultrasound energy was then transmitted through the chest to the receiver, the acoustic energy was converted to electrical energy, and the electrodes contacting endocardium stimulated pacing. The electrode catheter was a modified steerable bipolar EP catheter with receiver-transducers incorporated near the tip between the bipolar pacing electrodes. Proximal connections on the catheter allowed either direct electrical pacing using a Pacing System Analyzer or monitoring of the receiver output during ultrasound-mediated pacing. The catheter itself was not directly involved in or required for ultrasound-mediated pacing. The acute porcine study demonstrated feasibility of leadless ultrasound-mediated pacing in five animals at 30 selected sites in the right atrium, right ventricle, left ventricle, and simultaneously in both left and right ventricles.⁶

Following the animal experiments, human studies were performed.⁷ Twenty-four patients were tested during or after completion of clinical electrophysiology procedures (Figure 1). A total of 80 pacing sites in the right atrium, right ventricle, and left ventricle were tested. The transmit-to-receive distance was 11.3 ± 3.2 cm. Ultrasound-mediated pacing was achieved at all 80 sites with consistent capture at 77 sites. There was no adverse event related to ultrasound-mediated pacing. No patient experienced discomfort during pacing. This novel technology targeting cardiac resynchronization therapy was also tested in heart failure patients.⁸ In these patients, the acoustic window on the chest wall that allows efficient ultrasound transmission was defined and found to be large and sufficient for subcutaneous implantation of the ultrasound generator, even after accommodating changes with respiratory movement and body positioning (Figure 2).

Incorporating this novel technology, the future implantable leadless pacing system is being designed. The system includes an ultrasound generator to be implanted subcutaneously in the acoustic window of the chest wall and an endocardial receiver-electrode. The receiver electrode is delivered by a steerable transvascular catheter to the target heart chamber, and be detached and implanted onto the endocardium directly. The receiver and transmitter are programmed

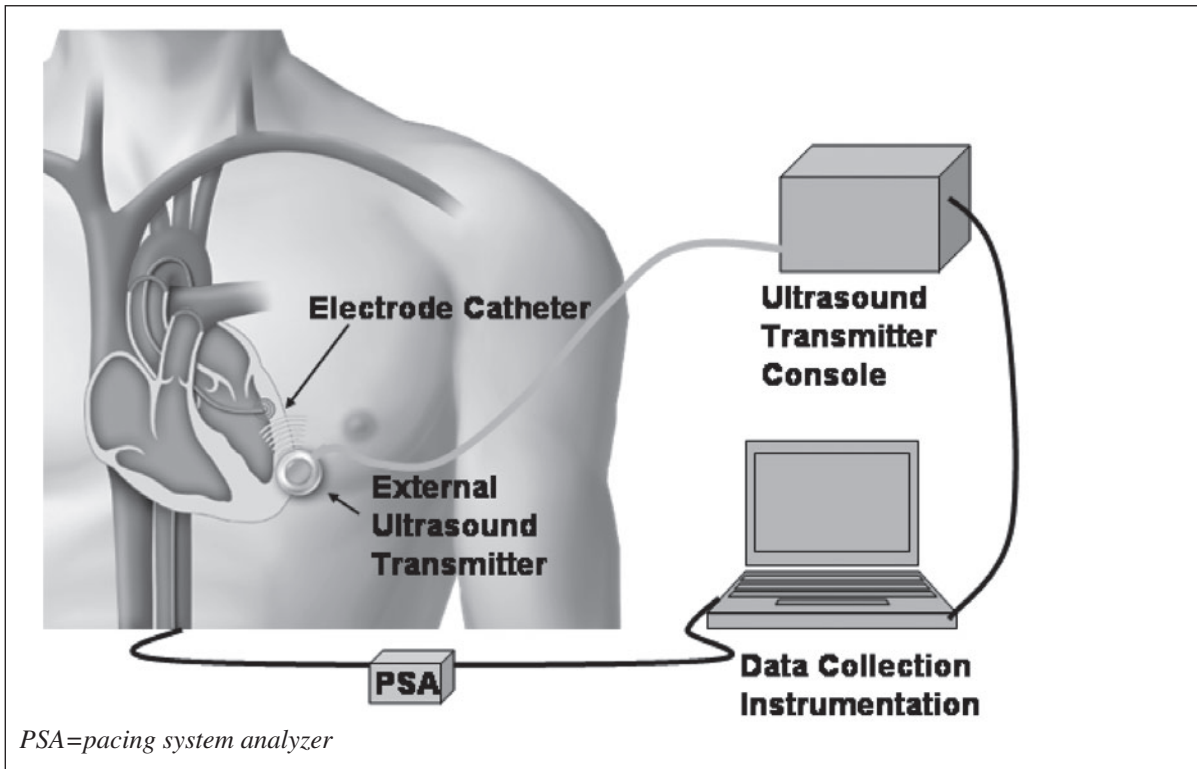


Figure 1. Equipment set up of the first study of leadless pacing in humans.

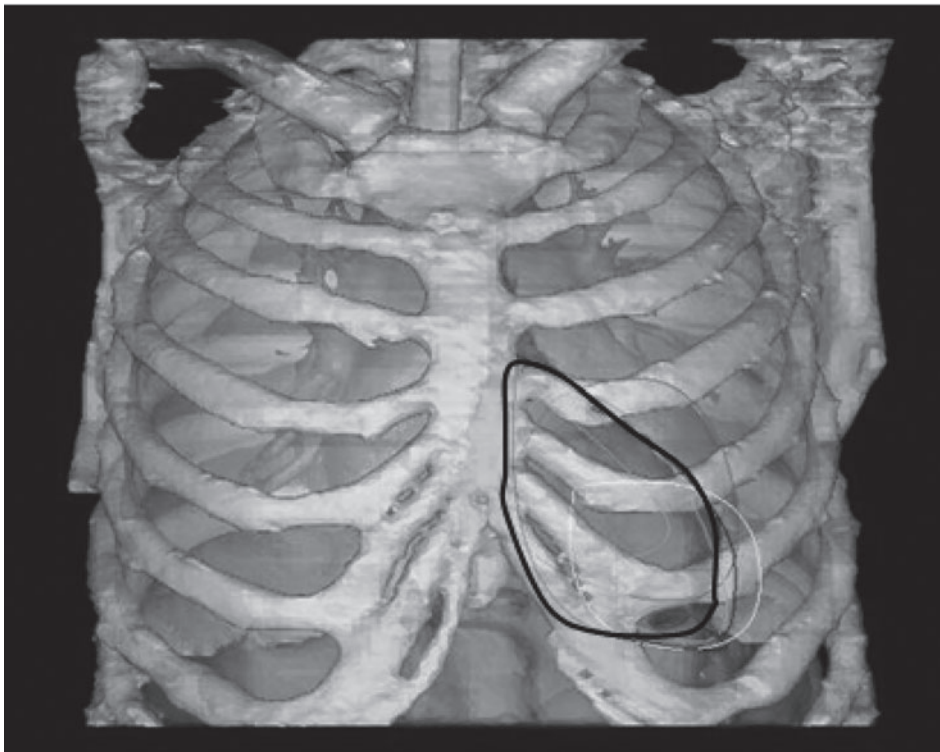


Figure 2. Acoustic window outlined on a reconstruction CT thorax.

to operate at the same frequency of ultrasound pulses to avoid external interference. The ultrasound beam will be optimized and focused onto the receiver electrode in order to improve the efficacy of energy transfer. The estimated device longevity will need to be comparable to that of a conventional pacemaker to make it commercially viable. The acoustic window suitable for implant on the patient's chest wall can be localized preoperatively by conventional echocardiography, a convenient method that has been shown to predict efficient ultrasound transmission for leadless pacing.⁸

There are of course other unresolved issues that ongoing research is aiming to answer (Table 1).^{9,10} For example, many will doubt the long term safety of continuous ultrasound exposure. Although the lack of effect of short term ultrasound-mediated pacing has been shown histologically in animal experiments, tissue injury secondary to heating with continuous exposure is theoretically possible. Similar studies on long term exposure when ultrasound energy was applied in continuous pacing will address the safety concern. Another challenge of this technology is environmental interference which may be ubiquitous. Although precise formatting and fine tuning of the device can be done, little is known about its actual performance in the real world. This is another big safety issue that requires extensive evaluation. Apart from energy delivery,

transmission of sensed endocardial electrograms is an important function of a pacing lead. There is so far no available information on how the sensing circuit of the future leadless pacemaker is going to operate. As for the risk of infection, the receiver electrode is small, embedded in the endocardial followed by complete endothelialization. It is expected to carry minimal risk of infection, probably comparable to that of coronary stents and atrial septal defect occluders.

Alternative Energy Sources

Apart from ultrasound, an alternating magnetic energy source has been tested in a pig model for leadless cardiac stimulation.¹¹ The system consists of two components, an external transmitter unit and a receiver unit in contact with the myocardium. The subcutaneous primary coil generates an alternating magnetic field, which is converted by the secondary coil inside the heart to a voltage pulse for pacing stimulation. In the pig experiment described, an alternating magnetic field of approximately 0.5 mT was generated by the transmitter unit in a distance of 3 cm. Voltage pulses with a duration of 0.4 ms and voltage amplitude of 0.6-1.0 V were generated. Continuous stimulation of the heart was demonstrated for 30 minutes. The advantage of this

Table 1. Challenges in the development of leadless pacing technology

- Low efficiency of energy conversion significantly shortens battery longevity
 - Problem with consistent capture with a constantly changing pacing window
 - Long term safety of prolonged exposure to ultrasound with possible heating effect and tissue injury
 - Reliability of a leadless sensing circuit
 - Operation and integration of multiple sensing and pacing sites for CRT
 - Potential interference from external environment
 - Unconventional parasternal site for implanting the pacemaker generator
 - Development of a reliable delivery and anchoring system for implanting the endocardial receiver-electrode
 - Integration with an implantable defibrillator
-

technology is that the efficiency of energy conversion is relatively high, making device longevity less of a problem. However, the transmitter coil is of considerable measurement, 60 mm in diameter, 10 mm in width, and 80 g in weight, while the receiver is 15 mm in length and 2.5 mm in diameter. Apart from the size of the device, one major concern of this technology is potential external interference by environmental magnetic fields. The long term safety with heat production in the receiver unit generated with continuous exposure is also uncertain.

Other wireless energy sources have been used for stimulation to a limited extent. Radiofrequency energy transmission is employed in an implantable micro-stimulator device currently under clinical investigation for neuro-modulation applications.¹² However, the implant incorporates a battery requiring frequent recharging using an inductive link from an external device. In general, the use of radiofrequency power for wireless applications in the body has the disadvantages of being unfocused and having a shallow depth of penetration. Therefore, it is less efficient than ultrasound energy. It also requires the transmitter and receiver to be in close proximity, which essentially precludes its use for many applications including cardiac pacing.

Ultrasound energy transduction is currently being used in a clinical application of a wireless sensor.¹³ In this application, the receiver is coupled to a pressure transducer implanted within an abdominal aortic aneurysm graft for post procedure monitoring after endovascular repair. The transmitter is an external device placed on the anterior abdomen to communicate with the implanted receiver to acquire real time intrasac pressure data.

Future Perspectives

Leadless pacing with ultrasound-mediated energy was demonstrated in animals and humans in acute studies. This is by far the most advanced development with proven technological feasibility. More scientific

data will be collected from ongoing animal and human research. With refinement of the technology, challenges identified will be addressed. Currently, a prototype device and the implantation equipment are being developed, and clinical trial of the permanent implantable system is being planned. At the same time, various competing technologies are being developed and evaluated. Although it is not certain if the intended progress will be accomplished, the favorable result achieved has aroused significant interest in the field of cardiac pacing. Many including those in the device industry are keen to be involved in the development of a commercially viable leadless implantable pacemaker. When this is materialized, lead complications and lead extractions will be minimized.

Summary and Conclusions

Leadless pacing will also open up a new arena for cardiac resynchronization therapy. Some non-responders may be converted to responders with optimization of the left ventricular stimulation site. Multi-site pacing will no longer be limited by problems in vascular access or coronary venous anatomy. Responders may obtain maximal benefit from cardiac resynchronization therapy with endocardial left ventricular stimulation.

Although leadless pacing is only in the stage of proof-of-concept, subcutaneous implantable defibrillator has been developed and tested in clinical trial.¹⁴ The preliminary result is encouraging. The integration of leadless pacemaker and subcutaneous implantable defibrillator is important as heart failure patients frequently require cardiac resynchronization therapy in combination with an implantable defibrillator.

Despite all the advances achieved, much more work needs to be done before we can accomplish this major breakthrough in the development of leadless implantable cardiac devices (Table 2). By that moment, implanting a pacing lead will become a procedure of history.

Table 2. Key issues in development of leadless pacing

- Pacemaker lead complications and failures are important clinical problems.
- The indication of device therapy has been expanded with the advent of cardiac resynchronization therapy; multiple pacing leads are now implanted in non-bradycardiac heart failure patients.
- Coronary vein anatomy may limit implant success and the clinical response to cardiac resynchronization therapy. The benefit of cardiac resynchronization therapy may be maximized with endocardial selected-site and multi-site pacing.
- The idea of energy transfer utilizing an external energy source to stimulate the heart directly was conceived as the basis of leadless pacing.
- Leadless cardiac stimulation with ultrasound-mediated energy was demonstrated to be feasible in acute studies in animal models, human patients and heart failure patients.
- Alternative technology of leadless cardiac stimulation with alternating magnetic field for energy induction has also been successful in an animal experiment.
- The future leadless pacemaker consists of a subcutaneously implanted ultrasound transmitter and an endocardially implanted receiver. A percutaneous catheter delivery and anchoring system will be used for the implantation procedure.
- The challenges of leadless pacing include but are not limited to the efficacy of energy conversion, potential external interference, sensing issues, and long term safety.
- A prototype device for leadless pacing with ultrasound energy has been developed for clinical testing. The first human implant may be feasible in the near future.

References

1. Borek PP, Wilkoff BL. Pacemaker and ICD leads: strategies for long-term management. *J Interv Card Electrophysiol* 2008; 23:59-72.
2. Bax JJ, Abraham T, Barold SS, et al. Cardiac resynchronization therapy: Part 2--issues during and after device implantation and unresolved questions. *J Am Coll Cardiol* 2005;46:2168-82.
3. Barold SS, Herweg B. Pacing in heart failure: how many leads and where? *Heart* 2008;94:10-2.
4. Van Deuren C, van Hunnik A, Kuiper M, et al. Endocardial left ventricular pacing improves cardiac resynchronization therapy in canine LBBB hearts. *Circulation* 2007;116(II), 222.
5. Rademakers LM, van Hunnik A, Lampert A, et al. Electrical and hemodynamic benefits of endocardial CRT with chronic infarction and LBBB. Presented in: *Heart Rhythm* 2009, Boston, USA, 13-16 May, 2009.
6. Echt DS, Cowan MW, Riley RE, et al. Feasibility and safety of a novel technology for pacing without leads. *Heart Rhythm* 2006;3:1202-6.
7. Lee KL, Lau CP, Tse HF, et al. First human demonstration of cardiac stimulation with transcutaneous ultrasound energy delivery: Implications for wireless pacing with implantable devices. *J Am Coll Cardiol* 2007;50(:877-83. * (First report of leadless pacing in humans)
8. Lee KL, Tse HF, Echt DS, et al. Temporary leadless pacing in heart failure patients with ultrasound-mediated stimulation energy and effects on acoustic window. *Heart Rhythm* 2009;6: 742-8.
9. Sweeney MO. In a footnote, at least. *Heart Rhythm* 2006;3: 1207-9.
10. Benditt DG, Goldstein M, Belalcazar A. The leadless ultrasound pacemaker: a sound idea? *Heart Rhythm* 2009;6:749-51. ** (Critically identifies the technological challenges ahead)
11. Wieneke H, Konorza T, Erbel R, et al. Leadless pacing in the heart using induction technology: a feasibility study. *Pacing Clin Electrophysiol* 2009;32:177-83.
12. Loeb GE, Richmond FJR, Singh J, et al. RF-powered BIONS's™ for stimulation and sensing. Presented in: 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society. San Francisco, USA, 1-5 September, 2004.
13. Ellozy SH, Carroccio A, Lookstein RA, et al. First experience in human beings with a permanently implantable intrasac pressure transducer for monitoring endovascular repair of abdominal aortic aneurysms. *J Vasc Surg* 2004;40: 405-12.
14. Bardy GH, Smith WM, Hood MA, et al. An Entirely Subcutaneous Implantable Cardioverter Defibrillator. *N Engl J Med* 2010;363:36-44.

Institute of Cardiovascular Science and Medicine
Faculty of Medicine
The University of Hong Kong

The Fourteenth Annual Scientific Meeting

18 December 2010
Hong Kong Convention and Exhibition Centre
Hong Kong

Meeting Committee

Co-Chairmen: Prof. Paul M. Vanhoutte
Prof. Bernard M.Y. Cheung
Dr. Kathy L.F. Lee

Secretary-General: Dr. Heather J. Ballard

Scientific Faculties: Prof. Michael R. Rosen
Prof. Hung-Fat Tse
Prof. Bernard M.Y. Cheung
Prof. Cheuk-Man Yu
Dr. Carman W.S. Chan
Dr. David C.W. Siu

Meeting Secretariat

Dr. Jeethan Bendoor, MedEd Initiatives

Address: 2/F, Room A-B, Lee Kee Commercial Building, 221-227 Queen's Road Central, Hong Kong

Tel: (852) 2527 3588; Fax: (852) 2527 3788; E-mail: icsm@mededin.com

Website: <http://www.icsm-hk.org>

SCIENTIFIC PROGRAMME

18 DECEMBER 2010 (SATURDAY)

- 08:30 - 09:00 **Registration**
- 09:00 - 10:00 **Oral presentations for Young Investigator Award**
Chairmen: Prof. M.R. Rosen, University of Columbia, New York, USA and Prof. P.M. Vanhoutte, The University of Hong Kong, HKSAR
- 10:00 - 11:30 **Coffee break, poster presentations for Young Investigator Award**
Chairmen: Prof. Y. Huang, Chinese University of Hong Kong, HKSAR and Dr. G.R. Li, The University of Hong Kong, HKSAR
- 11:30 - 12:30 **Oral presentations for Young Investigator Award**
Chairmen: Prof. X.Q. Yao, Chinese University of Hong Kong, HKSAR and Dr. H.J. Ballard, The University of Hong Kong, HKSAR
- 12:30 - 12:45 **Opening Ceremony**
Guest of Honour: Prof. G.M. Leung, Food and Health Bureau, HKSAR
- 12:45 - 14:15 **Lunch symposium - New therapy to prevent coronary thrombosis**
*Prof. C.M. Yu, Chinese University of Hong Kong, HKSAR
Prof. B.M.Y. Cheung, The University of Hong Kong, HKSAR*
- 14:15 - 15:45 **Afternoon session I**
Chairmen: Dr. C.S. Chiang, Hong Kong College of Cardiology, HKSAR and Dr. C.K.Y. Wong, Hong Kong College of Cardiology, HKSAR
Biological pacing: promises and challenges
Prof. M. R. Rosen, University of Columbia, New York, USA
Cardiac resynchronization therapy
Prof. C.M. Yu, Chinese University of Hong Kong, HKSAR
- 15:45 - 16:15 **Coffee break, poster viewing and booth visit**
- 16:15 - 17:45 **Afternoon session II**
Chairmen: Prof. C.Q. Jiang, Guangzhou No.12 Hospital, China; Dr. A.S.P. Wong, private cardiologist, HKSAR and Dr. K.L.F. Lee, Queen Mary Hospital, HKSAR
The Guangzhou Biobank Cohort Study
Prof. C.Q. Jiang, Guangzhou No.12 Hospital, China
New findings from the Hong Kong Cardiovascular Risk Factor Prevalence Study
Prof. B.M.Y. Cheung, The University of Hong Kong, HKSAR
State-of-the-art imaging of coronary arteries
Dr. C. Chan, Queen Mary Hospital, HKSAR
Implantable cardioverter debribrillator - who should have it?
Dr. C.W. Siu, The University of Hong Kong, HKSAR
- 17:45 - 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 Invited Lectures:

IL1.

CARDIOVASCULAR RISK FACTORS IN HONG KONG – AN UPDATE

BMJ Cheung

Department of Medicine, The University of Hong Kong, Hong Kong

The Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) was initiated in 1995-96, when 2895 Hong Kong adults aged 25-75 were recruited from the general population in Hong Kong using random domestic telephone numbers. This became a cohort study (CRISPS2) when 1944 participants were contacted and returned for follow-up in 2000-04. The third round of follow-up was conducted in 2005-08 (CRISPS3). The overall aim of the cohort study is to determine the prevalence of cardiovascular risk factors, including hypertension, diabetes and dyslipidaemia, and to find out the factors that predispose to the development of these conditions in Hong Kong Chinese. Previously, we have found that impaired glucose tolerance confers an overwhelming risk of developing diabetes. We have also traced the natural history of the development of the metabolic syndrome, which usually starts with central obesity, followed by dyslipidaemia and then diabetes and hypertension. We reported that the metabolic syndrome predicts the development of hypertension and is associated with increased mortality.

In CRISPS3, the prevalence of general obesity, defined as a body mass index (BMI) of 27.5 kg/m² or greater, has not increased since CRISPS1 (16.8% in CRISPS1, 15.7% in CRISPS2 and 14.9% in CRISPS3). However, the percentage of the cohort with abdominal obesity, defined as a waist circumference ≥90 cm in men or ≥80 cm in women, increased significantly from 25.4% in CRISPS1 to 41.4% in CRISPS3. At the same time, the

prevalence of hypertension increased from 18.1% in CRISPS1 to 39.6% in CRISPS3. This increase remains significant after adjusting for age. Our findings confirm the importance of waist circumference in this population, as calculating the BMI alone may give a false sense of security. The prevalence of hypertension in Hong Kong is now approaching the level in developed countries such as the United States. 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. Our analysis of cancer incidence in CRISPS3 suggests a link between insulin resistance and risk of cancer, so tackling obesity may also be beneficial in terms of reducing cancer risk.

In CRISPS, DNA has been collected and analysed for association with the development of cardiovascular diseases. Some of these studies were done in collaboration with the Guangzhou Number 12 Hospital, where the Guangzhou Biobank Cohort Study – Cardiovascular Disease Subcohort study is carried out. One of the first fruits of this collaboration was the identification of a single nucleotide polymorphism in the APOA5 gene that is common in Chinese and has an important influence on plasma triglyceride level.

IL2.

BIOLOGICAL PACING: PROMISES AND CHALLENGES

MR Rosen

Center for Molecular Therapeutics, Departments of Pharmacology and Pediatrics, College of Physicians and Surgeons of Columbia University, USA

Electronic pacing has revolutionized the prevention and treatment of life-threatening bradyarrhythmias during the last half-century. Because electronic pacemakers are a palliative therapy that might be improved upon, investigators have begun using gene and cell therapy approaches to effect a cure. Strategies have coalesced to focus on achieving stable and autonomously responsive cardiac rhythms in a setting that ultimately would require no implanted hardware. Most groups working in the area are now using variations on the HCN (hyperpolarization-activated cyclic nucleotide-gated) family of genes to create biological pacemakers. Interventions have included the creation of mutant or chimeric HCN genes as well as co-incorporation of genes to modify sodium or potassium currents or cell-cell connectivity. In the gene therapy approach, adenoviral and lentiviral vectors are being explored. In cell therapy approaches, adult stem cells have been used to carry pacemaker genes to specific regions of the heart, and embryonic stem cells and induced pluripotent cells have been used to create pacemakers as well. Challenges to be met include many aspects of efficacy and safety. These include, but are not limited to, optimization of the pacemaker construct, understanding more regarding stability and duration of function and risk of proarrhythmia, and comprehending the extent to which biological pacing might or might not represent a clinically relevant supplement to or replacement for electronic pacing.

ABSTRACTS

Abstracts for Oral Communications:

OC1.

LOSS-OF-FUNCTION IN TOLL-LIKE RECEPTOR 4 RECEPTORS NITRIC OXIDE-MEDIATED RELAXATIONS IN LEPR^{db/db} MICE

CF Liang,¹ A Xu,^{1,2} PM Vanhoutte¹

¹Department of Pharmacology and Pharmacy and ²Department of Medicine, The University of Hong Kong, Hong Kong

The present study analyzes the role of toll-like receptor 4 (TLR4), a target for saturated fatty acids and lipopolysaccharides, in modulating endothelial function in the mouse aorta. A type-2 diabetes model with double knockout (DKO) of leptin receptors (Lepr) and TLR4, was obtained by crossing Lepr^{db/+} and TLR4^{-/-} mice. Mice were sacrificed and rings [with and without endothelium] of their aorta were studied for measurement of isometric tension in a Mulvany-Halpern myograph.

Acetylcholine-induced endothelium-dependent relaxations were potentiated in DKO mice compared with Lepr^{db/db} control mice. The eNOS dimer to monomer ratio, as well as the basal and acetylcholine-stimulated eNOS phosphorylation levels were higher in preparations from DKO mice. This difference in relaxations and eNOS activity [dimer to monomer ratio and phosphorylation levels] between Lepr^{db/db} and DKO mice was abolished by apocynin [non-specific NADPH oxidase inhibitor]. Lucigenin-enhanced chemiluminescence assay suggested a lower basal endothelial production of superoxide anions by NADPH oxidase in DKO mice preparations. Quantitative PCR results revealed that NADPH oxidase isoform 4 and isoform 1 was downregulated in DKO mice arteries. Administration of lipopolysaccharide inhibited acetylcholine-evoked relaxations in wild type mice but not in TLR4^{-/-} mice. Western blotting analysis revealed that lipopolysaccharide

decreased eNOS dimer to monomer ratio and phosphorylation levels, which was accompanied by phosphorylation of glycogen synthase kinase 3 β and increased expression of β -catenin and NADPH oxidase isoform 4 in wild type mice aortae and cultured endothelial cells (HUVEC EA.Hy926). The attenuated relaxations to acetylcholine and the changes in eNOS activity induced by lipopolysaccharide were inhibited by apocynin, LY294002 (PI3K inhibitor) and enhanced by LiCl [non specific inhibitor of glycogen synthase kinase 3 β].

In conclusion, TLR4 activation decreases eNOS-mediated relaxations by enhancing oxidative stress produced by NADPH oxidase and resulting in the stimulation of the glycogen synthase kinase 3 β / β catenin cascade. TLR4 inhibition or down-regulation may be a potential target for the treatment of vascular dysfunction.

OC2.

DEFICIENCY OF EP4 RECEPTOR ON BONE MARROW-DERIVED CELLS BOOSTED INFLAMMATION AND ABDOMINAL AORTIC ANEURYSM FORMATION INDUCED BY ANGIOTENSIN II IN HYPERLIPIDEMIC MICE

EHC Tang,^{1,2} E Shvartz,¹ K Shimizu,¹ VZ Rocha,¹ C Zheng,¹ D Fukuda,¹ GP Shi,¹ G Sukhova,¹ P Libby¹

¹Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, USA.; ²Department of Pharmacology and Pharmacy, The University of Hong Kong, Li Ka Shing Faculty of Medicine, Hong Kong

Objective: Chronic inflammation during abdominal aortic aneurysm (AAA) formation contributes to remodeling and eventual weakening of the vessel wall. Prostaglandin E₂ (PGE₂), through activation of its receptor EP4, can mute inflammation. Whether EP4 participates directly in the pathogenesis of aneurysm remains unknown. We tested the hypothesis that a lack of EP4 receptor on bone marrow-derived cells would increase local inflammation and enhance the formation of AAA *in vivo*.

Methods and Results: Hypercholesterolemic low-density lipoprotein receptor knockout (LDLR^{-/-}) mice transplanted with either EP4^{+/+} [EP4^{+/+}/LDLR^{-/-}] or EP4^{-/-} [EP4^{-/-}/LDLR^{-/-}] bone marrow received infusions of angiotensin II to induce AAA. Deficiency of EP4 on bone marrow-derived cells increased the incidence and severity of AAA, increased monocyte chemoattractant protein-1 (MCP-1), and enhanced infiltration of macrophages and T cells into the AAA lesions. Lack of EP4 also augmented elastin fragmentation, increased the number of cells bearing markers of apoptosis, and decreased smooth-muscle cell accumulation within the AAA lesions.

Conclusions: Deficiency of EP4 receptor boosted inflammation and AAA formation induced by angiotensin II in hyperlipidemic mice. This study affirms the pathophysiologic importance of PGE₂ signaling through EP4 as an endogenous anti-inflammatory path involved in AAA formation.

ABSTRACTS

Abstracts for Oral Communications:

OC3.

IDENTIFICATION OF CHANNELS ON SKELETAL MYOBLASTS THAT MAY CONTRIBUTE TO ACIDOSIS-INDUCED ATP RELEASE

L.Lu, GR Li, HJ Ballard

Department of Physiology and Institute of Cardiovascular Science & Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Low pH stimulated ATP efflux from skeletal myoblasts, and inhibition of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) abolished the acidosis-induced ATP release. CFTR is a chloride channel that has been reported to mediate ATP efflux from some cell types. Several other chloride channels or stretch-activated channels are also reported to conduct ATP. In this study, I aim to identify channels on skeletal myoblasts that are firstly opened at low pH, and secondly contribute to ATP release.

RT-PCR was used to determine which of the channels reported to conduct ATP in other cell types were present on skeletal myoblasts. The chloride channels CFTR, Clcn-2, Clcn-3, Clcn-7, Ca²⁺ activated chloride channel and volume-sensitive outwardly-rectifying channels, and the stretch-activated channels, Connexin 36, Connexin 43 and Pannexin 3, were expressed on the cells.

Whole-cell patch clamp was performed on skeletal myoblasts using potassium-free solutions: the pipette (intracellular) solution contained 110 mM Cs-Aspartate, 20 mM CsCl, 5 mM Na₂-Phosphocreatine, 1.0 mM MgCl₂·6H₂O, 10 mM HEPES, 5 mM Cs₂-EGTA, 0.1 mM GMP and 5 mM Mg₂-ATP, pH adjusted to 7.2 with CsOH, while the bath (extracellular) solution contained 140 mM NaCl, 5 mM CsCl, 1.0 mM MgCl₂, 5.0 mM HEPES and 1 mM

CaCl₂, pH adjusted to 7.3-7.4 with NaOH. The reversal potential of the whole-cell current was -28.3 ± 1.34 mV (n=6), which suggests that the current recorded was the chloride current. The current increased when the medium pH was reduced from 7.4 to 6.8. A specific inhibitor of CFTR, CFTR_{inh}-172, had no effect on the current at pH 7.4, but reduced it at pH 6.8; application of forskolin (10 μM), which elevates intracellular cAMP, produced a similar increase in whole-cell current to that produced by low pH.

These data suggest that the CFTR Cl⁻ channels were opened at low pH. Further experiments are in hand to determine which of the other channels contribute to acidosis-induced ATP release.

OC4.

DIPEPTIDYL-PEPTIDASE 4 INHIBITOR IMPROVES ENDOTHELIAL FUNCTION OF SPONTANEOUSLY HYPERTENSIVE RATS THROUGH ACTIVATION OF GLP-1/GLP-1 RECEPTOR/AMPK/NO CASCADE

L.Liu,¹ WT Wong,¹ XY Tian,¹ J Liu,¹ CW Lau,¹ YX Wang,² G Xu,³ A Xu,^{4,5} KSL Lam,⁴ ZY Chen,⁶ X Yao,¹ Y Huang¹

¹Institute of Vascular Medicine, Li Ka Shing Institute of Health Sciences, and School of Biomedical Sciences; Departments of ²Radiology and ³Medicine and Therapeutics, The Chinese University of Hong Kong; Departments of ⁴Medicine and ⁵Pharmacology and Pharmacy, The University of Hong Kong; ⁶Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong

Objectives Sitagliptin, a highly selective dipeptidyl peptidase 4 (DPP-4) inhibitor, is a new anti-diabetic drug through inhibiting inactivation and degradation of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide. This study investigated whether sitagliptin could protect endothelial function in spontaneously hypertensive rats (SHRs).

Methods SHRs and normotensive WKYs were treated orally with sitagliptin (10 mg.kg⁻¹.d⁻¹) or vehicle for 2 weeks. Renal blood flow was measured by magnetic resonance imaging. Intrarenal arteries were suspended in myograph for force measurement and levels of marker proteins were assayed by Western blotting. Nitric oxide (NO) production was determined by confocal microscopy in primary culture of SHR aortic endothelial cells.

Results Relaxation to GLP-1 receptor agonist exendin-4 was impaired in SHR arteries, which was restored by sitagliptin. The improved relaxation and sitagliptin- or exendin-4-stimulated rises in [NO]_i was reversed by GLP-1 receptor antagonist exendin 9-39, AMPK inhibitor compound C and NOS inhibitor L-NAME. Overexpression of AMPKα2 further increased while expression of dominant negative AMPK inhibited phosphorylation of AMPKα at Thr¹⁷² and eNOS at Ser¹¹⁷⁷ in response to sitagliptin and exendin-4 in endothelial cells. 12h incubation with sitagliptin and exendin-4 augmented endothelium-dependent relaxation (EDR) in SHR arteries, which was antagonized by exendin 9-39 and compound C. In addition, two-week administration of sitagliptin improved EDR, increased phosphorylation of AMPKα and eNOS, up-regulated GLP-1 and GLP-1 receptor in SHR arteries, and restored renal blood flow in SHR.

Conclusions Sitagliptin improves endothelial function in hypertensive rats by restoring NO bioavailability via AMPK/eNOS activation. Stimulating GLP-1/GLP-1 receptor signaling pathway could be another therapeutic option in controlling hypertension-related vascular events.

ABSTRACTS

Abstracts for Oral Communications:

OC5.

INTERMITTENT HYPOXIA INDUCES PARADOXICAL CARDIO-PROTECTIVE EFFECTS IN AN ANIMAL MODEL VIA HEME OXYGENASE-1 UPREGULATION

Q HAN,¹ SC Yeung,¹ MSM Ip,^{1,2} JCW Mak^{1,3}

¹Department of Medicine; ²Research Centre of Heart, Brain, Hormone and Healthy Aging; ³Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong

Background: Obstructive sleep apnea (OSA), with intermittent hypoxia during sleep, is increasingly recognized as an independent risk factor of cardiovascular diseases (CVD). Oxidative stress and inflammation are pathogenic mechanisms in CVD, but majority of studies have focused on the systemic status. Heme-oxygenase-1 (HO-1) is a stress inducible protein and catalyzes rate-limiting step of degradation of cellular heme into free iron, carbon monoxide (CO) and biliverdin/bilirubin, all of which may exert anti-oxidative and anti-inflammatory effects. The aim of this study was to use an animal model to evaluate oxidative stress and inflammation in response to intermittent hypoxia (IH) with or without diet-induced hyperlipidemia, with special reference to any difference in the systemic and cardiac response, and the mechanistic pathways involved.

Methods: Male Sprague-Dawley rats were divided into four groups: regular chow diet or high fat high cholesterol (HFHC) diet plus intermittent air (IA) or IH treatment, and rats were sacrificed at 2 or 4 weeks. Serum and cardiac levels of oxidative and pro-inflammatory markers were assayed with ELISA and semi-quantitative PCR, the expression of HO-1 and activation of signaling pathways in the heart were analyzed by Western blot.

Results: IH and HFHC diet alone or together caused time-dependent elevation in serum malondialdehyde (MDA) and CINC-1 and reduction in serum adiponectin levels. In contrast, elevation of cardiac adiponectin level and suppression of the cardiac levels of oxidative and pro-inflammatory markers were seen, accompanied by upregulation of expression of cardiac HO-1 at 4 weeks when cardiac activation of ERK and Akt signaling pathways were also observed.

Conclusions: Oxidative stress and inflammation resulted from IH and hyperlipidemia may serve as a potential mechanism underlying OSA-related CVD. However, the upregulation of HO-1 expression may exert a local protective function in the heart via activation of ERK and Akt signaling pathway from systemic oxidative and inflammatory insults.

Acknowledgement: This research is supported by Hong Kong RGC General Research Fund (HKU 771908M).

OC6.

BETA1 SUBUNIT-DEPENDENT MODULATION OF BK CHANNEL BY MEMBRANE CHOLESTEROL

W Wu, CP Lau, HF Tse, GR Li

Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Background: The large conductance Ca²⁺-activated K⁺ (BK, or slo) channels are ubiquitously expressed in different tissues without (brain, liver, etc) or with (smooth muscle and heart) regulatory beta-subunit, and play an important role in regulating various physiological processes such as cell excitability, hormone secretion, vascular activity, etc. Recent studies have shown that membrane cholesterol is a major regulator of several potassium channels including Kir and Kv1.5 channels. However, the regulation of BK channels by cholesterol is not fully understood.

Methods: Whole cell BK current and BK single channel current were recorded in whole-cell patch clamp mode and cell-attached single channel recording, respectively, in HEK 293 cells stably expressing Maxi-K with beta1-subunit. Western Blotting was performed to detect the protein expression of BK channel.

Results: We found that whole-cell BK current was significantly suppressed with cholesterol enrichment by cholesterol saturated methyl-beta-cyclodextrin (MβCD), whereas cholesterol depletion by MβCD had no effect on the current amplitude. Low-density lipoprotein (LDL), a class of lipoprotein particles carrying cholesterol around the body, also largely decreases BK current. Single channel recording showed that cholesterol enrichment significantly reduced the open probability of BK channel, suggesting that cholesterol increase likely decreases the membrane channel number. In the opposite, in the cells stably

expressing BK channel without beta1-subunit, cholesterol-saturated-MβCD has no significant effect on the current amplitude of BK channels. Western Blotting data shows that BK channel α subunit expression was reduced by cholesterol-saturated-MβCD or LDL while the β1 subunits expression did not alter. However, both α and β1 subunits expression of BK channel in cultured HCASMCs (Human Coronary Artery Smooth Muscle Cells) was suppressed by cholesterol-saturated-MβCD or LDL.

Conclusion: Our results demonstrate the important evidence that BK channels exhibit beta1-subunit-dependent responses to cholesterol. The enriched-cholesterol and LDL reduce the activity of BK channels co-expressed with α and β1 subunit, which may at least in part accounts for the occurrence of hypertension in patients with high plasma cholesterol level, since both of α and β1 subunit transcripts are abundant in vascular smooth muscle.

ABSTRACTS

Abstracts for Oral Communications:

OC7.**LIPOCALIN-2 DEFICIENCY PROTECTS HEARTS AGAINST ISCHEMIA/REPERFUSION INJURY**B Yang,¹ A Xu,^{1,2} PM Vanhoutte,¹ HF Tse,² Y Wang¹¹Department of Pharmacology and Pharmacy, ²Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Lipocalin-2 is a pro-inflammatory adipokine causally involved in the development of obesity-associated metabolic and vascular diseases. Clinical evidences suggest that there are close associations between circulating levels of lipocalin-2 and cardiac dysfunction. However, little is known about the detailed roles of lipocalin-2 in regulating pathophysiological functions of the heart. The present study has used a Langendorff system to evaluate the heart function of mice lacking lipocalin-2. The results demonstrate that in response to a 20-min global ischemia injury and during the 60-min reperfusion, hearts from lipocalin-2 knockout mice (Lcn2-KO) show a better recovery and improved myocardial contractile functions, compared to those littermates with normal lipocalin-2 expressions. These phenomena can be observed in mice under both standard chow and high fat feeding conditions. Deficiency of lipocalin-2 significantly reduced heart infarct size and lactate dehydrogenase release. These protective functions are partly attributed to the enhancement of mitochondrial respiratory chain activities in Lcn2-KO mice. Furthermore, lipocalin-2 treatment is able to block the recovery of heart function during ischemia/reperfusion injury and acutely damage the mitochondrial functions. In summary, the results support a potential role of lipocalin-2 in the pathogenesis of obesity-related cardiac disorders.

OC8.**UP-REGULATION OF HEME OXYGENASE-1 IMPROVES ENDOTHELIAL FUNCTIONS BY IMPAIRING ENDOTHELIUM-DEPENDENT CONTRACTIONS AND ENHANCING ENDOTHELIUM-DEPENDENT HYPERPOLARIZATIONS**

Z Li, PM Vanhoutte

Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong

Objective: Heme oxygenase (HO) attenuates the production of reactive oxygen species (ROS) through its ability to degrade heme and produce carbon monoxide (CO), biliverdin/ bilirubin, and to release free iron. Up-regulation of HO lowers blood pressure in animals. However, the underlying mechanism is still unknown. The present study was designed to investigate whether or not up-regulation of HO by the pharmacological agent hemin improves endothelial function in arteries of the spontaneous hypertensive rat (SHR).

Methods: 36 weeks old SHR were divided into two groups. One group received an intraperitoneal injection of hemin (50 mg•kg⁻¹, 24 hours before sacrifice) while; the control group was injected with normal saline. Rings of aorta and mesenteric arteries were suspended in organ chambers for isometric tension recording. The intracellular reactive oxygen species (ROS) concentration was measured by confocal microscopy. The release of prostanoids was measured by enzyme immunoassay. Expression of HO-1, cyclooxygenase (COX) and prostaglandin I₂ synthase in the aorta was measured by Western blotting.

Results: HO-1 protein expression was significantly higher in the hemin treatment group than in controls, implying that HO-1 is induced by hemin. This up-regulation of HO-1 resulted in an impairment of both acetylcholine- and A23187-induced endothelium-dependent contractions. A lower expression level of COX-1, a lower intracellular ROS production and a lower release of prostaglandin F_{1 α} (the major stable metabolite of prostacyclin) were observed in the hemin treatment group as compared to the controls. The sensitivity of thromboxane-prostanoid (TP) receptors to prostacyclin and iloprost (prostacyclin analog) were significantly attenuated in hemin-treated rats. Moreover, hemin treatment potentiated acetylcholine-evoked relaxations in mesenteric arteries in the presence of L-NAME and indomethacin, implying a facilitation of endothelium-dependent hyperpolarizations. These enhanced EDHF response were abolished by ouabain (Na⁺-K⁺-ATPase blocker) and barium chloride (Kir channel blocker), indicating an involvement of Na⁺-K⁺-ATPase and Kir channel.

Conclusions: Up-regulation of HO-1 improves endothelial function by attenuating endothelium-dependent contractions as well as potentiating endothelium-dependent hyperpolarizations. The impairment of endothelium-dependent contractions can be explained by an impairment of the expression of COX-1 resulting in the attenuation of the production of ROS and vasoconstrictor prostaglandins combined with a reduced TP receptor sensitivity. The enhancement of endothelium-dependent hyperpolarizations is related to Na⁺-K⁺-ATPase and Kir channel.

ABSTRACTS

Abstracts for Posters:

P1.

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

ALS Au,¹ CCW Poon,¹ Q Zhang,¹ GPH Leung,² SK Kong,³ HP Ho,⁴ YW Kwan¹

¹School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong; ²Department of Pharmacology and Pharmacy, Faculty of Medicine, The University of Hong Kong, ³School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong and ⁴Department of Electronic Engineering, Faculty of Engineering, The Chinese University of Hong Kong, Hong Kong

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

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

determined (ELISA).

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

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

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

P2.

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

WS Cai, FK Ng, HJ Ballard

Department of Physiology and Institute of Cardiovascular Science & Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

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

Experiments were performed in rats anaesthetised with sodium pentobarbitone and breathing spontaneously. A microdialysis probe was placed in one gastrocnemius muscle and samples of interstitial microdialysate were analysed by HPLC for the ATP breakdown products, AMP and adenosine. An electrode was placed on the sciatic nerve, which was stimulated to induce two bouts of muscle contractions, separated by a recovery period

of 40 mins. In the test group of rats, KT5720, a protein kinase A inhibitor which prevents activation of CFTR, was administered between the two bouts of contractions; in the control rats, no drug was given.

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

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

ABSTRACTS

Abstracts for Posters:

P3.

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

YC Chan¹, YK Lee¹, KM Ng¹, WH Lai¹, D Yang², HF Tse^{1,3}, CW Siu^{1,3}

¹Cardiology Division, Department of Medicine, Queen Mary Hospital; ²Department of Chemistry; ³Research Center of Heart, Brain, Hormone and Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

The electrophysiological hallmark of cells and tissues isolated from failing hearts is prolongation of action potential duration (APD), resulted from down-regulation of repolarizing K⁺ currents and/or alterations in depolarizing Na⁺ and Ca²⁺ currents, which predisposes the failing heart to lethal ventricular tachyarrhythmia (ventricular tachycardia (VT) and ventricular fibrillation (VF)). C11, a small synthetic Cl⁻ channel, exhibits membrane-repolarizing power. Therefore, we hypothesize C11 corrects the delayed repolarization and shortens APD at cellular level, thus modifying ventricular arrhythmogenic substrate at whole heart level. First, we demonstrated APD reduction upon C11 application (30 μM) at 37°C to isolated guinea pig ventricular cardiomyocytes with patch-clamp experiments in whole cell configuration.

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

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

P4.

VASCULAR MODULATION EFFECT OF GINSENG EXTRACTS

GHH Chan,¹ SW Chan,² CW Lau,³ Y Huang,³ PYK Yue,¹ RNS Wong¹

¹Department of Biology, Hong Kong Baptist University; ²Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University; ³School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

Background: It is known that endothelial damage is the primary cause of the vascular complications of diabetes mellitus. Moreover, the endothelial damage is usually not reversible.

Materials and methods: In the present study, we examine the effects of ginseng extracts (PPD and PPT) on acetylcholine-induced endothelium dependent vasorelaxation pre-contracted with phenylephrine. Studies were performed in adult Sprague-Dawley male rats and the responses were examined in vitro using isolated thoracic aortic rings.

Result: Severe impairment of vasorelaxation was found in diabetic group (62.4% of control in maximum dosage of ACh-induced vasorelaxation) while the groups fed with ginseng extracts restored the vasorelaxation (no significant difference with control). Blood profile exhibited that the ginseng extracts could not reduce blood glucose and blood cholesterol level caused by diabetes mellitus. However, ginseng extract PPD improved nitric oxide (in terms of nitrite) production stimulated by acetylcholine.

Conclusion: Ginseng may have vascular protective effect on diabetic condition.

Acknowledgement: The funding support is provided by the Collaborative Research Fund (HKBU 1/06C) of Research Grant Council of HKSAR.

ABSTRACTS

Abstracts for Posters:

P5.

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

WS Cheang,¹ XY Tian,¹ WT Wong,¹ SY Tsang,² CW Lau,¹ X Yao,¹ Y Huang¹

¹Institute of Vascular Medicine and School of Biomedical Sciences, ²Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong

Objectives: H₂S may act through the opening of ATP-sensitive K⁺ channels or 4-aminopyridine-sensitive voltage-gated K⁺ channels to cause vasodilatation, depending on the type of blood vessels. It is however unclear how H₂S acts on cerebral arteries. The present study investigates whether NaHS, a H₂S donor, inhibits voltage-sensitive Ca²⁺ channels and thus relaxes cerebral arteries.

Methods: Middle cerebral arteries of Sprague-Dawley rats were suspended in wire myograph or pressurized myograph for measurements of vascular reactivity under isometric or isobaric conditions. Single myocytes were enzymatically isolated from the rat cerebral arteries for measuring whole-cell L-type Ca²⁺ currents by a patch-clamp method. Ca²⁺ movement in isolated cerebral arteries was determined using a fluo-4 fluorescence dye under confocal microscope.

Results: NaHS relaxed both phenylephrine and 60 mM KCl pre-contracted rat cerebral arteries with the same potency. The relaxations were unaffected by several K⁺ channel blockers, NOS inhibitor N^G-nitro-L-arginine methyl ester, nor cyclo-oxygenase inhibitor indomethacin. H₂S precursor L-cysteine induced dilatation in cerebral arteries, which was inhibited by cystathionine γ-lyase inhibitor DL-propargylglycine. NaHS concentration-dependently

inhibited CaCl₂-induced contraction in Ca²⁺-free Krebs solution. NaHS reduced the amplitude of L-type Ca²⁺ currents in isolated myocytes and directly inhibited Ca²⁺ influx in rat cerebral arteries. NaHS also caused relaxation of myogenic tone in cerebral arteries under pressurized condition. **Conclusions:** NaHS causes relaxations, independent of K⁺ channel activation, and suppresses CaCl₂-induced contractions in rat cerebral arteries. It reduced L-type Ca²⁺ currents in myocytes and inhibited Ca²⁺ influx in rat cerebral arteries. These suggest that H₂S relaxes cerebral arteries primarily through inhibiting Ca²⁺ influx via L-type Ca²⁺ channels.

P6.

EXPRESSION OF TRP CHANNELS IN ARTERIAL BARORECEPTOR NEURONS

OC Lau, CO Wong, Y Huang, X Yao

School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

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

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

There are two major baroreceptors, aortic and carotid baroreceptors. In the present study, immunohistochemistry was employed to explore the expression of mechanosensitive TRP isoforms in the rat aortic baroreceptor.

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

ABSTRACTS

Abstracts for Posters:

P7.

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

KL Ong,¹ CQ Jiang,² B Liu,² YL Jin,² AWK Tso,¹ S Tam,³ KS Wong,⁴ B Tomlinson,⁴ BMY Cheung,¹ JM Lin,² XJ Yue,² KSL Lam,¹ TH Lam,⁵ GN Thomas⁶

¹Department of Medicine, The University of Hong Kong, Hong Kong; ²Guangzhou No. 12 Hospital, China; ³Department of Clinical Biochemistry, Queen Mary Hospital, Hong Kong; ⁴Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong; ⁵Department of Community Medicine and School of Public Health, The University of Hong Kong, Hong Kong; ⁶Public Health, Epidemiology and Biostatistics, University of Birmingham, UK

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

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

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

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

P8.

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

XY Tian,¹ WT Wong,¹ J Tian,² P Zhang,² N Wang,² Y Huang¹

¹Institute of Vascular Medicine and School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong; ²Institute of Cardiovascular Science, Peking University, China

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

Methods: Mouse aortas were treated with rosiglitazone for 24 hours, and ET-1-induced vasoconstriction was assessed by wire myography. The results showed that rosiglitazone attenuated ET-1-induced contraction in mouse aortas; this effect was abolished by ET-B receptor (ET_BR) antagonist A192621, NO synthase inhibitor (L-NAME), and by the removal of endothelium. Western blotting, and immunohistochemistry showed that rosiglitazone upregulated expression of ET_BR in mouse aortas. Rosiglitazone also enhanced selective ET_BR agonist sarafotoxin 6c-induced vasodilatations in mesenteric resistance arteries which were abolished by L-NAME or

A192621. In vivo treatment with rosiglitazone also attenuated the ET-1-induced vasoconstrictions and increased the ET_BR expression without affecting the expression of ET_AR in mouse aortas and mesenteric arteries.

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

ABSTRACTS

Abstracts for Posters:

P9.

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

J Zhang, YC Chan, HF Tse

Department of Medicine, The University of Hong Kong, Hong Kong

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

The expression of various common ion channels for sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and chloride (Cl^-) in human iPS-MSCs were examined by reverse transcription-polymerase chain reaction (RT-PCR). Those functional ion channels identified by RT-PCR were confirmed by whole-cell patch clamp technique. RT-PCR revealed the molecular identities (mRNAs) of some ion channels and their possible existence in human iPS-MSCs, including *KCa1.1* (responsible for the big conductance Ca^{2+} -activated K^+ current, BK_{Ca}), *Kv10.1* (for the delayed rectifier K^+ current, IK_{DR}), *Kir2.1* and *Kir2.3* (for the inwardly-rectifying K^+ current, I_{Kir}), *KCa3.1* (for the intermediate conductance Ca^{2+} -activated K^+ current, IK_{Ca}), *Clcn3* (for the chloride current, I_{Cl}), *SCN9A* (for the tetrodotoxin-sensitive sodium current, $\text{I}_{\text{Na,TTX}}$), *CACNA1C* (for the nifedipine-sensitive L-type Ca^{2+} current, $\text{I}_{\text{Ca,L}}$)

and *Kv4.3* (for the transient outward potassium current, I_{to}), but not *Kv1.4* and *Kv4.2* (for I_{to}). Patch clamp experiments were conducted to verify the existence of functional ion channels, five types of currents (BK_{Ca} , IK_{DR} , I_{Kir} , IK_{Ca} and I_{Cl}) were found in human iPS-MSCs, but not the three ($\text{I}_{\text{Na,TTX}}$, $\text{I}_{\text{Ca,L}}$ and I_{to}) reported in BM-MSCs.

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

P10.

EXPRESSION OF TRPC AND TRPM CHANNELS IN HUMAN ATRIAL MYOCYTES

YH Zhang, CP Lau, HF Tse, GR Li

Department of Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong

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

information TRPC1, TRPC3, and TRPM7 channels are present in human atrial myocytes. Activation of TRP channels likely contribute to the genesis of human atrial fibrillation, and therefore TRP channels may be a target for the development of anti-atrial fibrillation approach.

ABSTRACTS

Abstracts for Posters:

P11.

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

YZ Zhao, SWS Leung, PM Vanhoutte

Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong

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

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

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

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

P12.

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

Q Zou, PM Vanhoutte

Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong

Acetylcholine causes both endothelium-dependent relaxations and contractions in the rat aorta. Both muscarinic (mAChRs) and nicotinic (nAChRs) acetylcholine receptors are expressed in endothelial cells. It is generally accepted that mAChRs [of the M_3 -subtype] are responsible for both endothelium-dependent relaxations and contractions evoked by acetylcholine. To study the expressions of acetylcholine receptors in rat aorta, PCR primers for different subunits of nAChRs and mAChRs were designed. To study whether or not nAChRs are involved in endothelium-dependent relaxations evoked by the cholinergic transmitter, 36 weeks old spontaneous hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, and 64 weeks old WKY rats were used. Rings with endothelium were suspended in organ chambers for isometric tension recording. Quiescent rings were incubated with vehicle, mecamylamine (nAChRs inhibitor, 10^{-4} M), atropine (mAChRs inhibitor, 10^{-5} M), or mecamylamine plus atropine. The concentration of mecamylamine and atropine was the lowest concentration that gave the most significant inhibitory effect in organ chamber experiments. All the rings

were incubated with indomethacin (non-selective cyclooxygenase inhibitor, 10^{-5} M) to prevent endothelium-dependent contractions. After 40 minutes of incubation, the rings were contracted with phenylephrine (10^{-5} M) and then relaxed with cumulatively increasing concentrations of acetylcholine (10^{-9} to 10^{-5} M). The PCR results revealed that $\alpha 2$, 3, 4, 5, 6, 7, 9, 10 subunits of nAChRs and M1-M5 subunits of mAChRs were expressed in 36 weeks old SHR aorta. The organ chamber experiments demonstrated that in both SHR and WKY aortae, acetylcholine-induced relaxations were similar in control and mecamylamine-treated rings, while the relaxations were significantly reduced in atropine-treated preparations. In rings of 36 weeks old SHRs, the remaining acetylcholine-induced relaxations in the presence of atropine approximated 50% of those observed in untreated control preparations, while in both 36 weeks and 64 weeks old WKY aortae the remaining response in atropine-treated preparations was minimal. The response to acetylcholine was nearly abolished in rings which had been incubated with mecamylamine plus atropine. Thus in the SHR and WKY aorta, mAChRs are mainly responsible for endothelium-dependent relaxations to acetylcholine under control conditions. However, when mAChRs are inhibited by atropine, nAChRs mediate relaxation to the cholinergic transmitter in the SHR but not the WKY aorta.

ABSTRACTS

Abstracts for Posters:

P13.

CALCITRIOL PROTECTS RENOVASCULAR FUNCTION OF OVARIECTOMIZED RATS THROUGH THE DOWN-REGULATION OF CYCLOOXYGENASE-2 AND TP RECEPTORJ Dong,¹ SL Wong,¹ CW Lau,¹ WT Wong,¹ ZY Chen,² X Yao,¹ Y Huang¹¹Institute of Vascular Medicine and School of Biomedical Sciences;²Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong

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

showed that calcitriol reduced the elevated expression of COX-2 and TP receptor in renal arteries from ovariectomized rats. The present results suggest that the impaired endothelium-dependent relaxations in renal arteries of ovariectomized rats are possibly attributed by the release of COX-2-derived prostaglandin(s) that activate the TP receptor and that calcitriol normalizes the renovascular function at least in part through COX-2 and TP receptor down-regulation.

P14.

DOSE-RESPONSE RELATIONSHIP BETWEEN SMOKING, SMOKING CESSATION STATUS AND CAROTID ATHEROSCLEROSIS: THE GUANGZHOU BIOBANK COHORT STUDY-CVDCQ Jiang,¹ L Xu,^{1,2} TH Lam,² JM Lin,¹ WS Zhang,¹ B Liu,¹ YL Jin,¹ KK Cheng,³ GN Thomas³¹Guangzhou No.12 Hospital, China; ²Department of Community Medicine, School of Public Health, The University of Hong Kong, Hong Kong; ³Public Health and Epidemiology, University of Birmingham, UK

Objective: To examine the dose-response relationship between smoking and quitting smoking status with carotid atherosclerosis in 959 relatively healthy Chinese men.

Methods: 959 men aged 50-85 years were randomly selected from phase III (2006-2007) of the Guangzhou Biobank Cohort Study into this cross-sectional study. Common carotid artery intima-media thickness (CCA-IMT) was measured by B-mode ultrasonography, and carotid arterial plaques were identified. Major cardiovascular risk factors, including fasting triglyceride, LDL- and HDL-cholesterol, glucose, and systolic and diastolic blood pressure were assessed.

Results: 1) Composition of the cases: 39.1% were non-smokers, 25.7% were former smokers and 35.2% were current smokers. The mean (95% confidence interval) carotid IMT was 0.78 (0.77-0.79) mm. 18.4% of the subjects had a carotid IMT \geq 1.0 mm, while 34.1% had carotid plaque. 2) After adjusting for age, sex, physical activity, body mass index, fasting glucose, triglyceride, HDL-cholesterol, systolic and diastolic blood pressure, compared to never smokers, current smokers had significantly increased risk for thicker IMT

and carotid plaque [odds ratio(OR)=1.82, 95%CI: 1.30-2.55 and OR=1.95, 95%CI:1.38-2.75, respectively, all P<0.001]. The risk for thicker IMT and carotid plaque increased with the increasing amount (cigarettes/day) and duration of smoking (years) as well with cigarette pack-years (P for trend all \leq 0.01). 3) Compared to current smokers, after adjustment for cigarette pack-years and other potential confounders, the adjusted ORs (95% CI) for the duration following quitting for 1-9, 10-19 and 20+ years were 0.77 (0.47 to 1.26), 0.45 (0.26 to 0.79) and 0.37 (0.17 to 0.77) for the presence of CCA atherosclerosis, and 0.69 (0.43 to 1.12), 0.47 (0.27 to 0.82) and 0.45 (0.23 to 0.96) for the presence of carotid plaques, respectively.

Conclusion: An elevated risk with a clear dose-response relationship was found between cigarette smoking and carotid atherosclerosis. Smoking cessation was beneficial in attenuating the risk of carotid atherosclerosis associated with a dose-response relationship with quitting time.

Acknowledgement: This project was supported by grants from the National Natural Science Foundation of China (No. 30518001/CO301070202) and the Hong Kong Research Grants Council (No. N-HKU720/05)

ABSTRACTS

Abstracts for Posters:

P15.

THE POTENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPS) DERIVED ENDOTHELIAL PROGENITOR CELLS (EPC) IN THERAPEUTIC ANGIOGENESISWH Lai,¹ JCY Ho,^{1,2} YK Li,¹ KW Au,¹ CW Siu,^{1,2} HF Tse^{1,2}¹Cardiology Division, Department of Medicine; ²Research Centre of Heart, Brain, Hormone & Healthy Aging, Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Experimental and clinical studies have shown that endothelial progenitor cells (EPCs) can enhance angiogenesis in ischemic hindlimb muscles and myocardium. However, the use of autologous EPCs transplantation isolated from patients' blood are limited by their number and proliferative potential. Human induced pluripotent stem cell (hiPS) is a potential alternative cell source for EPC generation due to their autology, high power of proliferation and pluripotency.

Methods: We characterize the phenotype and function of hiPS-derived EPCs and compare them with human endothelial cell line (HUVEC) and human embryonic stem cell derived EPC (H1-EPC). Donor specific Induced pluripotent stem cells were generated from their skin fibroblast in feeder free, serum free culture system and subsequently differentiated into EPCs.

Results: Two EPCs were generated (SIU1-EPC and IMR90-EPC) and have similar morphology as H1 HES derived EPC. Positive vWF, α -SMA, Di-acetyl-LDL-DiI and Lectin staining was observed in both iPS-EPC and H1-EPC. Tube formation assay revealed similar potential in forming capillary with IMR90-EPC and SIU1-EPC as H1-EPC and HUVEC (13450 \pm 882,

15108 \pm 984.6 vs 14867.12 \pm 934.54 and 15349.59 \pm 1034.67 AU/well). Animal model showed that the iPS derived EPSs can have comparable potential that resume part of regional blood flow 28 days after left femoral artery occlusion (IMR90-EPC and SIU1-EPC 35.5 \pm 6.2%, 37.9 \pm 5.5% vs H1-EPC and HUVEC 38.5 \pm 4.5%, 32.8 \pm 2.8%).

Conclusions: Our results demonstrate that hiPS-derived EPCs resemble normal human endothelial cells with similar phenotypes and angiogenic function but unlimited proliferation capacity. These findings suggest that hiPS-derived EPC can be used as patient specific cell source in therapeutic angiogenesis.

P16.

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

ZF Lai

Department of Pharmacology and Molecular Therapeutics, Graduate School of Medical Sciences, Kumamoto University, Japan

Objectives: TNNI3K is a novel cardiac-specific and troponin I (cTnI)-interacting MAP kinase. Recently, we investigated the effects of TNNI3K on P19CL6-derived cardiomyogenesis in vitro, and results indicated that TNNI3K specifically interacted with cTnI by inhibiting cTnI phosphorylation and played important roles in cardiac myogenesis. The aim of this study is to evaluate whether TNNI3K overexpression can reduce myocardial ischemic injury in mouse cardiac infarction model and whether plasma TNNI3K levels can predict the occurrence of acute cardiac infarction (AMI) in patients or not.

Methods and Results: A myocardial infarction (MI) model of mice was made by ligation of left anterior descending branch of the coronary artery, and the differentiated P19CL6 cells (induced 4 days in 1% DMSO) with overexpressing TNNI3K was injected at four points, with 20 microliter per point, in the border zone surrounding the infarcted area. TNNI3K-overexpression attenuated ventricular remodeling, improved impaired UCG ejection fraction, left ventricular end-diastolic and end-systolic dimensions, decreased the infarct size when compared with the medium-only or the vector-only groups (P<0.05). To measure the circulating TNNI3K level in AMI patients, a polyclonal anti-human TNNI3K antibody was raised in rabbit

by injecting recombinant TNNI3K and purified as IgG by using protein A-conjugated sepharose column. Standard curve gained by enzyme-linked immunosorbent assay (ELISA) was linear through the range from 0.011 to 11.00 ng/ml of TNNI3K protein. Plasma TNNI3K concentrations of healthy volunteers, acute renal failure, chronic heart failure and acute myocardial infarct patients measured by ELISA were 221.38 \pm 12.8 (n=21), 218.26 \pm 6.8 (n=6), 320.7 \pm 8.9 (n=18) and 2025.0 \pm 200.7 (n=18) ng/ml, respectively. Results indicated that circulating TNNI3K level in AMI patients was significantly higher than that in any other group (p<0.001). Specificity and sensitivity to differentiate AMI from the other two was evaluated using receiver operating characteristic curve analysis (MedCalc software) and 82.4% of specificity and 86.7% of sensitivity was gained by setting a cut-off value at 366 ng/ml.

Conclusions: Over-expressing the TNNI3K level would be a useful therapeutic approach for ischemic cardiac diseases, and measurement of plasma TNNI3K level may be a novel useful diagnostic tool for acute myocardial infarction. Using TNNI3K as a molecular target may be a new potential approach for developing remedies or diagnosis agents for ischemic cardiac diseases.

ABSTRACTS

Abstracts for Posters:

P17.

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

CW Lau,¹ FP Leung,¹ SL Wong,¹ WT Wong,¹ X Yao,¹ Y Li,² Y Huang¹

¹Institute of Vascular Medicine and School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong; ²Department of Pharmacology, School of Pharmaceutical Science, Central South University, Hunan, China

Rutaecarpine, a bioactive alkaloid extracted from the dried fruit *Evodia rutaecarpa*, is widely used to treat hypertension. However, its effect on peripheral resistance arteries remains elusive. The present study characterizes the vascular action of rutaecarpine in resistance arteries. Alterations in isometric tension of the third-order Sprague-Dawley rat mesenteric resistance arteries were studied in myograph. Field stimulation was applied to trigger neurogenic relaxations. Real-time changes in intracellular calcium concentration ($[Ca^{2+}]_i$) in native mesenteric endothelial cells and NO production ($[NO]_i$) in primary cultured mesenteric endothelial cells were determined by confocal microscopy. Rutaecarpine caused potent relaxations which were partially inhibited by N^G-nitro-L-arginine methyl ester (NOS inhibitor), ODQ (guanylate cyclase inhibitor) or in rings without endothelium. Rutaecarpine-induced relaxations were attenuated by CGRP receptor antagonist, CGRP (8-37) or depletion of CGRP from sensory nerves by capsaicin. Field stimulation-induced relaxations were eliminated by

CGRP (8-37) and capsaicin. Rutaecarpine only partially relaxed mesenteric arteries of eNOS knockout mice to an extent similar to the L-NAME-treated arteries from the wild type mice, and the relaxations in eNOS knockout mice were abolished by CGRP (8-37). Rutaecarpine stimulated rises in both endothelial cell $[Ca^{2+}]_i$ and $[NO]_i$. The present results demonstrate that rutaecarpine exerts potent vasodilatory effects in resistance arteries by (1) increasing eNOS activity and calcium-dependent NO formation in endothelial cells, and (2) stimulating the neurogenic release of capsaicin-sensitive CGRP which causes NO-independent relaxations. The concomitant neurovascular effects in the resistance arteries are likely to account for the reported blood pressure-lowering action of rutaecarpine.

P18.

CALCIUM HANDLING IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

YK Lee,^{1#} KM Ng,^{1#} WH Lai,¹ YC Chan,¹ Q Lian,^{1,2} CP Lau,¹ HF Tse,^{1,2} CW Siu^{1,2}

¹Cardiology Division, Department of Medicine, Queen Mary Hospital, The University of Hong Kong; ²Research Center of Heart, Brain, Hormone and Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

#contribute equally to this work

Although human induced pluripotent stem cells (hiPSCs) has brought optimism and excitement to the field of cardiac regenerative medicine due to its unquestioned potential to differentiate into cardiomyocytes, many questions remain to be answered before clinical application can be contemplated. The crucial first step is the characterization of the functional properties of hiPSC-derived cardiomyocytes. However, the calcium handling properties, the key process for excitation-contraction coupling, has not been studied. In fact, the functional immaturity in calcium homeostasis may at best result in poor graft-host integration, and at worst lead to potential lethal arrhythmias, thus raising important safety issues for regenerative purposes. This Here, we studied the calcium homeostasis of hiPSC-derived cardiomyocytes in comparison with H7 hESC-derived cardiomyocytes with fluorescence confocal microscopy. Cardiac differentiation of hiPSCs and hESCs were induced with standard mesoderm induction protocol. Beating outgrowth suggestive cardiomyocytes were first appeared on day 15.

Compared with differentiated hESCs, differentiated hiPSCs appeared to have a lower expression level of a panel of cardiac specific genes including Nkx2.5, α -MHC and β -MHC. In addition, hiPSC-derived cardiomyocytes exhibited an more immature calcium handling property in comparison with hESC-derived cardiomyocytes with a significantly smaller amplitude and slower maximal upstroke velocity (V_{max} upstroke) in the spontaneous calcium transients as well as caffeine induced calcium transient suggestive poorly developed sarcoplasmic reticulum function. Consistently, the expression level of the a few key calcium-handling proteins including sodium-calcium exchanger (NCX-1) and sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) as well as the sarcoplasmic reticulum (SR) junctional candidate, triadin (Trd), were significantly lower in hiPSC in comparison with hESCs.

Conclusions: Taken collectively, hiPSCs exhibited immature calcium handling properties, which may hamper its potential for future clinical application.

ABSTRACTS

Abstracts for Posters:

P19.**HEMIN RESTORES THE IMPAIRED ENDOTHELIUM-DEPENDENT VASODILATATION IN DIABETIC *db/db* MICE THROUGH PI3K/Akt PATHWAY**

J Liu, WT Wong, XY Tian, LM Liu, SL Wong, CW Lau, J Yu, X Yao, Y Huang

Institute of Vascular Medicine and School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

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

P20.**MELATONIN ATTENUATES INTERMITTENT HYPOXIA INDUCED OXIDATIVE STRESS AND TISSUE INFLAMMATION IN RAT ADRENAL MEDULLA**

Y Liu, ML Fung

Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Patients with sleep-disordered breathing suffer from intermittent hypoxia (IH). Previous studies have shown that chronic exposure to IH associated with recurrent apnoea induces oxidative stress and pathophysiological changes in the cardiovascular system. The adrenal medulla is responsive to acute hypoxia and plays an important role in the cardiovascular response to hypoxia. Yet, the chronic effect of IH on the adrenal medulla is currently undefined. We hypothesized that the free radical scavenger melatonin can attenuate the IH-induced oxidative stress and local injury and inflammation in the adrenal medulla. Adult Sprague-Dawley rats were exposed to air or IH mimicking a severe condition of sleep apnoea for 14 days. An intraperitoneal injection of melatonin (10 mg/kg) or vehicle was given daily prior to the IH treatment. The adrenal medulla was harvested for the measurement of markers for oxidative stress, malondialdehyde (MDA) and nitrotyrosine, and for the histological analysis of macrophages infiltration and TUNEL staining for apoptosis. Also, the protein expression of iNOS was examined by western blot. Our results showed that the MDA level was significantly increased in the IH group, when compared with the Nx control and melatonin-treated IH group. Image analysis also showed significantly

more percentage area of the adrenal medulla with positive immunostaining of NTR than that of the Nx group and the melatonin-treated IH group. In addition, macrophage marker ED1-immunoreactivity was remarkable and the protein expression of iNOS was also significantly increased in the IH group, suggesting a local inflammation induced by IH in the adrenal medulla. Moreover, there was an increase in the number of apoptotic cells in the adrenal medulla of IH rats, and the increase was ameliorated in the IH group treated with melatonin. In conclusion, our results support the hypothesis that IH-induced oxidative stress is involved in the local inflammation and apoptosis in the adrenal medulla. The antioxidant melatonin can effectively attenuate the IH-induced tissue injury and inflammation in the rat adrenal medulla.

ABSTRACTS

Abstracts for Posters:

P21.

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

CCW Poon,¹ ALS Au,¹ Q Zhang,¹ SK Kong,² HP Ho,³ GPH Leung,⁴ YW Kwan¹

¹School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong; ²School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong; ³Department of Electronic Engineering, Faculty of Engineering, The Chinese University of Hong Kong; ⁴Department of Pharmacology and Pharmacy, Faculty of Medicine, The University of Hong Kong, Hong Kong

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

Experimental approaches: The pancreatic islets of age-matched (female; ~6 month-old) lean/control (*db+/m+*) and obese/diabetic (*db+/db+*) of C57BL/KsJ mice were isolated using collagenase; single pancreatic β -cells of both strains of mice were further isolated by trypsin. Effects of L-NAC (20 mM; 24-hr incubation) on mitochondrial ROS generation, insulin release/

content and cortical F-actin cytoskeleton levels of pancreatic islets/single β -cells (bathed in normal (5 mM) and high (15 mM) glucose culture medium) of both strains of mice were evaluated and compared.

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

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

Acknowledgements: This project is financially supported (to YWK) by RGC Research Grant of Hong Kong SAR (Reference no: 2140565). Provision of post-graduate (MPhil) studentship to Ms. CCW Poon by School of Biomedical Sciences (The Chinese University of Hong Kong) is acknowledged.

P22.

ERGOTHIONEINE PROTECTS ENDOTHELIAL CELLS FROM OXIDATIVE STRESS

ASM Sit,¹ EYW Ho,¹ RWS Li,¹ RYK Man,¹ YW Kwan,² M Hausman,³ PM Vanhoutte,¹ GPH Leung¹

¹Department of Pharmacology and Pharmacy, The University of Hong Kong; ²School of Biomedical Sciences, The Chinese University of Hong Kong; ³Total Nutraceutical Solutions, Hong Kong

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

In this study, we aimed to investigate whether or not ergothioneine can enter endothelial cells and protect endothelial cells against cellular damage induced by oxidative stress. Human brain microvascular endothelial cells (HBMECs) were used in this study. The results of RT-PCR demonstrate the expression of OCTN1 in HBMECs. Consistently, [³H]ergothioneine could be taken up by HBMECs through a sodium-dependent and transporter-dependent system. MTT assay shows that the viability of HBMECs was decreased when the

cells were incubated in medium containing 25 mM (which mimics hyperglycemic condition in diabetes) instead of 5 mM glucose. Interestingly, ergothioneine (in a concentration as low as 10 nM) was able to reduce the detrimental effect of 25 mM glucose. In addition, 1 mM ergothioneine could reduce the cytotoxic effect of 600 nM hydrogen peroxide on HBMECs.

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

ABSTRACTS

Abstracts for Posters:

P23.

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

YY Tam,¹ WT Wong,¹ XY Tian,¹ WS Cheang,¹ CW Lau,¹ AM Xu,² Y Huang¹
¹Institute of Vascular Medicine and School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong; ²Department of Medicine and Department of Pharmacology, China

Objectives: Homocysteine is a well-known risk factor of cardiovascular disease. Hyperhomocysteinemia is found in patients with atherosclerosis and myocardial infarction. Homocysteine also impairs endothelium-dependent vasodilatation. Tea consumption is known to reverse endothelial dysfunction in patients with cardiovascular disease. This study aims at investigating whether homocysteine-induced endothelial dysfunction involves endoplasmic reticulum (ER) stress, and whether black tea polyphenols can improve endothelial function impaired by homocysteine. **Methods:** Vasoreactivities of rat aortas were assessed in wire myograph. Protein expressions were examined by Western blotting.

Results: Homocysteine impairs endothelium-dependent relaxations (EDRs) of rat aortas in a concentration-dependent manner. ER stress alleviator sodium 4-phenylbutyrate (PBA) increases EDRs impaired by Homocysteine. A mixture of theaflavins (TE) and theaflavin-3,3'-digallate (TF3) also improves EDR impaired by Homocysteine. In addition, Western blotting showed homocysteine increased eIF2 α phosphorylation in rat aortic endothelial cells, which was inhibited by PBA, and theaflavins.

Conclusions: The present study provides evidences for the possible involvement of ER stress in homocysteine-induced endothelial dysfunction, it also suggests that theaflavins improve endothelial function impaired by homocysteine.

P25.

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

HJ Wu, W Wu, GR Li
 Department of Medicine, The University of Hong Kong, Hong Kong

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

P24.

ROLE OF JUMI EXTRACTION IN ACUTE RENAL FAILURE RATS

M Wang, ML Xia, XM Zhou, XY Xie
 Department of Physiology, Jiaying University, School of Medicine, China

Objective: The purpose of this study is to investigate the effect of jumi extraction on acute renal failure (ARF) rats: role of tyrosine hydroxylase (TH) in hypothalamus paraventricular (PVN) and kidney.

Methods: Male SD rats were randomly divided into four groups: control group, ARF group, Jumi extraction group and ARF+ Jumi extraction group. Glycerol-induced acute renal failure in rats was employed. Malondialdehyde (MDA) and reduced glutathione hormone (GSH) in renal cortex homogenate were measured by commercial kits. Electron microscope were used to examine the pathological changes. Meanwhile the expressions of TH in kidney were evaluated by immunohistochemistry.

Results: ARF rats administrated orally with 2 mL of normal saline for 48 h showed a significant increase in MDA ($P<0.05$), but GSH markedly decreased ($P<0.05$), when compared with that in control group. After treatment of ARF rats with jumi extraction for 48 h, MDA were significantly decreased ($P<0.05$), but GSH markedly increased ($P<0.05$), and the severity of tubular necrosis was alleviated when compared with that in ARF group. Immunohistochemistry showed obvious increase of tyrosine hydroxylase-immunoreactivity (TH-IR) in PVN and kidney in ARF group ($P<0.05$), but TH-IR was further enhanced in ARF+ Jumi extraction group ($P<0.05$).

Conclusion: The results indicated that jumi extraction could have a strong renal protective effect against ARF. TH in PVN and kidney may contribute to renal protective effect of jumi extraction against ARF.

acacetin blocks both closed and open channels of hKv1.5 by binding to the S6 domain of hKv1.5 channels. The use- and rate-dependent blocking property of hKv1.5 by acacetin indicates that this natural compound could exert a strong suppressive effect on atrial fibrillation in man.

ABSTRACTS

Abstracts for Posters:

P26.

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

ML Xia, M Wang, YY Huang

Department of Physiology, College of Medicine, Jiaying University, China

Objective: To observe the differences of mean arterial blood pressure (MABP), heart rate (HR) and thoracic aorta tension induced by two septic shock models in rats and explore the possible mechanism.

Methods: we used cecal ligation and puncture (CLP) 20 hours and lipopolysaccharide (LPS) 6 hours to establish septic shock in rats. The carotid artery was cannulated and connected to a pressure transducer to determine mean arterial blood pressure (MABP). Ventricular dynamic parameters were determined following intraventricular cannulation via the carotid artery, including heart rate (HR), left ventricular developed pressure (LVDP), maximal rise/fall velocity of ventricular pressure ($\pm dp/dt_{max}$). Isolated thoracic rings were mounted on an organ bath and the tension of the vessel was recorded.

Results: (1) The mortality was 65.2% (30/46) in CLP shock rats, but no rats dead in LPS shock rats (0/24); (2) The two models showed significant decrease in MABP and HR, and CLP model's decrease more ($P < 0.01$) (CLP decrease 55.7% and 71.5%, LPS decrease 41.5% and 58.3%); (3) Constriction by high K^+ (60 mmol/L) or 10^{-6} mol/L phenylephrine (PE) in endothelium-intact aortic rings were all decrease, and LPS model's

decrease more ($P < 0.01$) (CLP decrease 22.8% and 26.4%, LPS decrease 70.1% and 72.9%). And constriction by high K^+ or PE in endothelium-denuded aortic rings had the similar decrease.

Conclusion: The ventricular-dynamic parameters and vasoconstriction responsiveness of aorta were all decrease in two septic shock models in rats. CLP model's decrease more in the ventricular-dynamic parameters, though LPS model's decrease more in vasoconstriction responsiveness of aorta.

P27.

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

CY Yuen,¹ SL Wong,¹ CW Lau,¹ B Tomlinson,² X Yao,¹ Y Huang¹

¹Institute of Vascular Medicine, Li Ka Shing Institute of Health Sciences, School of Biomedical Sciences and ²Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong

Telmisartan robustly activates peroxisome proliferator-activated receptor- γ (PPAR γ) besides serving as an angiotensin II type 1 receptor (AT₁R) blocker. It has been suggested that the activation of PPAR γ exerts beneficial effects on the vascular function. However, the PPAR γ agonistic effect of telmisartan on resistance arteries is unclear. The present study aimed at investigating whether telmisartan promotes relaxations in mouse mesenteric resistance arteries by stimulating the expression and activity of endothelial nitric oxide synthase (eNOS), which in turn augments nitric oxide (NO) production via the PPAR γ -dependent mechanism. Second-order mesenteric arteries from male C57BL/6J were isolated and cultured overnight with telmisartan, after which changes of their isometric tension were measured in myographs. Expression and activation of relevant proteins were analyzed by Western blotting. Real time changes in intracellular NO level in human endothelial cells was monitored by confocal microscopy. Telmisartan (10 μ M) enhanced acetylcholine (ACh)-induced endothelium-dependent relaxations, which were inhibited by a PPAR γ antagonist, GW9662 (300 nM). Telmisartan up-regulated GW9662-sensitive eNOS expression and phosphorylation, which were absent in PPAR γ knockout (KO) mice. Additionally, telmisartan

increased the basal and ACh-stimulated NO production, and both were prevented by GW9662. Taken together, the present results indicate that telmisartan enhances endothelium-dependent relaxations in resistance arteries, which is mediated through PPAR γ -dependent elevation of NO production as a result of the increased eNOS expression and activity, possibly independent of AT₁R blockade. PPAR γ agonistic activity of telmisartan may offer additional vascular benefits in addition to the known AT₁R antagonistic effect.

ABSTRACTS

Abstracts for Posters:

P28.

ROLE OF AMPK AND HMG CoA REDUCTASE IN SIMVASTATIN-MEDIATED INSULIN RELEASE FROM PORCINE ISOLATED PANCREATIC ISLETS OF LANGERHANSQ Zhang,¹ MF Wong,¹ FY Kwok,¹ ALS Au,¹ CCW Poon,¹ GPH Leung,² SK Kong,³ HP Ho,⁴ CH Cho,¹ YW Kwan¹¹School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong; ²Department of Pharmacology and Pharmacy, Faculty of Medicine, The University of Hong Kong, ³School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong and ⁴Department of Electronic Engineering, Faculty of Engineering, The Chinese University of Hong Kong, Hong Kong**Background:** Diabetes mellitus (DM) is a medical disorder characterized by a persistent hyperglycemia especially after meals. In DM patients, there is a deficiency/absence of insulin release. The ability of HMG CoA reductase inhibitors (statins) to decrease the incidence of diabetes in patients with dyslipidemia has also been documented. However, the underlying mechanisms involved for the beneficial effects of the uses of statins in treating DM have not been elucidated in details.**Methods:** Fresh porcine pancreatic islets of Langerhans were isolated from porcine pancreases. Then, isolated islets were incubated with or without simvastatin (a commonly used HMG CoA reductase inhibitor) in medium with different glucose levels (5 and 25 mM). The protein expression of HMG CoA reductase, p-HMG CoA reductase-Ser⁸⁷¹, AMPK and p-AMPK-Thr¹⁷² was determined. Insulin release was measured using ELISA kits.**Results:** The biochemical existence of HMG CoA reductase was demonstrated in pancreatic islets of Langerhans, and the expression of HMG CoA reductase was glucose (5 mM and 25 mM)-dependent (~40% increase). Simvastatin (10 µM, 24 hr incubation) caused a decrease in HMG CoA reductase expression irrespective of glucose levels. There was no apparent change of p-HMG CoA reductase expression caused by both glucose levels except with simvastatin (10 µM, with 25 mM glucose). An increase of p-AMPK-Thr¹⁷², but not AMPK, expression was detected under high glucose conditions which was reduced by simvastatin. Simvastatin elicited a concentration (0.01, 0.1 and 10 µM)-dependent insulin release (3.6-fold and 8.5-fold increase with 0.1 and 10 mM simvastatin, respectively) from isolated islets bathed in 5 mM glucose medium. No apparent change caused by simvastatin was observed under high glucose (25 mM) conditions.**Conclusions:** Our results demonstrate the biochemical existence of HMG CoA reductase in porcine's pancreatic islets. Simvastatin elicited a concentration-dependent insulin release under normal glucose conditions which is related to an inhibition of p-AMPK expression.**Acknowledgements:** Provision of fund to Ms. Lori FY Kwok (summer of 2010) by the School of Biomedical Sciences (Faculty of Medicine, CUHK) is acknowledged. This project was financially supported by GRF Grant (to YWK) (Reference number: 2140565).

P29.

MONOAMINE OXIDASE-A-MEDIATED GENERATION OF REACTIVE OXYGEN SPECIE BY 5-HYDROXYTRYPTAMINE IN HUVECSQ Zhang,¹ ALS Au,¹ CCW Poon,¹ GPH Leung,² SK Kong,³ HP Ho,⁴ YW Kwan¹¹School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong; ²Department of Pharmacology and Pharmacy, Faculty of Medicine, The University of Hong Kong, ³School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong and ⁴Department of Electronic Engineering, Faculty of Engineering, The Chinese University of Hong Kong, Hong Kong**Background:** 5-Hydroxytryptamine (5-HT), a potent vasoactive neurotransmitter, after released is terminated at the nerve terminals mainly via enzymatic metabolism such as monoamine oxidases (MAOs), resulted in the generation of different metabolites (e.g. 5-HIAA, 5-HTOL and H₂O₂). In HUVECs, generation of H₂O₂ and the role(s) of MAOs in response to 5-HT challenge is unknown.**Methodology:** Cultured human umbilical vein endothelial cells (HUVECs) (passages: 4-6) were used. ROS generation elicited by 5-HT in the absence or presence of L-NAME (an eNOS inhibitor) was evaluated using H₂DCF fluorescence dye by flow cytometry. Mitochondrial H₂O₂ levels were estimated by MitoTracker Red (reduced form) (a selective fluorescence probe for mitochondrial H₂O₂ measurement) using confocal laser scanning microscope. The participation of a particular isoform of MAOs (MAO-A and MAO-B) was evaluated using selective MAO inhibitors (MAO-A: clorgyline; MAO-B, selegiline).**Results:** In the presence of L-NAME (100 µM), 5-HT consistently elicited a concentration (0.3, 0.6, 1, 3 and 10 µM)- and time (10-30 min)-dependent increase in ROS generation. Antimycin A (10 µM, a positive control) caused ROS generation in HUVECS as by 5-HT. 5-HT-induced ROS generation was sensitive to clorgyline (10 µM) and citalopram (10 µM, a selective 5-HT transporter inhibitor) but not to selegiline (10 µM) or GR127935 (10 µM, a 5-HT_{1B/1D} receptor antagonist). 5-CT (a 5-HT analogue), 5-HIAA (10 µM), 5-HTOL (10 µM) and acetylcholine (10 µM) failed to cause ROS generation.**Conclusions:** Our result clearly demonstrates that 5-HT elicits mitochondrial ROS production (a 5-HT_{1B/1D} receptor-independent manner) probably via MAO-A-mediated 5-HT metabolism in HUVECs.**Acknowledgements:** Provision of a post-graduate (PhD) studentship to Ms. Q Zhang by School of Biomedical Sciences (Faculty of Medicine, The Chinese University of Hong Kong) is acknowledged.

ABSTRACTS

Abstracts for Posters:

P30.

GENDER-SPECIFIC RELAXATION OF FLAVONOIDS IN RAT MESENTERIC ARTERIES

Y Zhang, SWS Leung, RYK Man

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

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

P31.

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

Z Li,¹ LB Qian,^{1,2} ML Xia,³ HP Wang,¹ M Treiman,² Q Xia¹

¹Department of Physiology, Zhejiang University School of Medicine, Hangzhou 310058, China; ²Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; ³Department of Physiology, Medical School, Jiaying College, Jiaying 314001, China

Background: Glucagon-like peptide-1 (GLP-1), usually recognized as a gut hormone with potent plasma glucose-lowering action, is rapidly cleaved by the widely expressed dipeptidyl peptidase-4 enzyme at the N terminus to generate GLP-1 (9-36) amide. More recently, both GLP-1, its analogues exendin-4 (Exe-4) and GLP-1(9-36) amide have been showed cardioprotective actions in a number of experimental models. However, the mechanisms underlying the actions of GLP-1 and GLP-1 (9-36) amide remain poorly understood.

Objective: To investigate the signaling pathways of GLP-1 (9-36) amide and Exe-4 (both in the concentration of 1 nM) protecting the isolated cardiomyocyte from adult rats against ischemia-reperfusion (IR) injury.

Methods: When administered, the agents were presented for 10 min before 25 min simulated ischemia (glucose oxidase-catalase oxygen-scavenging system) and the whole reperfusion period.

Results: After 5 minutes reperfusion, both GLP-1 (9-36) amide and Exe-4 strongly reduced trypan blue-sensitive cells (from 53.57±7.68% in IR group to 39.29±3.02% and 41.53±6.73%, *P*<0.01). This cell death-limiting

effect of GLP-1(9-36) amide was abolished by endothelial nitric oxide synthase (eNOS) inhibitor L-NAME, MEK1/2 inhibitor U0126 or protein kinase A (PKA) inhibitor H89, but not by GLP-1 receptor antagonist exendin (9-39), phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 or wortmannin. In contrast, such effect of Exe-4 was abolished by exendin (9-39), L-NAME, or LY294002 and wortmannin. All of the inhibitors alone had no effect on IR cardiomyocytes.

Conclusion: The results indicate that separate actions for GLP-1 (9-36) amide vs. GLP-1 analogues Exe-4 against ischemia-reperfusion death in cardiomyocytes isolated from adult rats, and reveal that the cardioprotective effect of GLP-1 (9-36) amide is independent on GLP-1 receptor, but through MEK, eNOS and PKA signaling pathways, while Exe-4 exerts cardioprotective effect through GLP-1 receptor, PI3K and eNOS signaling pathways.

ABSTRACTS

Abstracts for Posters:

P32.

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

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

Objectives: Increased production of advanced glycation end-products (AGEs) contributes to endothelial dysfunction in diabetes. The present study examines whether AGE could exert a direct vascular action and the possible mechanisms involved.

Methods: Aortas from *db/m*⁺ mouse were treated with AGE-BSA at 10 µg/ml for 24 hours in the absence or presence of pharmacological inhibitors. Wire myograph was used to assess changes in the vascular reactivity of arteries. Production of mitochondria-derived reactive oxygen species (ROS) was determined using MitoSOX fluorescence under confocal microscope. Western blot was used to assay the p47 level in *db/m*⁺ mouse aortas after AGE treatment with or without inhibitors.

Results: The present study shows that AGEs reduced acetylcholine-induced endothelium-dependent relaxations (EDRs) in aortas from *db/m*⁺ mice and AGE-induced impairment of EDRs was reversed by co-incubation with AGE inhibitor aminoguanidine, NADPH oxidase inhibitor DPI and apocynin, mitochondrial ROS scavenger mitoQ, and protein kinase C inhibitors GF 109203X and chelerythrine. AGE treatment increased the mitochondrial ROS production in native endothelium of intact mouse aortas and in primary mouse aortic endothelial cells, which were inhibited by DPI, apocynin, mitoQ, and ROS scavenger Tiron+DETCA. Western blot data show that a p47 up-regulation by AGE was reversed by aminoguanidine, DPI, apocynin, mitoQ, and also PKC inhibitors.

Conclusions: The present study suggests that AGEs impair endothelial function through NADPH oxidase- and PKC-dependent increase of mitochondrial ROS, which may contribute to diabetes-related vascular dysfunction. (supported by Hong Kong GRF)

P33.

IN VITRO SYNERGISTIC BONE ANABOLIC EFFECTS OF 1,25α DIHYDROXYVITAMIN D₃ AND CU409B1 IN RAT OSTEOBLASTS

LCM Lau,¹ WYW Lee,² AKF Chan,¹ CCW Poon,¹ PPY Lui,² CKM Chan,² SK Kong,³ GPH Leung,⁴ YW Kwan¹

¹School of Biomedical Sciences; ²Department of Orthopaedics and Traumatology, Faculty of Medicine; ³School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong; ⁴Department of Pharmacology and Pharmacy, Faculty of Medicine, The University of Hong Kong, Hong Kong

Background: Osteoporosis is a disease of bones with increased risk of bone fracture because of reduced bone mineral and disrupted bone micro-architecture. The disease is mainly due to increased osteoclasts activity and/or reduced osteoblasts activity. Vitamin D₃ (Vit. D₃) is one of the medications for osteoporosis as it enhances Ca²⁺ absorption from diets. Previous findings in our laboratory demonstrated that CU409B1 (patent pending on chemical structure) improved rat osteoblasts differentiation. In this study, we tested the hypothesis that a combination of CU409B1 and Vit. D₃ (at the lowest plasma concentration, 10 nM) provided synergistic bone anabolic effects.

Methods: Primary rat osteoblasts were isolated from Sprague Dawley (SD) rats (female, 6 weeks old) (sacrificed using an over-dose of pentobarbital). Iliac crests collected were immediately immersed in PBS (for 10 min) supplemented with 10X antibiotics, and bone marrow was removed and the crests were washed thoroughly twice with plain low glucose-DMEM (LG-DMEM). Trabecular bones were harvested, cut into small pieces, and immersed in LG-DMEM (10% fetal bovine serum with 1X antibiotics) for cultured in a humidified incubator (37°C and 5% CO₂). Cells reached over 90% confluence were treated with either 1,25α dihydroxyvitamin D₃ (10 nM, the active form of Vit. D₃), CU409B1 (10 nM) or a combination of Vit. D₃ (10 nM) plus CU409B1 (10 nM) for 7 days before subjecting to analysis of various biomarkers. mRNA was isolated and subjected to quantitative real-time reverse

transcription polymerase reaction (qRT-PCR) to determine the levels of osteogenesis-related mRNAs, including alkaline phosphatase (ALP), bone morphogenetic protein 2 (BMP2), osteopontin (OPN), osteocalcin (OCN), pro-collagen type I, and Runt-related transcription factor 2 (RunX2). The extent of osteogenesis was determined by measuring the activity of extracellular ALP and Alizarin Red staining of Ca²⁺ deposits.

Results: At day 7, a combination of CU409B1 (10 nM) and 1,25α dihydroxyvitamin D₃ (10 nM) significantly enhanced the mRNA levels of ALP, OCN, BMP2, and pro-collagen type I (*P*<0.05) whereas no apparent change was observed in osteoblasts treated with either CU409B1 (10 nM) or 1,25α dihydroxyvitamin D₃ (10 nM) alone. However, no significant change of ALP activity and calcium deposition by all drug treatments was observed.

Conclusions: Our results demonstrate that a combination of CU409B1 and 1,25α dihydroxyvitamin D₃ treatment (7 days) have osteogenesis effects. Current study is underway to elucidate the cellular signaling pathways involved.

Acknowledgements: Fund provided to Mr. Lawrence CM Lau (summer of 2010) by the School of Biomedical Sciences (Faculty of Medicine, CUHK) is acknowledged.

List of Reviewers 2010

CHAN Ngai Yin

CHAU Mo Chee, Elaine

CHENG Cheung Wah, Boron

LAM Tse Fun, Cathy

LAU Suet Ting

LEE Lai Fung, Kathy

LEUNG Wai Suen, Albert

SIU Chung Wah, David