ABSTRACTS

Abstracts for Oral Communications:

OC1.

Tanshinone IIA Attenuated Cardiac Fibrosis in Rats via Inhibition of Oxidative Stress: In Vivo and In Vitro Investigations

Jie Gao, Ruifang Li, Guoqing Yang, Tieqiao Wang, Kang Le, Yingxia Bao, Fangyang Zhang, Wenjie Tang, Peiqing Liu
Laboratory of Pharmacology and Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, Zhongshan No.2 Road #74, Guangzhou 510080, People's Republic of China

Aim: It has been reported that an enhanced ROS production and decreased antioxidant defenses in cardiac tissue is one of the causes of cardiac fibrosis. In this study, we examined whether Tanshinone IIA (TSN), an anti-oxidant, could attenuate cardiac fibrosis in.rats and the mechanisms probably involved in it.
Methods: We used rat abdominal aortic coarctation (AAC) model as an in vivo model and AngII-treated adult rat cardiac fibroblasts as an in vitro model. HE and VG staining, echocardiographic study, haemodynamic study, serum SOD and MDA assay, DHE detection, MTT assay, and hydroxyproline assay were performed in our experiments.
Results: Pretreatment of AAC rats with TSN (35, 70 mg/kg) dose-dependently reversed the elevated heart weight/body weight ratio and attenuated cardiac fibrosis accompanied by improved LV diastolic function without significant changes in heart rate and blood pressure. Further studies revealed that TSN increased serum SOD, reduced serum MDA and heart superoxide anion production in AAC rats. Moreover, TSN significantly inhibited AngII-stimulated collagen synthesis in cultured rat cardiac fibroblasts. And this was also associated with the inhibition of superoxide anion production.
Conclusions: TSN attenuates fibrosis in AAC rats and prevents AngII-stimulated collagen synthesis in cultured rat cardiac fibroblasts via inhibition of oxidative stress, making it a promising drug for the treatment of cardiac fibrosis.

OC2.

Effects of Green Tea on Hypercholesterolemia Induced Hypertension in Rats

LY Song,[1] MWL Koo,[2] BMY Cheung,[1] CP Lau[1]
[1]Department of Medicine; [2]Department of Pharmacology, The University of Hong Kong, Hong Kong SAR, China

Background and purpose: Green tea was reported to have cardioprotecive effects. In this study, the effects of green tea on hypercholesterolemia-induced hypertension, vascular responses in isolated aortic rings, and serum lipid profile in rats were examined.
Experimental approach: Male Sprague-Dawley rats were divided into 4 groups of 6 rats each. The normal control group was fed with a normal diet while the remaining 3 groups were given a diet high in cholesterol. Two of the hypercholesterol diet treated groups were administered with green tea or tea powder solution. The treatments were given for 2, 4 or 8 weeks before blood pressure and vascular function evaluation and serum lipid profile measurement.
Results: High cholesterol diet treatment for 2 and 4 weeks induced significantly higher total serum cholesterol level ($p<0.01$) and blood pressure ($p<0.05$). The cholesterol level was still elevated in disease control group at 8 weeks ($p<0.05$). High density lipoprotein (HDL)-cholesterol level was significantly reduced by cholesterol feeding at 2 and 8 weeks in the disease control group ($p<0.05$). Green tea and tea powder treatments for 2 to 4 weeks significantly lowered the serum cholesterol ($p<0.05$) which became normal at the end of 8 weeks. HDL-cholesterol was not suppressed in the tea and tea powder groups except at 2 weeks of the tea group ($p<0.05$). There was no increase in blood pressure in tea and tea powder treated groups when compared to the normal control group. The blood pressure of the tea leaf group, and tea powder group was significantly lowered than the disease control group at 4 weeks and 4 to 8 weeks, respectively. Tea and tea powder solutions at physiologically attainable concentrations enhanced vasorelaxation induced by physiological concentrations of acetylcholine (10^{-8} M-10^{-7} M) in rat aortic rings.
Conclusions: Green tea lowered blood pressure that was increased by hypercholesterolemic diet. It decreased serum total cholesterol and prevented reduction of HDL-cholesterol level, thus tea consumption may exert beneficial effects on the cardiovascular system.

ABSTRACTS

Abstracts for Oral Communications:

OC3.

Expression Pattern of Genes Related to the Cholesterol Uptakes and Efflux in Age-dependent Atherosclerotic Development of ApoE$^{-/-}$ Mice

FT Tang, TQ Wang, XQ Wu, SR Chen, P Wang, RF Li, HJ Zhang, J Gao, LP Bao, PQ Liu , HQ Huang
Laboratory of Pharmacology and Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510080, People's Republic of China

Lipid deposition in vessel is one of the characteristics of early atherosclerosis and imbalances between uptakes and efflux of cholesterol by macrophages might be responsible for this phenomena. At molecular levels, expressions of genes related to this uptakes and efflux might behave differently in the development of atherosclerosis. We evaluated in the present study the mRNA expression pattern of uptake receptor CD36 and SR-A and PPARγ, LXRα, ABCA1, which are involved in the cholesterol efflux in the age-dependent atherosclerotic development of ApoE$^{-/-}$ mice. On normal chow, these mice showed age-dependent, spontaneous development of atherosclerotic lesions assessed by both oil red O staining in aortic arch and H&E staining in aortic valves. Anti-oxidative vitamin E complementation to mice significantly attenuated the early atherosclerotic lesion at ages up to 24 weeks and had no affection on advanced lesion after 24 weeks compared with age-matched mice. Serum level of oxLDL increased in an age dependent manner at weeks of 6 to 24 and maintained relatively stable after 24 weeks. Likely, vitamin E decreased serum oxLDL content at ages up to 24 weeks and had no affection after that age. The vascular expressions of CD36 and SR-A, two scavenger receptors recognizing and taking up oxLDL, and PPARγ, LXRα, ABCA1, which are involved in the cholesterol efflux, increased at or before 24 weeks in an age dependent manner. However, expressions of CD36 and SR-A maintained relatively stable whereas that of PPARγ, LXRα, ABCA1 decreased after 24 weeks throughout the experiment. In comparison with the age-matched mice, vitamin E decreased the expressions of those genes at or before 24 weeks and had no affection on them after that age. Our results suggest that at mRNA level, relatively more uptakes of oxLDL contribute to the early atherosclerosis whereas less efflux of cholesterol is mainly responsible for the advanced atherosclerosis. In addition, vitamin E was shown to inhibit early rather than advanced atherosclerosis.

OC4.

Polyol Pathway Contributes to the Impairment of Calcium Homeostasis in Adult Rat Cardiomyocytes Subjected to Simulated Ischemia

WH Tang, M Sauert, SK Chung, SSM Chung
Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

Cardiovascular disease represents the major cause of morbidity and mortality in developed countries. Recently, a number of studies have shown that the activation of aldose reductase (AR), the first and rate-limiting enzyme of the polyol pathway, is crucial to ischemia/reperfusion (I/R)-induced myocardiac infarction. To clarify the pathophysiological role of AR in the heart, we investigated the effect of blockade of the polyol pathway on calcium (Ca^{2+}) homeostasis in the rat cardiomyocytes subjected to simulated I/R. We determined the following: 1) calcium homeostasis by measuring electrically induced Ca^{2+} transient, which provides the overall picture for the regulation of Ca^{2+} signal; 2) expression of RyR2 and SERCA2a by Western blot analysis in the isolated sacroplasmic reticulum (SR); and 3) activities of RyR2 and SERCA2a in isolated SR vesicles. We found that I/R led to significantly reduced amplitude of the Ca^{2+} signal, prolonged the time to reach peak amplitude as well as the time for signal to decay. In addition, the activities of RyR2 and SERCA2a in the isolated SR from cardiomyocytes subjected to I/R were found to be impaired, but there was no change in their protein levels. Inhibition of AR or sorbitol dehydrogenase (SDH), attenuated I/R-induced impairment of Ca^{2+} homeostasis and restored the activities of RyR2 and SERCA2a. The results are evidence that AR contributes to I/R injury of the rat hearts, at least partly, by impairing Ca^{2+} homeostasis at multiple sites (RyR2 and SERCA2a).

OC5.

Testosterone Augments β-Adrenergic Preconditioning in the Rat Heart

S Tsang, S Wu, TM Wong
Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR, China

Testosterone at a physiological concentration is required for delayed cardioprotection of ischaemic preconditioning (IPC). This shows the enhancing effect of the hormone on preconditioning. Considerable data suggest that transient activation of β-adrenoceptor elicited protection that mimics the cardioprotection of IPC. We previously showed that testosterone at a physiological concentration enhanced β-adrenergic stimulation. We therefore hypothesize that testosterone augments β-adrenergic preconditioning in the rat heart. Male Sprague-Dawley rats (7-8 weeks) underwent sham operation, orchidectomy without (ORX) or with testosterone replacement (ORX+T) for 8 weeks. We used both isolated heart and myocyte preparations in addition to cardiac myoblast cell line, H9C2. Isolated hearts, ventricular myocytes and H9C2 were preconditioned with isoproterenol, a β-adrenoceptor agonist (10^{-8} mol $L^{-}1$). Isolated hearts were subjected to global ischaemia, followed by reperfusion whereas isolated myocytes and H9C2 to metabolic inhibition and anoxia to induce injury. Isoproterenol induced β-adrenoceptor preconditioning improved contractile functions impaired by ischaemic insult in isolated hearts, increased viability and decreased lactate dehydrogenase release in the myocytes and H9C2. The protection was enhanced in control and testosterone-replacement groups. In H9C2, we showed that the protection was abolished by PKA and PI3K/Akt inhibitors. The inhibition of PKA and PI3K/Akt was enhanced by testosterone. The effect of testosterone replacement in cells was abolished by androgen receptor blockade. In conclusion, testosterone augments β-adrenergic preconditioning at least in part by enhancing PI3K/Akt pathway. The effect of testosterone is androgen receptor-mediated.

ABSTRACTS

Abstracts for Oral Communications:

OC6.

Lactic-Acid-Induced ATP Release from Rat Soleus Muscle Cells Through Adenylyl Cyclase-cAMP-PKA-Regulated CFTR

J Tu, HJ Ballard
Department of Physiology & Institute of Cardiovascular Science and Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

Background and Objective: We have previously shown that pH depression could elevate the interstitial ATP concentration ([ATP_i]). The increase in [ATP_i] could be blocked by inhibitors of the cystic fibrosis transmembrane conductance regulator (CFTR). This study was performed to address whether CFTR is expressed on muscle, whether it functions as a channel for ATP efflux from muscle and to explore how the activity of CFTR is regulated during acidosis challenge.
Methods: A decrease in pH of the buffer-perfused hindlimb muscleof anaesthetised rats was induced by infusion of lactic acid; interstitial microdialysate was collected from soleus muscle for determination of ATP concentrations by HPLC. Immunohistochemistry was used to determine whether CFTR was expressed on rat soleus muscle. Inhibitors or activators of the cAMP-protein kinase A (PKA) pathway were used to investigate the signal transduction pathway for CFTR-mediated ATP transport.
Results: The [ATP_i] was significantly increased by 20 minutes infusion of lactic acid at 5 or 10 mM. Infusion of $CFTR_{inh-172}$ (20 μM), a specific inhibitor of CFTR, blocked the lactic-acid-infusion-induced increase in [ATP_i]. When lactic acid infusion was withdrawn in the presence of $CFTR_{inh-172}$, [ATP_i] dropped below its pre-control level. Immunohistochemistry, using anti-CFTR antibody and ABC kit confirmed the existence of CFTR on the apical side of the rat soleus muscle cells. No CFTR staining was observed in the negative control which lacked the antibody for CFTR, indicating that the staining for CFTR was specific. In the presence of H-89, a non-specific inhibitor of PKA, interstitial ATP remained at the control level during infusion of 5 mM lactic acid. Similarly, the specific inhibitor of PKA, KT5720 (10 μM), blocked the increase in [ATP_i] during infusion of 5 mM lactic acid. Finally, 30 minutes infusion of forskolin (40 μM), an activator of adenylyl cyclase (AC), significantly increased [ATP_i], which suggested that the ATP-conductive CFTR channel might be activated by AC.
Conclusions: These data further demonstrated the involvement of CFTR in the lactic-acid-infusion-induced increase in [ATP_i], and suggested that this function of CFTR might be regulated by the AC-cAMP-PKA pathway.

OC7.

Cranberry Juice Consumption Ameliorates Endothelial Dysfunction during Estrogen Deficiency

LM Yung,[1] WT Wong,[1] XY Tian,[1] FP Leung,[1] CW Lau,[1] XQ Yao,[1] ZY Chen,[2] Paul M Vanhoutte,[3] Y Huang[1]
[1]Department of Physiology and [2]Department of Biochemistry, The Chinese University of Hong Kong; [3]Department of Pharmacology, The University of Hong Kong, Hong Kong SAR, China

Consuming vegetables and fruits can retard the onset of cardiovascular complications and relieve postmenopausal symptoms. Cranberries are rich in antioxidant polyphenols, such as flavanoids and related phenolic acids. This study was aimed to examine whether or not consumption of cranberry juice could ameliorate endothelial dysfunction in an animal model of estrogen deficiency. Female Sprague-Dawley rats were divided into three experimental groups: sham-operated, ovariectomized (OVX), and OVX rats receiving cranberry juice. Changes in vascular reactivity in isolated aortas were studied in organ baths, while the levels of protein expression of endothelial nitric oxide synthase (eNOS), angiotensin II type 1 (AT1R) and type 2 receptors (AT2R), gp91phox [membrane-bound subunit of NAD(P)H oxidase] and nitrotyrosine were detected by Western blot analysis. The endothelium-dependent relaxations to acetylcholine were significantly impaired in the aorta from OVX rats; which can be partially prevented by acute treatment with losartan (AT1R blocker). Chronic consumption of cranberry juice significantly ameliorated endothelial dysfunction in OVX rats. Vascular tension developed by phenylephrine was higher in OVX rats than in control rats. This increased contraction was attenuated in rats receiving cranberry juice. There is also an increased oxidative stress as indicated by increased levels of nitrotyrosine in OVX aortas, which was restored by cranberry juice consumption. The present study show that chronic consumption of cranberry juice (i) increases phosphorylation of eNOS at ser-1177 without affecting the total protein content of eNOS, (ii) decreases the protein expression for AT1R, gp91phox and nitrotyrosine in aorta from OVX rats. The results of the present study suggest that chronic oral administration of cranberry juice to OVX rats clearly augments the bioavailability of NO which is related to the reduced oxidative stress probably through inhibition of expression and function of AT1R-mediated vascular dysfunction.
(Supported by RGC grant, CUHK Li Ka Shing Institute of Health Sciences and Focused Investment Scheme)