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Secretariat, Room 1116, Bank of America Tower, 12 Harcourt Road, Hong Kong.
Tel: (852) 2899 2035, Fax: (852) 2899 2045
E-mail: enquiry@hkcchk.com
Ox-LDL, Lysophosphatidy Choline Platelet Activating Factor, IL-6 Expression Level and Foam Cell Number with Darappabdib Treatment in Early Development of Atherosclerosis: In Vivo Study for Type 2 Diabetes Mellitus Model

TEUKU HERIANSYAH,¹ BAMBANG BUDI SISWANTO,² ANWAR SANTOSO,³ DJANGGAN SARGOWO,³ IMAM SUBEKTI,⁴ AULANI' AM,⁵ RENAN SUKMAWAN,² NURJATI HAIRANI SIREGAR,⁶ SAPTAWATI BORDOSONO,⁷ TITIN ANDRI WIHASTUTI⁸

From ¹Department of Cardiology and Vascular Medicine, Faculty of Medicine, University of Syiah Kuala, Banda Aceh; ²Department of Cardiology and Vascular Medicine, Faculty of Medicine, University of Indonesia, Jakarta; ³Department of Cardiology and Vascular Medicine, Faculty of Medicine, University of Brawijaya, Malang; ⁴Department of Internal Medicine, Division of Metabolic and Endocrinology, Faculty of Medicine, University of Indonesia, Jakarta; ⁵Department of Anatomy Pathology, Faculty of Medicine; Indonesia University, Jakarta; ⁶Department of Nutrition, Faculty of Medicine, Indonesia University, Jakarta; ⁷Department of Biomedicine, Faculty of Medicine, Brawijaya University, Malang, Indonesia

HERIANSYAH ET AL.: Ox-LDL, Lysophosphatidy Choline Platelet Activating Factor, IL-6 Expression Level and Foam Cell Number with Darappabdib Treatment in Early Development of Atherosclerosis: In Vivo Study for Type 2 Diabetes Mellitus Model. Background: Insulin resistance in type 2 Diabetes Melitus (T2DM) is an extensive tissue damage condition due to vascular inflammation and oxidative stress. Lp-PLA2 has an antiinflammatory role as it hydrolyze atherogenesis mediators such as ox-LDL and platelet activating factor (PAF) and produces lysophosphatidylcholine (LysoPC) and oxidized fatty acid that have pro-inflammatory, proliferative and pro-atherogenic effect. Methods and results: This study aimed to discover the expression of ox-LDL level, LysoPC, PAF, IL-6 and foam cell numbers in aorta of T2DM in vivo model with darapladib treatment. True experimental laboratory and only post test with control group design using 30 Spraque Dowley rats that is divided into 3 main groups: normal, T2DM, and T2DM with the darapladib administration group. Each group consisted of 2 serials treatment time: 8-weeks and 16-weeks treatment groups. Parameter measured was IL-6, oxidized LDL and PAF, LysoPC, IL-6, foam cells and also blood glucose, lipid profile, and insulin plasma level. ANOVA test results showed that darapladib were significantly (p=0.000) lowering ox-LDL level, LysoPC, PAF, IL-6 expression and foam cells number in the aorta on T2DM in vivo model. Conclusions: This study concludes that dalapladib proved to have a role as anti atherogenesis on T2DM model. (J HK Coll Cardiol 2016;24:64-73)

Atherosclerosis, Darapladib, Type 2 DM

Address for reprints: Dr. Titin Andri Wihastuti
Jaksa Agung Suprapto I/47, Malang, East Java, Indonesia

Email: titinwihastuti@gmail.com

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EFFECT OF DARAPLADIB IN ATHEROGENESIS DEVELOPMENT

Introduction

Today, cardiovascular disease (CVD) remains as a serious global health problem and has become the leading cause of death in both developed and developing countries. CVD incidence in Indonesia turned out to be much higher than the other developed countries with cases number is 150.8 compared to 80.5 in the United States and 31.2 per 100,000 population in Japan. CVD death rate in Indonesia reaches 243,048 or 17.05% of total deaths. This condition puts CVD as the first cause of death from 20 leading causes of death in Indonesia. Atherosclerosis is the most common cause of CVD, including coronary heart disease (coronary artery disease/CAD), cerebrovascular disease (stroke) and peripheral blood vessel diseases (peripheral artery disease/PAD).

Type 2 Diabetes mellitus (T2DM) is one of major classical risk factors for atherosclerosis. Clinical condition with insulin resistance and T2DM is a condition of an extensive tissue damage due to vascular inflammation and oxidative stress. Hyperglycemia that occurred in DM type 2 increase Reactive Oxygen Species (ROS) production. Enzymatic activity and lipoprotein phospholipase A2 (Lp-PLA2) expression have been studied as a biomarker of CVD risk in people with CAD and healthy individual. The understanding about Lp-PLA2 role in the cardiovascular disease pathophysiology remains controversial and far from well established. Lp-PLA2 antiinflammatory role is demonstrated by this enzyme ability to hydrolyze mediators, such as ox-LDL and platelet activating factor (PAF), that could potentially have a role in atherogenesis. At the same time, hydrolysis products of these molecules that are mediated by Lp-PLA2, such as lysophosphatidylcholine (LysoPC) and oxidized fatty acid, have pro-inflammatory, proliferative and proatherogenic effect.

In the last decade, a new pharmacological therapy for atherosclerosis, which actively, selectively, and reversibly inhibit Lp-PLA2 is being developed and the result is darapladib. Darapladib is a substance that hydrolyzes Lp-PLA2 substrate. Darapladib forms non-covalent bonds and reversibly with human recombinant Lp-PLA2. Darapladib is lipophilic and has good membrane permeability. Darapladib reduce caspase-3 and caspase-8 activity, also inhibits macrophage apoptosis induced by ox-LDL.

Several studies stated that darapladib show significant results in inhibiting atherosclerosis process, both in vitro and in vivo experiments. Positive results are also shown in a trial study in patients who undergoing carotid endarterectomy. However, there are several opinions regarding darapladib function as atherosclerosis treatment, because some clinical trials show negative results of darapladib usage in reducing mortality from cardiovascular disease, myocardial infarction, or stroke. Based on that facts, the aim of this study is to determine the expression ox-LDL levels, LysoPC, PAF, IL-6 and foam cell number in aorta using in vivo T2DM model with darapladib treatment.
Materials and Methods

Study Group

This study used 4 weeks old male Sprague Dawley Rats and weigh around 150-200 grams as animal model. Samples were obtained from Bogor Agricultural University, Bogor, Indonesia. Samples were divided into three groups; normal group (N); type 2 DM model groups (DM) which fed with High Fat Diet (HFD) and given Streptozotocin (STZ) injection intraperitoneal low dose 35 mg/kgBW, and type 2 DM model with darapladib administration group (DMDP). Each group was divided into two serial time, 8 weeks (early phase) and 16 weeks (late phase). Darapladib was obtained from Glaxo Smith Kline. Samples were given darapladib via oral 20 mg/kgBW once per day with duration based on time serial groups. In short, there were 6 groups in total. N8 and N16 for normal groups (8 and 16 weeks), DM8 and DM16 for T2DM model groups (8 and 16 weeks), and DMDP8 and DMDP16 for T2DM model with darapladib administration groups (8 and 16 weeks).

Normal rats food contained 3.43 total energy calories (kcal/g) (67% carbohydrate, 21% protein, and 12% fat), while HFD contained 5.29 total energy calorie (kcal/g) (58% fat, 17% carbohydrate, and 25% protein). Each kilogram of vitamins and minerals mixture contained 2000000 IU Vitamin A, 400000 IU Vitamin D3, 0.8 grams Vitamin B2, 300 units Vitamin E, 0.4 grams Vitamin K, 1 gram calcium pantothenate, 4 grams Nicotinamide, 2.4 gram Vitamin B12, 60 grams Choline Chloride, 300 grams Calcium, 2.75 grams Manganese, 0.1 grams Iodine, 3 grams Iron, 6 grams Zinc, 0.8 grams Copper, and 0.18 grams Cobalt. 12 30 grams food were given for each rat everyday. Parameter measurement was done at the Central Laboratory of Biological Sciences, Brawijaya University, Malang, Indonesia. Slicing and staining samples were done in the Pathological Anatomy Laboratory, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.

Blood Glucose Measurement

STZ 20 mg/kgBW was administered before the first measurement of blood glucose to induce type 2 diabetes mellitus. Type 2 diabetes mellitus was diagnosed after blood glucose level measurement using GlucoDR blood glucose test meter (All Medicus Co. Ltd, Donganggu, Anyang-si, Korea). Type 2 diabetes mellitus was diagnosed after obtaining blood glucose levels >200 mg/dL in rats.

Lipid Profile Levels Measurement

Lipid profiles (total cholesterol, HDL (High Density Lipoprotein), and LDL (Low Density Lipoprotein)) were measured in rat blood serum using EnzyChrom™ kit.

Insulin Resistance Measurement

Insulin in rat’s blood plasma were measured using Rat INS (insulin) ELISA kit (Cat. No. E-EL-R2466). The obtained results are still in ng/mL units. Plasma insulin levels were converted into IU / L. WHO formula were used by dividing the result with 0.0347, as 1 IU is equivalent with 0.0347 mg/L.13 Insulin resistance can be measured with HOMA-IR (homeostatic model assessment-insulin resistance) formula especially in rats, which required some data, such as fasting glucose and plasma insulin levels by the following formula:14

\[
\text{HOMA-IR} = \frac{\text{FBG} \times \text{FINS}}{14.1}
\]

Explanation:

HOMA-IR: Homeostatic Model Assessment-Insulin Resistance
FBG : Fasting Blood Glucose (mmol/L)
FINS : Fasting Insulin Plasma (µgU/mL)

Interpretation of HOMA-IR calculation in rats is if the result >1.716 then it can be categorized as insulin resistance with 83.87% sensitivity and 80.56% specificity (95% confidence interval).15

IL6 Expression Measurement

IL-6 was measured using immunofluorescence. Aortic tissues were previously fixed with PHEMO buffer (68 mM PIPES, 25 mM, HEPES, pH 6.9, 15 mMEGTA, 3 mM MgCl2, 10% [v/v] dimethyl sulfoxide containing 3.7% formaldehyde and 0.05%
glutaraldehyde), then were processed by immunofluorescence double labeling with anti-rat antibody Lp-PLA2 using rhodamin secondary antibody and anti-rat antibody IL-6 using fluorescein isothiocyanate (FITC) secondary antibody (BIOS Inc., Boston, MA, USA). These parameter was observed using confocal laser scanning microscopy (Olympus Corporation, Tokyo, Japan) and was quantitatively analyzed using Olympus FluoView software (version 1.7A; Olympus Corporation).

**Ox-LDL Measurement**

Ox-LDL was measured using rats aorta tissue as samples. The rats had been fasted a day before the sample obtained. Ox-LDL level was measured by Sandwich ELISA method using Rat Ox-LDL ELISA kit (Cat. No. E-EL-R0710). The first step was antigen coating, 100 µgL standard and sample were put into well that are coated with antibody before then incubated in 37°C for 90 minutes. Residual antigen that was not bound with antibody were disposed. After that, add 100 µgL Biotin-antibody into each well. Incubated each well in 37 oC for 1 hour. The fluid in well was aspirated and washed by wash buffer for 3 times. Next, 100 µgL HRP avidin was added into each well and incubated in 37°C for 30 minutes. Repeat the aspiration and washing process 5 times. Add 90 µgL TMB substrate into each well then incubated in 37°C for 15 minutes in light-free area. 50 µgL stop reaction was added to stop the reaction. After 5 minutes read by ELISA reader at a wavelength of 450 nm.

**LysoPC Measurement**

The used samples were serum and rat’s aorta homogenates. The used tool was 6460 triplequadrupole LCMS / MS System, Agilent Technologies, USA. Standard LysoPC material (18:0) used was 1-Stearoyl-2-Hydroxy-sn-Glycero-3-Phosphocholine (16:0), cat 85567P, lot 16LPC-79, Avanti Polar Lipids, Inc., 700 Industrial Park Drive, Alabaster, Alabama 35007-9105, USA. Standard LysoPC internal material (16: 0) used was 1-palmitoyl (D31)-2-Hydroxy-sn-Glycero-3-Phosphocholine (16: 0), paint 85567P, lot 16 LPC-79, Avanti PolarLipids, Inc., 700 Industrial Park Drive, Alabaster, Alabama 35007-9105, USA. Standards calibration range used were 250-16000 ng/mL. Dilution factors of aorta homogenates samples 16-7 for LysoPC Stearoyl was 2X. Serum sample dilution factors was 20X, except palmitoyl (16:0) Code 8-1 and 8-2 it was 40X, and Stearoyl (18: 0) Code 8-1 was 80X. Standard material used specifically for research (Research Use Only). LysoPC analysis procedure refers to journals: Takatera A, et. al. Blood LysoPhosphatidylcholine (LPC) levels and Characteristic Molecular Species in Neonates: Prolonged Low Blood LPC Levels in Very Low Birth Weight Infants. Pediatr Res:477-482, 2007.

**PAF Measurement**

PAF concentrations measurement in rat blood plasma samples were conducted by competitive ELISA method using Rat Platelet Activating Factor ELISA kit (Cat. No. MBS722041). Competitive ELISA begins with coating antigen. Standard and 100 µgL sample were inserted into well (to form using PBS). Then, 50 µgL conjugate was added into each well (except blank well) and incubated it in 37°C for 1 hour. Aspirated and washed with wash buffer 5 times. Add 50 µgL substrate A and 50 µgL substrate B to each well and incubated for 10-15 minutes in 37°C (avoid light). 50 µgL stop solution was added to stop reaction in each well. After 5 minutes read by ELISA reader at a wavelength of 450 nm.

**Foam Cells Measurement**

Aortic tissues were prepared using a frozen section technique and stained with hematoxylineeosin. After aortic tissues preparation, foam cells were measured using a microscope with 400 magnification and dotSlide Olyvia version 2.4 software (Olympus America Inc., Center Valley, PA, USA). Examination and calculation of foam cells number were performed for each 10 microscope fields after taking images of the aortic tissues.
Ethics
We obtained ethical approval for animal treatment and experimental processes in this study from Animal Care and Use Committee Brawijaya University Number 229-KEP-UB.

Statistical Analysis
One-way analysis of variance (ANOVA) test was used to determine the effects of time series factor, darapladib treatment, and the interaction between time and darapladib treatment on mRNA Lp-PLA2 expression, ox-LDL, PAF, LysoPC, PAF and foam cells in Spraque Dowley rats with type 2 diabetes mellitus. This analysis was continued by post hoc test using Duncan method to detect parameter differences in each treatment group. SPSS software version 20 (IBM Corporation, New York, NY, USA) was used for data analysis.

Results
Total cholesterol level range was observed from 56.560 to 123.002 (mg/dL). The highest total cholesterol level was found in DM8 group (123.002 mg/dL). Meanwhile, the lowest total cholesterol level was found in N16 group (56.560 mg/dL). HDL level range was observed from 4.958 to 35.767 (mg/dL). The highest HDL level was found in N16 (35.767 mg/dL). Meanwhile, the lowest HDL level was found in DM8 group (4.958 mg/dL). VLDL/LDL level range was observed from 19.241 to 95.531 (mg/dL). The highest VLDL/LDL level was found in DM8 group (95.531 mg/dL). Meanwhile, the lowest VLDL/LDL level was found in N16 group (19.241 mg/dL). Fasting glucose level range was observed from 79.6 to 147.8 (mg/mL). The highest fasting glucose level was found in DM16 group (147.8 mg/mL). Meanwhile, the lowest fasting glucose level was found in N16 (79.6 mg/mL). Plasma insulin level range was observed from 4.664 to 40.220 (ng/mL). The highest plasma insulin levels was found in DM16 (40.220 ng/mL). Meanwhile, the lowest plasma insulin levels was found in N8 group (4.664 ng/mL). HOMA-IR value range was observed from 0.462 to 3.789. The highest HOMA-IR value was found in DM16 (3.789). Meanwhile, the lowest HOMA-IR value was found in N16 group (0.462).

Ox-LDL level range was observed from 1.347 to 34.049 (ng/mL). The highest ox-LDL level was found in DM16 group (34.049 ng/mL). Meanwhile, the lowest ox-LDL level was found in N8 group (1.347 ng/mL). ANOVA test showed that ox-LDL level was significantly (p<0.050) influenced by time series, darapladib treatment, and also an interaction between time series and darapladib treatment (Figure 1A).

LysoPC level was observed from 28.427 to 266.777 (ng/g aorta). The highest LysoPC level was found in N8 group (266.777 ng/g aorta). Meanwhile, the lowest LysoPC level was found in DMDP16 group (28.427 ng/g aorta). ANOVA test showed that LysoPC level was significantly (p<0.050) influenced by time series, darapladib treatment, and also an interaction between time series and darapladib treatment (Figure 1B).

PAF level range was observed from 0.726 to 1.963 (ng/mL). The highest PAF level was found in DM16 group (1.963 ng/mL). Meanwhile, the lowest PAF level was found in N8 group (0.726 ng/mL). ANOVA test showed that PAF level was significantly (p<0.050) influenced by time series, darapladib treatment, and also an interaction between time series and darapladib treatment (Figure 1C).

IL-6 expression range was observed from 634.60552 to 792.131 (AU). The highest IL-6 expression was found in DM16 group (792.131 AU). Meanwhile, the lowest IL-6 expression was found in DMDP8 group (634.60552 AU). ANOVA test showed that IL-6 expression was significantly (p<0.050) affected by darapladib treatment factor. Meanwhile, time series factor and interaction between time series and darapladib did not significantly (p>0.050) influenced IL-6 expression (Figure 1D). Foam cells number range was observed from 80 to 138.8 (cell). The highest foam cells number was found in DM16 group (138.8 cells). Meanwhile, the lowest foam cells number was found in N8 group (80 cells). ANOVA test showed that foam cells number was significantly (p<0.050) affected by darapladib administration factor. Meanwhile, time series factor and interaction between time series and darapladib treatment did not significantly (p>0.050) influenced the amount of foam cell (Figure 1E).
Figure 1. The expression of ox-LDL level, LysoPC, PAF, IL-6 and foam cell numbers in aorta for each group, (A) ox-LDL tissue, (B) LysoPC serum, (C) PAF, (D) IL-6, (E) Foam cell.
Discussion

High-Fat Diet (HFD) can increase dyslipidaemia and insulin resistance incidence that can cause a rise in blood sugar.\textsuperscript{16} In DM model, Total Cholesterol (TC) and Low-Density Lipoprotein (LDL) increasing level and High-Density Lipoprotein (HDL) decreasing level were observed. Dyslipidaemia that occur in this study lead to insulin resistance. All cells insulin receptor can decrease in downregulation system event due to high glucose in blood. If these events occur continuously and chronically, insulin receptor desensitization will happen. It leads to a higher need blood plasma insulin to incorporate glucose into cell tissue.

In this research, an increase in plasma insulin levels in DM8 and DM16 groups occurred. That conditions may increase insulin resistance incidence. By using HOMA-IR rat formula it was found that insulin resistance occurred in DM16 and DM8 groups (CI 95%; sensitivity 83.87%; specificity 80.56%), DM8 group has shown an increase in HOMA-IR value compared with other groups, but has not been able to reach the cut-off value. This shows that DM8 group is almost in insulin resistance state or pre-diabetic condition. Abnormalities in lipoprotein metabolism are also associated with type 2 diabetes and trigger atherosclerosis development.\textsuperscript{17} Glucose and lipid metabolism disorders are synergistic in atherosclerosis development.\textsuperscript{18} In atherosclerosis, vascular damage occurs as a result of inflammation and oxidative stress.\textsuperscript{4,5} Metabolic disorders associated with type 2 diabetes such as hyperglycaemia, AGEs formation, increased FFA, and lipoprotein abnormalities can induce vascular inflammation and promote atherosclerosis progressivity.\textsuperscript{19}

Lp-PLA2 is an enzyme that able to catalyze many important biological reactions and allegedly played an important role in atherosclerosis pathogenesis. Lp-PLA2 expression in this study tend to increase in type 2 diabetes condition and tend to decrease overtime increment. This scheme shows Lp-PLA2 role in early stages of atherosclerosis. Darapladib administration in this study had no significant effect on Lp-PLA2 expression. The increasing of Lp-PLA2 expression is associated with ox-LDL level. A study shows that ox-LDL play an important role in atherosclerosis pathogenesis and it is easy to form and increase in T2DM state.\textsuperscript{19} Ox-LDL level in this study is significantly influenced by time series, darapladib treatment, and also interaction between time series and darapladib treatment. An increasing of ox-LDL level occurred in adipocytes development, insulin resistance, and type 2 diabetes.\textsuperscript{20} LysoPC, the main products of Lp-PLA2 activity, is a proatherogenic property of ox-LDL which can induce proteoglycans synthesis and play an important role in the intimal layer thickening of the endothelium.\textsuperscript{21} LysoPC in this study is significantly influenced by time series, darapladib treatment, and interaction between time series and darapladib treatment. The increased of Lp-PLA2 activity are also increase LysoPC levels that are cytotoxic on blood vessels and smooth muscle cells.\textsuperscript{22} Based on preclinical studies, darapladib is proven to reduce atheroma which has LysoPC and some expression of multiple genes related to macrophage and T lymphocyte function in large enough quantities to the plaque and necrotic core area.\textsuperscript{23} Research conducted by Melissa et al in 2012 stated that the increased levels of LysoPC occur at type 2 diabetes mellitus onset and obesity condition.\textsuperscript{24}

Lp-PLA2 has an anti-inflammatory role through its ability to hydrolyze oxidized phosphatidylcholine and PAF that are pro-inflammatory and pro-thrombotic. PAF is a potent inflammatory agent that has a very important role in atherosclerosis formation.\textsuperscript{25} In this study, PAF levels were significantly (p<0.050) influenced by time series, darapladib treatment, and interaction between time series and darapladib treatment. Previous study explained, darapladib and rilapladib can inhibit PAF receptor, therefore reducing PAF biological activity, as well as interfere with PAF biosynthesis.\textsuperscript{26}

IL-6 is one of pro-inflammatory cytokine that involved in atherogenesis. IL-6 in type 2 diabetes mellitus condition can increase basal glucose uptake, alter insulin sensitivity, improve adhesion molecule expression by endothelial cells, have pro-coagulant effects on platelets, and increase fibrinogen release by liver. In addition, IL-6 is also capable of blocking lipoprotein lipase action and stimulate lipolysis process.\textsuperscript{27} In this study, IL-6 expression were significantly (p<0.050) affected by
darapladib treatment. While time series factor and interaction between time series and darapladib treatment does not significantly affect IL-6 expression (p>0.050) (Figure 2). Increased Lp-PLA2 expression followed by increased expression of cytokines IL-6 in type 2 diabetes.

Ox-LDL stimulate macrophages to phagocyte lipid, then form a foam cell that signed the ongoing process of atherogenesis. Ox-LDL stimulate macrophages to phagocyte lipid, then form a foam cell that signed the ongoing process of atherogenesis. Ox-LDL stimulate macrophages to phagocyte lipid, then form a foam cell that signed the ongoing process of atherogenesis. Ox-LDL stimulate macrophages to phagocyte lipid, then form a foam cell that signed the ongoing process of atherogenesis. Ox-LDL stimulate macrophages to phagocyte lipid, then form a foam cell that signed the ongoing process of atherogenesis.28 This study shows that foam cells number was significantly (p<0.050) affected by darapladib treatment. While the time series factor as well as the interaction between time series and darapladib treatment does not significantly affect the amount of foam cell (p>0.050) (Figure 3). Foam cells formation induced glucose indicates that glucose uptake is required by cells, namely D-glucose (which is taken up by cells) but not L-glucose (which can not be attached to cell membrane) which then could induce macrophage oxidative stress.29

Figure 2. Three immunofluorescence staining result of IL-6 using Fluorescein Isothiocyanate (FITC) secondary antibody.

Figure 3. Foam cells in different treatment groups of rats: (A) normal diet group 8 weeks; (B) T2DM 8 weeks; (C) T2DM + darapladib (20 mg/kgBW) 8 weeks; (D) normal diet group 16 weeks; (E) T2DM 16 weeks; and (F) T2DM + darapladib (20 mg/kgBW) 16 weeks
Conclusion

Darapladip is Lp-PLA2 inhibitor, it is lipophilic and has a stupendous membrane permeability. Recent studies explained that darapladip showed significant result in inhibiting atherosclerosis process, whether by \textit{in vitro} or \textit{in vivo} test. \cite{7,9} Darapladib were significantly affected ox-LDL level tissue, PAF plasma, IL-6 expression and foam cells number.

References

EFFECT OF DARAPLADIB IN ATEROGENESIS DEVELOPMENT


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SCIENTIFIC PROGRAMME (as of 25 October 2016)

19 NOVEMBER 2016 (SATURDAY)

08:30-09:00  Registration

09:00-10:40  Free Oral Communications & Oral Presentations for Young Investigator Award
Sponsored by Sun Chieh Yeh Heart Foundation
Chairpersons: Dr. Susan W.S. Leung, The University of Hong Kong, HKSAR
             Prof. Xiao-qiang Yao, The Chinese University of Hong Kong, HKSAR

10:40-11:10  Coffee break, poster viewing and booth visit

11:10-12:00 Poster Presentations for Young Investigator Award
Sponsored by Sun Chieh Yeh Heart Foundation
Chairpersons: Dr. Man-lung Fung, The University of Hong Kong, HKSAR
             Dr. George P.H. Leung, The University of Hong Kong, HKSAR

12:00-12:30  Lunch Symposium
Sponsored by AstraZeneca HK Ltd.
Chairperson:  Dr. Kelvin K.H. Yiu, The University of Hong Kong, HKSAR

New Paradigm for Double Anti Platelet Therapy (DAPT) – For Who and for How Long?
Prof. David C.W. Siu, The University of Hong Kong, HKSAR

12:30-14:00  Lunch

14:00-14:15  Opening Ceremony
Prof. John Kao, Vice-President & Pro-Vice-Chancellor (Global), The University of Hong Kong, HKSAR
Prof. Chu-pak Lau, Sun Chieh Yeh Heart Foundation, HKSAR

14:15-15:15  Symposium 1: Cardiac Regeneration
Chairpersons: Prof. Kenneth R. Boheler, The University of Hong Kong, HKSAR
             Dr. Shu-kin Li, President, Hong Kong College of Cardiology, HKSAR

Keynote Lecture 1: Cardiomyocyte Transplantation in Animal Models and Its Significance for Regenerative Medicine
Prof. Bernd Fleischmann, University of Bonn, Germany

Keynote Lecture 2: New Approaches to Treating Heart Failure
Sponsored by Novartis Pharmaceuticals (HK) Ltd.
Prof. Hung-fat Tse, The University of Hong Kong, HKSAR
15:15-16:15 Symposium 2: Two Decades of Excellence in Cardiovascular Research
Chairpersons: Prof. Chu-pak Lau, Sun Chieh Yeh Heart Foundation, HKSAR
Prof. Bernard M.Y. Cheung, The University of Hong Kong, HKSAR

Invited Lecture 1: Surgery of Congenital Heart Diseases: Is Structure Repair Associated with Molecular Changes
Prof. Guo-wei He, TEDA International Cardiovascular Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, China

Invited Lecture 2: Personal Viewpoints about Cardiovascular Sciences and Medicine during the Past Two Decades
Prof. David C.Y. Kwan, Shantou University, China / McMaster University, Canada

Invited Lecture 3: Zero Time Exercise and Anti-inertia Reminder (AIR) Model: Hong Kong Jockey Club FAMILY Project
Prof. Tai-hing Lam, The University of Hong Kong, HKSAR

16:15-16:45 Coffee break, poster viewing and booth visit

16:45-17:45 Symposium 3: Cardiovascular Signaling in Health and Disease
Chairpersons: Dr. Heather J. Ballard, The University of Hong Kong, HKSAR
Dr. Sheila Q. Yang, The Chinese University of Hong Kong, HKSAR

Keynote Lecture 3: Treatment of Cardiac Fibrosis by Targeting TGF-beta/Smad Signalling
Prof. Hui-yao Lan, The Chinese University of Hong Kong, HKSAR

Keynote Lecture 4: Tricuspid Valve in the Spotlight
Dr. Kelvin K.H. Yiu, The University of Hong Kong, HKSAR

17:45-18:00 Closing Remarks and Young Investigator Award Ceremony
Prof. Bernard M.Y. Cheung, The University of Hong Kong, HKSAR

18:00 Annual General Meeting
IL2.
CARDIOMYOCYTE TRANSPLANTATION IN ANIMAL MODELS AND ITS SIGNIFICANCE FOR REGENERATIVE MEDICINE
BK Fleischmann
Institute of Physiology I, University of Bonn, Life & Brain Center, Germany

Myocardial infarction is characterized by an irreversible loss of cardiomyocytes and this can result in heart failure. This disorder has a poor prognosis and the only causal treatment currently available is heart transplantation. Because of the shortage of donor organs, cell replacement approaches using progenitors and/or stem cells are considered a promising alternative approach. We have explored the potential of various progenitors and stem cells to differentiate into cardiac muscle cells, their physiological integration and contribution to pump function after engraftment into the infarcted mouse heart. First, the plasticity of bone marrow-derived cells was assessed and we found that these do neither differentiate into functional cardiac muscle- nor endothelial cells nor strongly enhance left ventricular function. Similar results were obtained using bone marrow-derived mesenchymal stem cells. In addition, the differentiation of these cells was not restricted by the heart tissue as often calcifications and bone formation were observed. In contrast, engrafted fetal cardiomyocytes and/or ES cardiomyocytes displayed accelerated differentiation, intact cellular function at the single cell level; these cells led to an improvement of left ventricular function and a reduction of mortality rates of mice. Next, the electrical integration of grafted cardiomyocytes into the cardiac syncytium was determined. The experiments revealed that engrafted cardiac muscle cells can electrically couple with the native heart muscle via connexin 43. Their engraftment results in an increased conduction velocity within the scar area and a striking reduction of post-infarction ventricular tachycardia. Having said this, a major shortcoming of current approaches are low rates of stable engraftment of cells. We are addressing this by combining nanomedicine with cell replacement, in addition we are using optogenetic approaches to modulate the electrical activity of the heart with light and strategies to assess and enhance endogenous repair.

IL4.
SURGERY OF CONGENITAL HEART DISEASES: IS STRUCTURE REPAIR ASSOCIATED WITH MOLECULAR CHANGES?
GW He,1 HT Hou,1 C Xuan,1 J Wang,1 XC Liu,1 Q Yang1,2
1TEDA International Cardiovascular Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin & The Affiliated Hospital of Hangzhou Normal University & Zhejiang University, China; 2The Chinese University of Hong Kong, Hong Kong; 3Department of Surgery, Oregon Health and Science University, USA

Purpose: The final goal for treatment of congenital heart diseases (CHD) is to resume not only the normal heart structure but also normal life towards "Precision Medicine". The present study explores the surgical results at molecular basis on the proteomic pattern in the pre- and post-operative period in tetralogy of Fallot (TOF) and ventricular septal defect (VSD) in order to find whether structure repair is associated with clinically important molecular changes in CHD.

Methods: Differential protein analysis by using two-dimensional gel electrophoresis and mass spectrometry followed by ELISA was performed in the plasma samples of patients with TOF (n=82) or VSD (n=82) preoperatively, 6-month postoperatively, and normal controls (n=82).

Results: A total of 473 protein spots in preoperative patients and 515 in postoperative patients were detected. Significantly (p<0.01) downregulated and upregulated protein spots were detected. Validation of proteins in the new group of patients demonstrated that in VSD patients, postoperative gelsolin (p<0.05) was partially and alpha-1-antitrypsin (p<0.01) was completely recovered. In TOF patients, postoperative C3c (p<0.05) was partially recovered. In TOF patients, postoperative complement Component C3c (p<0.05) was partially and serum amyloid p-component (p<0.05) was completely recovered. In TOF patients, postoperative gelsolin (p<0.05) was partially and alpha-1-antitrypsin (p<0.01) was completely recovered. In contrast, the elevated fibrinogen gamma chain level in preoperative patients (p<0.01) became normal postoperatively (p=0.1 vs. control).

Conclusions: We have for the first time, by using proteomic methods demonstrated that repair surgery for CHD not only corrects the structure malformation of the heart but also resumes the molecular normality. The degree of the recovery of protein or other molecules may provide useful information for "Precision Medicine" in the management of these patients.
Solving problems in cardiovascular (CV) diseases has always been a core objective in clinical medicine, which requires integration of research and education, in analogous to the functional coordination of our left and right limbs. My 30-year satisfying research life in vascular biology in a successful Smooth Muscle Research Program at McMaster has taught me to appreciate such an integration. Research dominated a large part of my young adult life, struggling through ladders of academic promotion and achieving bits of fame here and there, which still remain the dreams, and also, nightmares for many young researchers these days. I had them all and felt satisfied with what I have got. I do not know how I did it. I simply worked hard, but not as a loner. I had many helping hands along the path. I could have done with greed for more, but I shifted my career path, unknowingly, perhaps due to a cultural inspiration, which prompted me to take a leave of absence from McMaster University in 1992, move to the East and take up the Physiology Chair position at the University of Hong Kong (HKU), and subsequently helped build ICSM there. This swift marked a milestone in my academic career, not just in CV sciences, but in medicine as a whole. Being academically trained in the West as a vascular biologist transforming my early self as a chemist, I developed a profound interest in Chinese medicinal herbs as folk medicine, especially those treating circulatory diseases. I began curious in herbal medicine that displayed vasoactive and antihypertensive actions and to relate I my research those treating circulatory diseases. I began curious in herbal medicine that displayed vasoactive and antihypertensive actions and to relate I my research that forgetfulness and/or inertia are major barriers. We propose the Anti-inertia Reminder (AIR) Model which states that, for simple actions and behavior changes that the participants understand, accept, have tried and enjoyed during the learning process, and have pledged to act to benefit themselves and their families, simple, direct and frequent reminders would motivate the participants to overcome the inertia, leading to immediate simple actions and more sustained behaviour changes with the simple actions integrated into daily life. We have conducted several simple and brief (from 15-60 minutes) one-session interactive workshops on ZTEx and used AIR (several reminders within a session) to motivate participants to do ZTEx (leg raising and/or pedaling) during the whole period of the session. We told the participants that our research staff would observe and count how many who did so. We also asked the participants to raise their hands if they had done ZTEx while sitting and listening. We recorded our observations and/or their responses and presented these results in charts showing ZTEx responses against time. Preliminary Results: Most participants responded actively, enjoyed the session and showed great appreciation. AIR increased ZTEx in varying percentages of participants at different time points during the workshops. The immediate effects were easily observed for the active participants, while a minority of them appeared to be quite non-responsive. Further analysis and follow-up are in progress.

IL.5.
PERSONAL VIEWPOINTS ABOUT CARDIOVASCULAR SCIENCES AND MEDICINE DURING THE PAST TWO DECADES
CY Kwan
Shantou University, China

Solving problems in cardiovascular (CV) diseases has always been a core objective in clinical medicine, which requires integration of research and education, in analogous to the functional coordination of our left and right limbs. My 30-year satisfying research life in vascular biology in a successful Smooth Muscle Research Program at McMaster has taught me to appreciate such an integration. Research dominated a large part of my young adult life, struggling through ladders of academic promotion and achieving bits of fame here and there, which still remain the dreams, and also, nightmares for many young researchers these days. I had them all and felt satisfied with what I have got. I do not know how I did it. I simply worked hard, but not as a loner. I had many helping hands along the path. I could have done with greed for more, but I shifted my career path, unknowingly, perhaps due to a cultural inspiration, which prompted me to take a leave of absence from McMaster University in 1992, move to the East and take up the Physiology Chair position at the University of Hong Kong (HKU), and subsequently helped build ICSM there. This swift marked a milestone in my academic career, not just in CV sciences, but in medicine as a whole. Being academically trained in the West as a vascular biologist transforming my early self as a chemist, I developed a profound interest in Chinese medicinal herbs as folk medicine, especially those treating circulatory diseases. I began curious in herbal medicine that displayed vasoactive and antihypertensive actions and to relate I my research that forgetfulness and/or inertia are major barriers. We propose the Anti-inertia Reminder (AIR) Model which states that, for simple actions and behavior changes that the participants understand, accept, have tried and enjoyed during the learning process, and have pledged to act to benefit themselves and their families, simple, direct and frequent reminders would motivate the participants to overcome the inertia, leading to immediate simple actions and more sustained behaviour changes with the simple actions integrated into daily life. We have conducted several simple and brief (from 15-60 minutes) one-session interactive workshops on ZTEx and used AIR (several reminders within a session) to motivate participants to do ZTEx (leg raising and/or pedaling) during the whole period of the session. We told the participants that our research staff would observe and count how many who did so. We also asked the participants to raise their hands if they had done ZTEx while sitting and listening. We recorded our observations and/or their responses and presented these results in charts showing ZTEx responses against time. Preliminary Results: Most participants responded actively, enjoyed the session and showed great appreciation. AIR increased ZTEx in varying percentages of participants at different time points during the workshops. The immediate effects were easily observed for the active participants, while a minority of them appeared to be quite non-responsive. Further analysis and follow-up are in progress.

IL.6.
ZERO-TIME EXERCISE AND ANTI-INERTIA REMINDER (AIR) MODEL: THE HONG KONG JOCKEY CLUB FAMILY PROJECT
TH Lam, A Wan, HCY Ho, G Lau, A Lai
School of Public Health, The University of Hong Kong, Hong Kong

Obesity is increasing globally and physical inactivity is still prevalent. We propose "Zero-time Exercise" as a new approach to promote physical activity (PA), family exercise and well-being in Phase Two of The Hong Kong Jockey Club FAMILY project. We use a public health approach and positive psychology, and collaborate with government and many NGOs to design, implement and evaluate many simple and low-cost community based projects which are evidence-based and evidence-generating, for thousands of participants (See www.family.org.hk, or YouTube for details). "Zero-time Exercises" (ZTEx) are easy, enjoyable and effective (3Es) and can be done with zero time, zero money and zero equipment (3 Zeros) by anybody, anytime and anywhere (3As). ZTEx are simple movements and stretches that can be done while sitting, standing and walking. One good example is to raise both legs above the ground, and do pedaling while sitting. We have been running several major projects in collaboration with government and NGO partners and the preliminary results are encouraging. We use a participatory and interactive approach to engage the participants in our presentations and workshops. To begin with, we ask and all or almost all participants reply that they do not have enough exercise or PA and the main reasons are being lazy or too busy (i.e. having no time). They understand the harms of physical inactivity such as sitting too much and the benefits of exercises. They all pledge to do ZTEx because they want to be healthier and fitter, and to share with their family members because they love them. Although ZTEx should be a foot-in-the-door approach for an easy behavioural change, we recognize that forgetfulness and/or inertia are major barriers. We propose the Anti-inertia Reminder (AIR) Model which states that, for simple actions and behavior changes that the participants understand, accept, have tried and enjoyed during the learning process, and have pledged to act to benefit themselves and their families, simple, direct and frequent reminders would motivate the participants to overcome the inertia, leading to immediate simple actions and more sustained behaviour changes with the simple actions integrated into daily life. We have conducted several simple and brief (from 15-60 minutes) one-session interactive workshops on ZTEx and used AIR (several reminders within a session) to motivate participants to do ZTEx (leg raising and/or pedaling) during the whole period of the session. We told the participants that our research staff would observe and count how many who did so. We also asked the participants to raise their hands if they had done ZTEx while sitting and listening. We recorded our observations and/or their responses and presented these results in charts showing ZTEx responses against time. Preliminary Results: Most participants responded actively, enjoyed the session and showed great appreciation. AIR increased ZTEx in varying percentages of participants at different time points during the workshops. The immediate effects were easily observed for the active participants, while a minority of them appeared to be quite non-responsive. Further analysis and follow-up are in progress.
ABSTRACTS

Abstracts for Invited Lectures:

**IL7.**

**TREATMENT OF CARDIAC FIBROSIS BY TARGETING TGF-BETA/SMAD SIGNALLING**

Hy Lan
Department of Medicine and Therapeutics, and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong

It is now well accepted that TGF-β/Smad signaling is a major pathway leading to cardiac fibrosis. Among this pathway, we found Smad3 is pathogenic, while Smad7 is protective because deletion of Smad3 protects against but disrupted Smad7 enhances hypertensive cardiac remodeling and functional injury in a mouse model of hypertension induced by subcutaneous angiotensin II infusion. Next, we examined the mechanism by which Smad3 mediates cardiac fibrosis by RNA-seq and found that deletion of Smad3 prevents a loss of miR-29 family, suggesting that the miR-29 family is the downstream target of Smad3 and Smad3 mediates cardiac fibrosis by downregulating miR-29 expression. We then develop novel therapeutic strategies by targeting Smad3 signaling with a Smad3 inhibitor or by overexpressing Smad7, or by restoring miR-29b. All therapeutic strategies demonstrate that blockade of Smad3 directly with a Smad3 inhibitor or by overexpressing Smad7 is capable of inhibiting cardiac inflammation and fibrosis in the hypertensive heart disease. In addition, we also find that overexpression of miR-29b is able to blocking angiotensin II-induced cardiac fibrosis by inhibiting TGF-β/Smad3 signaling, suggesting a feedback loop of TGF-β/Smad3-miR-29 in regulating Ang II-induced cardiac fibrosis. In summary, TGF-β/Smad3 signaling is a key pathway leading to cardiac fibrosis and targeting this pathway by a Smad3 inhibitor, Smad7, or miR-29 may represent a novel and specific therapy for cardiac fibrosis.

*This work is supported by RGC GRFs and CRF (CUHK3/CRF/12R) and Lui Che Woo Institute of Innovative Medicine - CARE Research Fund (8303307).*
ABSTRACTS

Abstracts for Oral Presentation:

OP1.

PROTEOMIC STUDY IN THE PLASMA OF PATIENTS WITH ISOLATED PATENT DUCTUS ARTERIOSUS AND POTENTIAL CLINICAL SIGNIFICANCE

HT Hou,1,2 J Wang,1 X Zhang,1,2 TX Chen,1 Q Yang,1,3 GW He1,2,4
1Department of Cardiovascular Surgery & Center for Basic Medical Research, TEDA International Cardiovascular Hospital, Chinese Academy of Medical Sciences, China; 2The Affiliated Hospital of Hangzhou Normal University & Zhejiang University, China; 3The Chinese University of Hong Kong, Hong Kong; 4Department of Surgery, Oregon Health and Science University, USA

Purpose: Patent ductus arteriosus (PDA) is the third most common congenital heart disease (CHD) in which the arterial duct, normally closed spontaneously after birth within 24–48h in full-term infants, remains permanently open. This study was aimed to reveal the differential plasma proteins in isolated PDA patients compared with normal controls.

Methods: Isobaric tags for relative and absolute quantitation (iTRAQ) and enzyme-linked immunosorbent assay (ELISA) were used to detect differential plasma proteins (n=60 respectively).

Results: A total of 172 differential plasma proteins were found to be statistically significant, five proteins (platelet factor 4, von Willebrand factor, complement 9, mannose binding lectin-associated serine protease-2 and fibronectin), seemed to be associated with PDA, were selected for validation. We found that fibronectin was up-regulated and the other four were down-regulated (p<0.05 vs. control).

Conclusion: The present study for the first time by using the iTRAQ proteomic technology in CHD-PDA patients identified a large number of differential plasma proteins that are related to molecular function, biological processes, and cellular components. The five validated proteins in PDA patients may play key roles in the platelet aggregation, innate and adaptive immune response, coagulation mechanism, and the activation of complement system, and therefore have clinical significance.

OP2.

ZINC MEDIATES MITOCHONDRIA-ENDOPLASMIC RETICULUM "CROSSTALK" IN MORPHINE-INDUCED CARDIOPROTECTION AGAINST ISCHEMIA/REPERFUSION INJURY

MY Xi, YG He, P Wang, Y Fu, ZX Xu, JK Xi
Heart Institute, North China University of Science and Technology, China

Our previous studies showed that reperfusion but not ischemia initiates endoplasmic reticulum stress (ER stress) and inhibition of ER stress protects the heart from reperfusion injury through prevention of the mitochondrial permeability transition pore (mPTP) opening via intracellular Zinc. We aimed to explore whether Zinc was involved in morphine-induced cardioprotection against ischemia/reperfusion injury and to explore the potential mechanism. Morphine given at reperfusion significantly decreased the expression of the ER stress chaperones GRP78 and GRP94 in isolated rat hearts, which were reversed by the Zinc chelator TPEN, indicating that morphine could suppress the reperfusion-induced ER stress through Zinc. The immunofluorescence study with confocal microscope also support this effect. Experiments with infarct size study revealed that morphine could mimic the ER stress inhibitor TUDCA by significantly reducing the infarct size (21.3±6.7%) in isolated rat hearts subjected to 30 minutes regional ischemia followed by 2 hours of reperfusion. Transmission electron microscopy and hematoxylin-eosin staining showed that morphine could prevent endoplasmic reticulum and mitochondrial damages at reperfusion, which were abrogated by TPEN, implying that morphine may protect the heart at reperfusion through inhibition of ER stress via Zinc. H9c2 cardiac cells treated with 2-DG, the ER stress inducer, showed a significant decrease (47.2±6.9%) in TMRE fluorescence compared to the normal group (96.4±5.1%), indicating that ER stress induces the mPTP opening. Morphine could mimic the effects of TUDCA, ZnCl2 and mPTP inhibitor cyclosporin A by significantly inhibiting of the loss of TMRE fluorescence. In support, morphine and TUDCA could mimic ZnCl2 by increasing the fluorescent Zinc indicator Newport green DCF. The effects of morphine on TMRE and Newport green DCF were inhibited by TPEN, indicating that morphine prevents the mPTP opening through inhibition of ER stress via increasing the intracellular free Zinc. In conclusion, these data suggest that morphine protects the heart from reperfusion injury through inhibition of ER stress by prevention of the mPTP opening. Increased intracellular free Zinc accounts for the mitochondria-endoplasmic reticulum "CROSSTALK".
Abstracts for Oral Presentation:

OP3.
ZERO TIME EXERCISE: A CLUSTERED RANDOMIZED CONTROLLED TRIAL TO PROMOTE PHYSICAL ACTIVITY, HEALTH, HAPPINESS, AND FAMILY HARMONY UNDER HONG KONG JOCKEY CLUB FAMILY PROJECT

AYK Lam1, ANT Wan,2 DPK LEE,3 TH Lam4
1School of Public Health, The University of Hong Kong; 2Family Service, Caritas, Hong Kong

Objectives: About half of Hong Kong people are physically inactive. "Zero-time Exercise" (ZTEX) is a new approach to motivate people to do simple exercises, which need no extra time (zero time), money or equipment, and suit anybody, anytime and anywhere (3As). This cluster randomized controlled trial examined the effects of a ZTEX community intervention.

Methods: All the eight Caritas Integrated Family Services Centres with their respective community participants were randomized into either a physical activity experimental (PA) group or a control group (4 centres per group). We aimed to enhance the PA participants' physical activity by increasing their knowledge, motivation and self-efficacy about ZTEX through a 2-hour interactive core session at baseline, a 1.5-hour booster session at 3 months, and six monthly mobile messages. The control group had healthy diet (HD) intervention with the same methods, number of sessions and duration. Primary outcome was the number of days per week in doing ZTEX. Secondary outcomes were personal health, happiness, and family harmony. Each item allowed response on a 0 to 10 scale ranging from "not at all healthy/happy/harmonious" to "very healthy/happy/harmonious". We analysed by intention-to-treat method.

Results (preliminary): 673 participants (92% female) with at least one child, were recruited into the PA experimental group (n=357) or HD control group (n=316). Both groups had increased ZTEX [PA group: from 2.3 to 5.0 days/week (Cohen's d=1.14, p<0.001) at three months, and at six months; HD group: from 2.6 to 3.7 days/week (Cohen's d=0.44, p=0.001) at three months and 3.8 days/week (Cohen's d=0.49, p<0.001) at six months]. Both groups also had better perceived personal health [PA group: from 4.8 to 5.8 and 6.0 units; HD group: from 4.8 to 5.5 and 5.8 units], happiness (PA group: from 5.8 to 6.5 and 6.6 units; HD group: 5.4 to 6.0 and 6.3 units), and family harmony (PA group: 6.2 to 6.8 and 6.9 units; HD group: 5.8 to 6.4 and 6.5 units) at three months, and at six months (all p<0.001), respectively. Comparing with the HD control group, the PA experimental group performed more ZTEX by 1.3 days/week (Cohen's d=0.75, p<0.001), and had greater improvements in perceived personal health by 0.2 unit (Cohen's d=0.21, p=0.007) and family harmony by 0.2 unit (Cohen's d=0.17, p=0.036) at three and six months, adjusting for age, gender, education, income, marital status and their baseline values.

Conclusions: Our ZTEX intervention showed preliminary evidence of effectiveness in increasing physical activity, personal health, happiness and family harmony.

OP4.
UPREGULATION OF ATROGIN-1 PERK IN RESPONSE TO ER STRESS MEDIATES BKCa CHANNEL DYSFUNCTION INDUCED BY HOMOCYSTEINE IN CORONARY ARTERIES

WT Sun,1 XC Wang,2 SK Mak, J Fu, Q Yang
1Division of Cardiology, Department of Medicine and Therapeutics, Institute of Vascular Medicine, Li Ka Shing Institute of Health Sciences, Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

Background & Objectives: Although previous studies reported the inhibitory effect of homocysteine on large-conductance Ca2+-activated K+ (BKCa) channels in vascular smooth muscle cells, the mechanisms by which homocysteine inhibits BKCa channels remain poorly studied. Endoplasmic reticulum (ER) stress plays a role in various cardiovascular diseases though the cellular and molecular bases of ER stress in vascular pathology are inadequately elucidated. Whether ER stress affects BKCa channels and BKCa channel-mediated vasodilatory function so far remains uninvestigated. This study aimed to reveal whether ER stress regulates smooth muscle BKCa channels to participate in coronary dysfunction induced by homocysteine, with further uncovering the molecular determinants involved in the regulation.

Methods: Primary cultured porcine coronary arterial smooth muscle cells (PCASMCs) were used for mRNA and protein analysis of BKCa channels, as well as patch-clamp recording of the BKCa channel current. Vasorelaxant response to the BKCa channel opener NS1619 was studied in endothelium-denudated porcine small coronary arteries in a myograph.

Results: Homocysteine lowered protein level of BKCa β1 subunit whereas showed no effect on α subunit in PCASMCs. NS1619-induced vasorelaxant response of coronary arteries was attenuated by homocysteine. Inhibition of ER stress preserved the protein content of BKCa β1 and NS1619-evoked vasorelaxation. The selective inhibitor of PKR-like ER kinase (PERK) GSK2606414 showed similar protective effect against homocysteine as ER stress inhibitors on BKCa β1 expression and NS1619-induced relaxation, along with a significant enhancement of BKCa channel current. The restoration of BKCa β1 by PERK inhibition was associated with reduced atrogin-1 expression and decreased nuclear localization of forkhead box O transcription factor 3a (FoxO3a). Silencing of atrogin-1 prevented homocysteine-induced loss of BKCa β1 and silencing of FoxO3a prevented atrogin-1 upregulation in homocysteine-exposed PCASMCs, accompanied by preservation of BKCa β1 protein level and BKCa channel current.

Conclusions: ER stress mediated homocysteine-induced BKCa channel inhibition in coronary arteries. Activation of FoxO3a by PERK branch underlies the ER stress-mediated BKCa inhibition through a mechanism involving ubiquitin ligase-enhanced degradation of the channel β1 subunit. (Supported by BGC/GRF CUHK14118414; Lui Che Woo Institute of Innovative Medicine - CARE theme 8303303, and CUHK Direct Grant 4054182)
ABSTRACTS

Abstracts for Oral Presentation:

**OP5.**

**OPTIMAL DURATION OF DUAL ANTIPLATELET THERAPY AFTER DRUG-ELUTING STENT IMPLANTATION: NETWORK META-ANALYSIS OF RANDOMISED CONTROLLED TRIALS**

Y Fei, MF Tsoi, TT Cheung, BMY Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

**Objectives:** The optimal duration of dual antiplatelet therapy (DAPT) after drug-eluting stent (DES) implantation has always been debated. Evidence from clinical trials directly comparing short-term (<12 months) and extended (≥12 months) dual antiplatelet therapy is limited. So we performed a network meta-analysis to assess the risks and benefits of different DAPT durations to guide clinical practice.

**Methods:** We searched MEDLINE, EMBASE, Scopus, ISI Web of Science, Cochrane Library, ClinicalTrials.gov and recent conference proceedings for clinical trials and included those randomising patients to receive different durations of DAPT after DES implantation and reporting frequencies of cardiovascular and bleeding events. Network meta-analysis was performed with both a frequentist approach and a Bayesian framework using R statistics. Heterogeneity was calculated using I² statistics; bias in the selection or publication of studies was assessed.

**Results:** Twelve randomised controlled trials with 34,920 patients were included. Four trials compared extended DAPT (>12 months) vs. 12 months' regimen, seven trials compared short-term DAPT (<12 months) vs. 12 months' regimen and one trial compared extended vs. short-term DAPT. Extended DAPT significantly reduced the frequencies of myocardial infarction (OR 0.56, 95% CI 0.46-0.68 and OR 0.58, 95% CI 0.44-0.77), and stent thrombosis (OR 0.44, 95% CI: 0.30-0.65 and OR 0.49, 95% CI 0.29-0.82) when compared to 12 months' and short-term DAPT, respectively. However, the risk of major bleeding (OR 1.53, 95% CI 1.21-1.93 and OR 2.58, 95% CI 1.62-4.10) was substantially increased. Extended DAPT also increased the risk of all-cause mortality (OR 1.27, 95% CI 1.03-1.57) when compared to 12 months' DAPT. No significant difference was found in cardiovascular mortality, stroke and repeat revascularisation.

**Conclusions:** Our network meta-analysis with the latest available evidence suggests that 12 months' DAPT appears to be a reasonable compromise between preventing stent thrombosis and increasing bleeding risk. The decreased major bleeding with short-term DAPT than extended DAPT is offset by increased myocardial infarction and stent thrombosis. Patients at high bleeding risk could have a shorter DAPT duration; those with low bleeding but high ischaemic risk could consider a longer duration.

**OP6.**

**DISTURBED FLOW ACTIVATES TLR4 AND INDUCES ENDOTHELIAL INFLAMMATION**

D Ou, CW Lau, L Wang, W Song, J Xu, XY Tian, Y Huang
School of Biomedical Sciences and Institute of Vascular Medicine, The Chinese University of Hong Kong, Hong Kong

**Objectives:** The endothelium governs the health of blood vessels, while its dysfunction plays a critical role in both the initiation and progression of cardiovascular diseases including atherosclerosis. Disturbed flow of blood at arterial branches and curvatures directly modulates endothelial physiology and predispose the region to endothelial dysfunction and development of atherosclerotic lesions. Emerging experimental and clinical evidences show that activation of the Toll-like receptors (TLRs), in particular TLR4, contributes to vascular inflammation and atherosclerosis. However, whether TLR4 participates in the disturbed flow-induced endothelial dysfunction and inflammation has not been studied yet.

**Methods:** *Tlr4*−/− mice were used to investigate the role of TLR4 under disturbed flow in vivo. Partial carotid ligation was performed to induce disturbed flow in left common carotid in mice. Proximity ligation assay was used to detect interaction between target proteins in endothelial cells.

**Results:** An expression profile and functional assessment of TLRs have been provided, demonstrating that TLR4 was the dominant among TLR family members in vascular endothelial cells. TLR4 expression, activation, and its downstream inflammatory markers were elevated in mice aortic arch compared to thoracic aorta, which were absent in the *Tlr4*−/− mice. The same results were observed in partial carotid ligation models where TLR4 signaling was activated in response to ligation-induced flow disturbance and also reversed by the TLR4 mutant. Disturbed flow in vitro increased TLR4 expression and activation in ECs and promoted monocyte-endothelial cell adhesion, which were inhibited in TLR4 deficient ECs. Moreover, of endogenous TLR4 ligands examined as candidate mediators of disturbed flow-induced TLR4 activation, fibronectin containing the extra domain A (FN-EDA) expressed by endothelial cells was increased by disturbed flow and identified to directly interact and activate TLR4.

**Conclusions:** Our results demonstrates for the first time an indispensable role of TLR4 in disturbed flow-induced endothelial inflammation which may serve as the critical initiating step in atherogenesis, and pinpointed FN-EDA as an endogenous mediator that transfers the haemodynamic force to TLR4 activation.

(Supported by CUHK2/CRF12G, GRF14105814, NSFC91339117 and CUHK Lui Che Woo Foundation)
ABSTRACTS

Abstracts for Oral Presentation:

OP7.

DEFICIENCY OF PROSTAGLANDIN E2 RECEPTOR SUBTYPE 4 CAUSES DYSREGULATION OF CHOLESTEROL HOMEOSTASIS IN MICE

Ying Fang,1 Y Cai,2 EHC Tang1,3
1Department of Pharmacology and Pharmacy; 2Department of Anesthesiology, 3School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Mice lacking prostaglandin E2 (PGE2) receptor subtype 4 (EP4), one of the four receptors identified for prostaglandin E2, developed spontaneous hypercholesterolemia. The present study investigated the cause of the elevated plasma cholesterol in EP4 knockout mice. 15-17 weeks old normal diet-fed EP4 knockout mice showed a 48% increase in total cholesterol, 84% increase in very low density lipoprotein (VLDL)-low density lipoprotein (LDL) and 15.6% increase in high density lipoprotein (HDL), as compared to their wildtype littermates. The increase in plasma cholesterol is unrelated to alteration in intestinal cholesterol absorption, as fecal neutral sterol excretion did not differ between the two stains of mice. Rather, it was due to an increase production and efflux of cholesterol from the liver. The absence of EP4 increased protein expression of hepatic cholesterol efflux transporters, including ATP-binding cassette (ABC) A1 (by 61.33%) and ABCG1 (by 64.73%) and reduced phosphorylation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase; by 81.52%) and in so doing increased HMG-CoA reductase activity (by 170.36%), the rate-limiting enzyme for cholesterol synthesis. These effects were confirmed in human hepatoma (HepG2) cell line with EP4 knockdown. EP4 activators (CAY10580 and PGE2), cyclic AMP (cAMP) and protein kinase A (PKA) activators (8-Br-cAMP and 6-MB-cAMP, respectively) increased HMG-CoA reductase phosphorylation, while pre-treatment with H89 (a selective PKA inhibitor) reversed the PGE2-mediated increase in HMG-CoA reductase phosphorylation. These findings reveal that EP4 regulate cholesterol levels through a cAMP-PKA signaling axis and modulate the phosphorylation status of HMG-CoA reductase. In summary, the hypercholesterolemia in EP4 deficient mice is caused by an increase in cholesterol synthesis and cholesterol efflux from the liver. EP4 deficiency results in an inability to maintain normal plasma cholesterol levels in mice, identifying a new metabolic dimension in the physiological role played by endogenous EP4.

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OP8.

INHIBITION OF sEH UPREGULATION BY ALLEVIATING ER STRESS CONTRIBUTES TO THE ENDOTHELIAL PROTECTIVE EFFECTS OF TETRAMETHYLPERAZINE

SK Mak, WT Sun, J Fu, Q Yang
Division of Cardiology, Department of Medicine and Therapeutics, Institute of Vascular Medicine, Li Ka Shing Institute of Health Sciences, Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

Background & Objectives: Soluble epoxide hydrolase (sEH) is a key endogenous enzyme in the metabolism of epoxyeicosatrienoic acids. Despite numerous studies reporting the role of sEH upregulation in endothelial dysfunction, the mechanisms by which sEH is upregulated are still inadequately understood. It remains uninvestigated whether endoplasmic reticulum (ER) stress is involved in sEH dysregulation. Tetramethylpyrazine (TMP) is a major active ingredient of Chinese herb Chuanxiong that has been long known for cardiovascular benefits. However, whether TMP may protect vascular endothelial cells from ER stress and whether it may regulate sEH expression remain unknown. The present study therefore aimed to investigate whether ER stress participates in angiotensin II (Ang-II)-induced sEH dysregulation in coronary endothelium and whether anti-ER stress mechanism is involved in the vasoprotective effect of TMP through the regulation of sEH.

Methods: Endothelial cells (PCECs) were isolated by enzymatic digestion from porcine coronary arteries (PCAs) and primary cell cultures were used for western blot and reverse-transcription PCR analysis. Isometric force study of PCAs was performed in a myograph for assessment of endothelial dilator function.

Results: Ang-II induced upregulation of sEH at mRNA and protein level in PCECs, associated with increased expression and phosphorylation of ER stress molecules, i.e., GRP78, ATF6, p-PERK, and p-IRE1α. The increases of sEH mRNA and protein were also observed in PCECs exposed to tunicamycin, a chemical ER stress inducer. Pretreatment of PCECs with ER stress inhibitors suppressed Ang-II-induced upregulation of sEH. TMP showed comparable inhibitory effect to that of ER stress inhibitors on Ang-II and tunicamycin-induced expression/phosphorylation of ER stress molecules and upregulation of sEH. PCAs subjected to Ang-II and tunicamycin exposure exhibited impaired endothelium-dependent relaxation, which was improved by inhibitors of ER stress and sEH. TMP was similarly efficient as ER stress inhibitor in the restoration of endothelium-dependent relaxation in Ang-II- and tunicamycin-exposed PCAs.

Conclusions: Our results reveal that TMP possesses anti-ER stress properties and inhibition of sEH upregulation by alleviating ER stress is involved in the protective effects of tetramethylpyrazine against Ang-II-induced coronary endothelial dysfunction. These findings added new mechanistic insight into the cardiovascular benefits of this traditional Chinese medicine.

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ICSM, THE TWENTIETH ANNIVERSARY SCIENTIFIC MEETING

ABSTRACTS

Abstracts for Chaired Posters:

CP1.

TREATMENT OF CALCIUM CHANNEL BLOCKER OVERDOSE: A CASE SERIES

MF Tsoi,1 TT Cheung,1,2 BMY Cheung1,2,3,4
1Division of Clinical Pharmacology and Therapeutics, Department of Medicine; 2Research Centre of Heart, Brain, Hormone and Healthy Aging; 3Institute of Cardiovascular Science and Medicine; 4The State Key Laboratory of Pharmaceutical Biotechnology, The University of Hong Kong, Hong Kong

Introduction: Calcium channel blocker is a class of anti-hypertensive medications. However, its overdose may induce hypotension, shock and death. There was limited evidence in efficacy of high dose insulin (HDI) as supportive therapy in patients with calcium channel blocker overdose. Therefore, there is a need to study the survival of HDI treatment in patients with calcium channel blocker overdose.

Method: The Poison Information and Clinical Management System was used to identify patients with calcium channel blocker overdose. For inclusion, patients must be documented with calcium channel blocker overdose, hypotension and failed intravenous calcium salt. Patients were stratified according to the treatment regimen received by patients. The outcome of this study was the survival after treatment. Results were analysed by SPSS version 23.0. Logistic regression was used to study association between baseline parameters and mortality.

Result: 17 patients were included into this study. The mortality rate of patients were 38.5% and 0% in patients with HDI treatment and patients without HDI treatment, respectively (p=0.152). There is no significant difference in terms time-to-event relationship in death of patients (p=0.594) between two groups. Compared to death patients, survived patients had higher baseline serum albumin level (p=0.019). However, baseline serum albumin level was not associated with mortality of patients (adjusted odds ratio: 0.670; 95% Confidence interval: 0.435-1.032).

Interpretation: Calcium channel blocker overdose may induce death. High dose insulin did not improve survival rate in patients with calcium channel blocker overdose and treatment failure by intravenous calcium salt infusion. Baseline serum albumin was not predictor for mortality.

CP2.

SERUM 25-HYDROXYVITAMIN D AND THE RISK OF STROKE IN HONG KONG CHINESE

RYH Leung,1,2 Y Han,1 CW Sing,1 BMY Cheung,2,3 ICK Wong,1,5 KCB Tan,2 AWC Kung,2 CL Cheung1,3,4
1Department of Pharmacology and Pharmacy; 2Department of Medicine; 3The State Key Laboratory of Pharmaceutical Biotechnology; 4Centre for Genomic Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong; 5Research Department of Practice and Policy, UCL School of Pharmacy, United Kingdom

Objectives: Vitamin D level has been linked to the risk of cardiovascular diseases, however, its association with stroke remains inconclusive. In this study, we aimed to evaluate the association between serum 25-hydroxyvitamin D and risk of stroke in Hong Kong Chinese.

Methods: This study included 3,458 participants from the Hong Kong Osteoporosis Study aged 45 and older at baseline. They were examined between 1995 and 2010 and followed up using electronic medical records. Ischemic and hemorrhagic stroke were defined using ICD-9 codes.

Results: Quintiles 1 and 4 of 25-hydroxyvitamin D were significantly associated with increased risk of stroke when compared to the highest quintile (Quintile 1: HR, 1.78; 95% CI, 1.16-2.74 and quintile 4: HR, 1.61; 95% CI, 1.07-2.43) using multivariable Cox-proportional hazard regression. A similar association was observed in both genders. In subgroup analysis, the association was specifically observed for ischemic stroke, but not hemorrhagic stroke.

The association between vitamin D and risk of stroke was in a reverse J-shape in penalized regression spline, with the lowest risk of stroke being observed at 25(OH)D levels between 70 and 80 nmol/L.

Conclusion: A low vitamin D level is associated with higher risk of ischemic stroke. Further study is needed to evaluate whether high vitamin D level is also associated with increased risk of stroke.
Homocysteine is associated with many cardiovascular and metabolic diseases including hypertension and type 2 diabetes mellitus. High circulating homocysteine can trigger endoplasmic reticulum stress and causes excessive reactive oxygen species (ROS) production in endothelial cells. Elevated oxidative stress inactivates the function of the major endothelium-derived vasodilator nitric oxide (NO), resulting in endothelial dysfunction which is the hallmark of numerous cardiovascular complications. Secreted by intestinal endocrine L-type cells, the proglucagon-derived hormone glucagon-like peptide 1 (GLP-1) binds to GLP-1 receptors on endothelial cell surface to restore normal endothelial function by lowering vascular oxidative stress. Nevertheless, GLP-1 is subjected to rapid dipeptidyl peptidase 4 (DPP-4)-mediated degradation. It thus demands the development of other stable GLP-1 analogues such as exendin-4. Although exendin-4 can repair endothelial injury by restoring NO bioavailability, it remains unclear whether exendin-4 can protect endothelial cells in the presence of the cardiovascular risk factor homocysteine. Therefore, the present study aims to investigate whether exendin-4 reduces homocysteine-induced endothelial dysfunction through inhibiting oxidative stress in aortas from SD rats. Exendin-4 (1-100 nM) reverses homocysteine-induced impairment of endothelium-dependent relaxations in response to acetylcholine. Exendin-4 also inhibits the elevated accumulation of ROS in endothelial cells and the vascular wall of rat aortas triggered by homocysteine (300 μM). Future experiments are needed to confirm the in vitro vascular benefit of exendin-4 in the rat model of hyperhomocysteinemia to better understand any new mechanisms by which GLP-1 analogues protect vascular function under pathological conditions. The new findings shall shed new light on the potential use of these agents in the treatment of homocysteine-related cardiovascular diseases.

(This study is supported by CUHK Direct Grant and CUHK Lui Che Woo Foundation)

CONFEXIN 43, PANNEXIN 1 AND CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR IN THE REGULATION OF ATP RELEASE FROM MUSCLE

ETF Wu, L Lu, YS Wang, HJ Ballard
School of Biomedical Sciences, The University of Hong Kong, Hong Kong

ATP release from contracting skeletal muscle cells makes an important contribution to the increased blood flow that accompanies muscle contractions. Muscle contractions stimulate the formation of lactic acid, and the resulting decrease in intracellular pH stimulates the release of ATP. We studied the mechanism of lactic-acid-induced ATP release in the L6 skeletal myoblast cell line (which is a model for neonatal muscle) and in myofibres isolated from adult rat skeletal muscle. Lactic acid stimulated ATP release from myoblasts or isolated muscle fibres; in myoblasts, acidosis-induced ATP release could be inhibited by niflumic acid, lanthanum, or 100 μM carbenoxolone, but not by probenecid or 10 μM carbenoxolone, suggesting that connexins mainly mediated the ATP release. Lucifer yellow uptake into myoblasts was strongly stimulated in a Ca-free medium, but not in high potassium, confirming that connexins did not contribute to ATP release in adult muscle. This pattern corresponds to the change in protein expression in muscle cells, since L6 myoblasts and neonatal muscle strongly express Cx43, but connexin expression decreases to a low level in adult muscle whereas Panx1 expression increases. In both L6 myoblasts and in vivo, acidosis-induced ATP release was inhibited by CFTRinh-172, a specific inhibitor of the cystic fibrosis transmembrane conductance regulator (CFTR), whereas CFTR activators, forskolin or apigenin, stimulated ATP release; forskolin or apigenin-stimulated ATP release from myoblasts was inhibited by 100 μM carbenoxolone, suggesting that CFTR may play a role in the regulation of connexin opening. Decreased opening of ATP-releasing channels, resulting in diminished muscle vasodilation in exercise, may help to explain the decreased exercise tolerance in patients with CFTR.
ABSTRACTS

Abstracts for Chaired Posters:

CP5.

EPICALLOTECHIN GALLATE (EGCG) ALLEVIATED CSM-INDUCED ROS-ACTIVATED INFLAMMATION AND APOPTOSIS IN AC16 CARDIOMYOCYTES

YM Liang,1 MSM Ip,1,2 JCW Mak1,2,3
Departments of 1Medicine; 2Pharmacology & Pharmacy; 3Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong

Objectives: Smoking has been recognized as a major risk factor for cardiovascular disease (CVD). Cigarette smoke (CS) delivers high concentration of free radicals to smokers. Reactive oxygen species (ROS) has been shown to play a critical role in promoting inflammation, inducing mitochondria damage and inducing apoptosis in the development of CVD. Thus, we hypothesized that epigallocatechin gallate (EGCG), the major catechin found in green tea, could attenuate cigarette smoke medium (CSM)-induced injury by targeting ROS generation in human AC16 cardiomyocytes in vitro.

Methods: The AC16 cell line was cultured in DMEM/F12 containing 12.5% fetal bovine serum, in a CO2 incubator at 37°C. CSM was prepared by bubbling smoke from two cigarettes into 20ml serum-free medium, which was regarded as 100%. After serum starvation with 1% fetal bovine serum for 24h, cells were pretreated with EGCG (10 µM), N-acetyl-L-cysteine (NAC, 10 mM), or NF-κB inhibitors SC514 (10 µM) for 30 min before 4% CSM was added and incubated for an additional 24h. Supernatant was collected for determination of interleukin (IL)-8 by ELISA. Cells were collected to perform H2DCF-DA and MitoSox Red assays to determinate cellular ROS and mitochondrial superoxide by flow cytometry. Apoptotic cells were measured with Annexin V apoptosis detection kit. Cell lysates were collected for Western blot analysis.

Results: Exposure of AC16 cells to CSM for 24h markedly induced injuries, as evidenced by the elevations of cellular ROS and mitochondrial superoxide production and an increase of IL-8 release in supernatant, as well as increased apoptotic cells. These injuries were significantly attenuated by the pretreatment of cells with either EGCG or NAC respectively. Furthermore, exposure of cells to CSM increased the phosphorylation of NF-κB, which was abolished in the presence of NF-κB inhibitor SC514, leading to inhibition of IL-8 release. The increased activation of NF-κB pathway was ameliorated by pretreatment with either EGCG or NAC, resulting in the reductions of IL-8 release and apoptotic cells.

Conclusions: EGCG protected against CSM-induced injury by attenuating oxidative stress, inflammation and apoptosis via inhibiting NF-κB activation in AC16 cardiomyocytes. These findings suggest EGCG as a promising cardioprotective agent against ROS-mediated cardiac injury.

CP6.

DEFECTIVE AUTOPHAGY PARTICIPATES IN ENDOTHELIAL DYSFUNCTION IN DIABETIC MICE

L Zhao, J Liu, L Wang, JY Luo, LS Gou, CW Lau, XY Tian, Y Huang
School of Biomedical Sciences and Institute of Vascular Medicine, The Chinese University of Hong Kong, Hong Kong

Objectives: Endothelial dysfunction is closely associated with the development of atherosclerosis and vascular complications in diabetic patients although the mechanisms underlying endothelial dysfunction are not fully understood. Recent evidence indicates that autophagic flux is important in maintaining vascular function. Alterations in autophagic flux have been implicated in diabetes mellitus and atherosclerosis. However, the role of autophagy in endothelial cell function is largely unclear. Thus, the present study aims at examining whether altered autophagy involves diabetic endothelial dysfunction.

Methods and Results: We detected an increased accumulation of P62 and LC3 in aortas of diabetic db/db mice compared with non-diabetic db/m+ mice. Blocking the terminal stage of autophagy with chloroquine increased LC3 and p62 in aortas of db/m+ mice, but not in aortas of db/db mice, indicating autophagic flux was defective in diabetes. In addition, in cultured human aortic endothelial cells exposed to oxidized low-density lipoprotein and advanced glycation end products lead to a similar increased accumulation of P62 and LC3. Reduced autophagic flux was closely associated with increased mitochondrial reactive oxygen species production, a marked reduced of agonist-stimulated endothelial nitric oxide bioavailability and acetylcholine-induced endothelium-dependent relaxations. Furthermore, we identified that the transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagic flux, nuclear localization signal was reduced in db/db mouse.

Conclusion: The present results suggest that defective autophagy is involved in endothelial dysfunction in diabetic mice. Restoring autophagic flux reduces mitochondria ROS and thus improves endothelial function, indicating that restoration of the impaired autophagy may represent another novel therapeutic strategy for treating diabetic vascular diseases. (Supported by CUHK2/CRF/12G, GRF14105814 and CUHK Lui Che Woo Foundation)
CP7.

miR199a-3p REGULATES P53 BY TARGETING CABLES1 IN MOUSE CARDIAC STEM CELLS TO PROMOTE PROLIFERATION AND INHIBIT APOPTOSIS THROUGH A NEGATIVE FEEDBACK LOOP

YS Wang, HJ Ballard
Institute of Cardiovascular Science and Medicine, The University of Hong Kong, Hong Kong

MicroRNAs (miRNAs) have emerged as crucial factors that regulate proliferation and apoptosis of cardiac stem cells (CSCs). Although much is known about their role in maintaining CSCs pluripotency, the mechanisms by which they affect cell fate decisions that as an essential part in repairment of heart failure remain poorly understood. Here, we demonstrated a significantly decreased expression of miR199a-3p in heart failure samples compared with healthy donors. Meanwhile, we identified miR199a-3p as a proliferation and apoptosis-associated regulator impacted through Cdk5 and Abl enzyme substrate 1 (CABLES1) targeting and also attributed to their repression on P53 protein expression. We further demonstrated that P53 induced miR199a-3p expression, and in turn, miR199-3p decreased P53 activity. Collectively, our findings uncover one new mechanism that P53 induced miR199a-3p expression, and in turn, miR199-3p decreased P53 activity. Therefore, miR199a-3p and P53 are coupled through CABLES1 and comprised a novel negative feedback loop that likely contributes to CSC proliferation and apoptosis.

CP8.

TETRAMETHYLPYRAZINE PROTECTS AGAINST HOMOCYSTEINE-INDUCED BK$_\alpha$ CHANNEL DYSFUNCTION VIA INHIBITION OF ER STRESS

WT Sun, XC Wang, SK Mak, J Fu, Q Yang
Division of Cardiology, Department of Medicine and Therapeutics, Institute of Vascular Medicine, Li Ka Shing Institute of Health Sciences, Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

Background & Objectives: Large conductance Ca$^{2+}$-activated K$^+$ (BK$_\alpha$) channels in smooth muscle cells play a key role in the regulation of vascular function. Our latest study showed that endoplasmic reticulum (ER) stress mediates homocysteine-induced BK$_\alpha$ channel dysfunction in coronary arteries. Tetramethylpyrazine (TMP) is a major active ingredient of Ligusticum chuanxiong (Chuanxiong) that is well known for cardioprotective benefits. Whether TMP protect vascular cells from ER stress and whether protection of BK$_\alpha$ channel function is involved however are barely studied. The present study was designed to investigate the effect of TMP on homocysteine-induced BK$_\alpha$ channel dysfunction in coronary arteries with understanding the role of ER stress.

Methods: The vasorelaxant response to BK$_\alpha$ channel activator was examined by isometric force study in porcine small coronary arteries. Expressions of ER stress molecules and BK$_\alpha$ channel subunits were evaluated by Western blot and RT-PCR, and BK$_\alpha$ channel currents were recorded by patch-clamp in porcine coronary arterial smooth muscle cells (PCASMCs).

Results: TMP preserved the vasodilatory function of BK$_\alpha$ channels in coronary arteries subjected to homocysteine exposure. The protective effect of TMP on BK$_\alpha$-mediated relaxation was also observed in arteries exposed to tunicamycin, which is a chemical ER stress inducer. In homocysteine-exposed PCASMCs, TMP downregulated the protein expression/phosphorylation of ER stress molecules, i.e., GRP78, ATF6, p-PERK, p-eIF2$\alpha$, and p-IRE1 and meanwhile restored the lowered protein level of BK$_\alpha$ $\beta_1$. The restoration of BK$_\alpha$ $\beta_1$ level by TMP against homocysteine is accompanied by an enhancement of BK$_\alpha$ channel current.

Conclusions: TMP protects BK$_\alpha$ channels of coronary arteries from homocysteine-induced function impairment through inhibition of ER stress-mediated loss of channel $\beta_1$ subunit.

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ABSTRACTS

Abstracts for Posters:

P01.

A RANDOMIZED CLINICAL TRIAL TO COMPARE THE EFFECTS OF ICE-WATER IMERSION WITH COLD-WATER IMERSION ON THE CARDIOVASCULAR RESPONSES IN FATIGUED RUGBY PLAYERS

SSM Fong,1 GCC Chow,1,2 JWY Chung3
1School of Public Health, The University of Hong Kong; 2Faculty of Liberal Arts and Social Sciences, The Education University of Hong Kong; 3Department of Health and Physical Education, The Education University of Hong Kong, Hong Kong

Objectives: To compare the effects of ice-water immersion (IWI) with cold-water immersion (CWI) on cardiovascular responses in fatigued rugby players.

Methods: Thirty-one amateur rugby players (mean age ± SD = 21.6 ± 2.9 years old) were randomly assigned to either the IWI or CWI groups. Participants in the IWI group (n=13) received intermittent, high-intensity treadmill running at 90% of maximum heart rate for 3.5 minutes followed by a 1-minute half-body ice-water immersion (5°C). Participants in the CWI group (n=18) underwent the same intervention except that they immersed in cold tap water instead (25°C). Measurements were taken at 4 time points: at rest (T1, baseline), after high-intensity treadmill running and just before IWI/CWI (T2), right after IWI/CWI (T3), and 30 minutes after IWI/CWI (T4). The primary outcome measure was systolic (SBP) and diastolic (DBP) blood pressure as measured by a digital blood pressure monitor. The secondary outcome measure was heart rate which was monitored continuously using a Polar heart rate monitor.

Results: Two-way repeated measures analysis of variance results revealed that both SBP and DBP dropped similarly in the two groups from T2 to T4 though the declines were not significant statistically (p>0.008, Bonferroni adjusted). As expected, heart rate increased from T1 to T2 (p<0.001), then it levelled off from T2 to T3 (IWI/CWI period, p>0.008), and it dropped significantly from T3 to T4 (p<0.001) in both groups. Of note, heart rate returned to the baseline value within 30 minutes after IWI (p=0.037), but not after CWI (p<0.001). In addition, no significant group or time-by-group interaction effects were found for any of the outcome measures.

Conclusions: Post-high-intensity running IWI and CWI elicited similar cardiovascular responses among amateur rugby players. However, it seems that IWI is more favorable than CWI as heart rate returned to the baseline value faster after ice-water immersion. The exact mechanism of this phenomenon has yet to be explored.

P02.

TRANILAST, ORALLY ACTIVE TRPV2 ANTAGONIST, AMELIORATES END-STAGE HEART FAILURE IN MICE WITH DILATED CARDIOMYOPATHY

K Komamura, Y Iwata
Kobe Gakuin University, Japan

Objectives: Expression of transient receptor potential vanilloid 2 (TRPV2), a calcium-permeable cation channel, increased in the sarcolemma of animal and human myocardium with DCM. We assessed whether a TRPV2 antagonist, tranilast, ameliorated heart failure of DCM mice.

Methods: We used 4C30 mice, which has abnormal myocardial calcium handling, as a model of DCM. Sixteen 4C30 mice of 25 weeks old with end-stage heart failure were given no drug (control) or 20 mg/kg/day of carvediol (group C) or 400 mg/kg/day of tranilast (group T) or both of them (group B) for 2 weeks.

Results: Blood pressure and heart rate were similar among the 4 groups. Echocardiography demonstrated tranilast improved fractional shortening (in %). Control: 6.2±2.5; Group C: 14.2±5.6, NS; Group T: 20.8±3.3, p<0.01; Group B: 17.2±3.2, p<0.05. Tranilast also improved cardiac hypertrophy measured with heart-to-body weight ratio (HW/BW in mg/g). Control: 12.4±2.5; Group C: 11.5±3.4, NS; Group T: 8.6±0.9, p<0.05; Group B: 5.9±1.1, p<0.01. Sarcolemmal expression of TRPV2 measured with immunostaining in 4C30 mice increased twice as much as syngeneic C57BL/6CrSlc. Tranilast, not carvediol, halved the expression of TRPV2, corresponding to reduction in [Ca2+]i of isolated cardiomyocytes.

Conclusion: Tranilast improved cardiomyopathy in 4C30 mice, possibly due to the inhibition of Ca2+ influx through TRPV2.
ABSTRACTS

Abstracts for Posters:

P03.
RELATIONS OF POTASSIUM CHANNEL AND CARDIAC DEPRESSION BY FLUOXETINE
MY Lee
Department of Occupational Therapy, College of Health Science, Honam University, Republic of Korea

Objectives: Fluoxetine is serotonin-selective reuptake inhibitor (SSRI) without anticholinergic effects. Although fluoxetine has been used in the field of anesthetic medicine, the cardiovascular effects of fluoxetine is still controversial. This study investigated the relation of potassium (K) for fluoxetine-induced cardiac depression in rat.

Methods: The isolated heart was retrograde-perfused with an oxygenated modified Krebs-Henseleit buffer. The experimental system was designed to measure simultaneously left ventricular development pressure (LVDP), velocity of the change of pressure (dP/dt) and heart rate (HR) of isolated perfused heart of the perfusate simultaneously. Left ventricular pressure (LVP) was measured isovolumetrically with a transducer, connected to a thin, water-filled latex balloon, which was inserted into the left ventricle through the mitral valve via a left atrial incision.

Results: Fluoxetine produced decreasing of LVDP, dP/dt and HR. And, Fluoxetine blocked that increasing of LVDP by increasing of potassium. Especially, the effect of fluoxetine was prolonged after washout.

Conclusions: These results suggest that fluoxetine-induced cardiac depression can be partly responsible to the block of potassium channel, but these reactions are prolonged after wash out.

P04.
INFLUENCE OF OBESITY AND OBSTRUCTIVE SLEEP APNEA SYNDROME ON PLATELET ACTIVITY: ROLE OF PHOSPHATIDYL INOSITOL 3-KINASE β
Y Liu,1,2 JF Tian,1 R Tian,1 JC Zhang,1,2 DZ Shi,1,2 KJ Chen1,2
1Centre of Cardiovascular Diseases, Xiyuan Hospital of China Academy of Chinese Medical Sciences; 2China Heart Institute of Chinese Medicine, China Academy of Chinese Medical Sciences; 3Beijing Anzhen Hospital, Capital Medical University, China

Objectives: Platelet function in obstructive sleep apnea syndrome (OSAS) and obesity has not been clearly elucidated. In this study, we record the distribution of CD62p and PI3Kβ expression of human platelets of obese patients with OSAS, and to analyze the correlation between platelet PI3Kβ level and platelet activation.

Methods: This study included 216 subjects totally, with 70 obese subjects (Body Mass Index ≥30kg/m², group A) without OSAS and comorbidities, 91 obese subjects with OSAS (moderate or severe, history of OSAS is more than 3 years, group B), and 55 healthy participants (group C). The following morning without breakfast, fresh blood (20 mL) was drawn from an antecubital vein and collected, and the initial 2 mL of blood was discarded to avoid spontaneous platelet activation. The fluorescence intensity of CD62p and cytoplasmic calcium (Ca²⁺) in human platelets of patients and healthy participants were measured with flow cytometry. The PI3Kβ level of human washing platelets among three groups was measured by western blotting.

Results: The groups were matched for age, with a mean age of 38.6±5.1 in obese subjects with OSAS and 40.2±4.9 in obese subjects without OSAS (p=0.45). Compared with group C, platelet CD62p, [Ca²⁺]i and platelet PI3Kβ expression of group A and B increased significantly (p≤0.05); Compared with group A, platelet CD62p, [Ca²⁺]i and platelet PI3Kβ expression of group B increased significantly (p≤0.05).

Conclusions: In obese patients with OSAS, platelet activation is associated with greater levels of platelet PI3Kβ expression, and OSAS can increase the degree of platelet activation of obesity.
ABSTRACTS

Abstracts for Posters:

P05.

INHIBITORY EFFECT OF CAVEOLIN-1 ON THE ER STRESS-INDUCED APOPTOSIS IN MACROPHAGES VIA VIBRATING THE p38 MAPK PATHWAY

W Yue, ZP Shang
Pathophysiology Department, Taishan Medical College, China

Objectives: To explore the control effects and probable mechanisms of caveolin-1 on endoplasmic reticulum (ER) stress-induced apoptosis in RAW264.7 cells.

Methods: RAW264.7 cells were incubated with TG for ER stress-induced apoptosis model, and then the expression of caveolin-1 was determined by Western blot. The cells were treated with filipin(III), and then flow cytometry assay determining the apoptotic rate; laser scanning confocal microscopy observing the morphological changes of the cells; Western blotting determining the protein levels of CHOP and p-p38 MAPK.

Results: (1) The expression of caveolin-1 was increased in RAW264.7 cell treated with thapsigargin; (2) After treatment of filipin(III), the apoptotic rate was increased; the expression of p-p38 MAPK was reduced; and the expression of CHOP remain unchanged as compared to TG group.

Conclusion: Caveolin-1 inhibits the process of the TG-induced apoptosis in RAW264.7 cells through ER stress, one of the mechanisms may being correlated with promoting the p38 MAPK prosurvival pathway.

P06.

IS ENDOTHELIAL FUNCTION RELATED TO PLASMA LEVEL OF LIPOCALIN-2 IN MAN?

BMY Cheung,1,3,4 Y Wang,2,3,4 KSL Lam,1,3,4 HF Tse1,3,4,5
1Department of Medicine; 2Department of Pharmacology and Pharmacy; 3Partner State Key Laboratory of Pharmaceutical Biotechnology; 4Research Centre of Heart, Brain, Hormone and Healthy Aging; 5Institute of Cardiovascular Science and Medicine; The University of Hong Kong, Hong Kong

Background: Recent animal studies suggested that lipocalin-2 plays a key role in the development of hypertension, atherosclerosis and endothelial dysfunction. Lipocalin-2 knockout mice are resistant to the harmful effects of high fat diet and do not develop hypertension or atherosclerosis. We therefore hypothesised that lipocalin-2 is also related to endothelial function in man.

Method: We measured plasma lipocalin-2 concentration in 245 subjects (201 men, 44 women; mean age±SD, 68±9 years old) who had measurements of brachial artery flow-mediated dilatation (FMD). Among these subjects, 151 had hypertension, 80 had diabetes mellitus and 240 were on lipid lowering therapy. Brachial artery diameter and flow velocity were measured using a 7.5 MHz ultrasound probe. Scans were taken at baseline, 5 minutes after tourniquet inflation and after sublingual glyceryl trinitrate spray. The percentage change in brachial artery diameter following reactive hyperaemia was calculated. The coefficient of variation of FMD determination was 5%.

Results: Plasma lipocalin-2 correlated with serum creatinine (p=0.23, p<0.001) but not FMD (p=0.005, p=0.94). FMD correlated inversely with age (p=-0.14, p=0.03). Diabetes was associated with a lower FMD (p=0.044).

Conclusions: Plasma level of lipocalin-2 is unrelated to FMD. Endothelial function is influenced by many factors, including ageing and diabetes as found in this study, and also diet and drug therapy. The correlation of lipocalin-2 with creatinine is consistent with it being a marker of renal injury.

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