

e0214 **N-ACETYL CYSTEINE INHIBITS OXIDISED LDL, METALLOPROTEINASES -9 SECRETION AND APOPTOSIS IN ATHEROSCLEROSIS**

doi:10.1136/hrt.2010.208967.214

Li Zhenxiao, Meng Xiaoping. *Cardiology, The Second Hospital of Jilin University, China*

Atherosclerosis is a complex disease, in which oxidative stress in atherosclerosis through the whole process, antioxidant treatment is imperative.

Objective Discuss the effect of N-acetyl cysteine (NAC) in the APOE gene knockout mice in anti-atherosclerosis.

Method In this study, select 8-week-old male Apoe knockout (Apoe^{-/-}) mice were studied, a total of 10, with an average weight of 20 g. Were randomly divided into high-fat diet group (control group), and NAC interfere with treatment group (experimental group), two groups of mice were treated with high-fat diet, NAC intervention group administered a dose of 300 mg/kg, according to body weight of mice in each of the group on the required dose, ministered through drinking water bottle means, and to ensure that set up a daily dose of drug intake. Modelling 60 days, to give a 24-h fasting mice, pick the eye blood, placed in test tubes containing EDTA-specific biochemical tests carried out centrifugation, separated plasma. Off mice were sacrificed, rapid separation of mouse aortic arch and access to paraffin-embedded specimens. By using situ immunofluorescence staining method for the determination of artery intima-mouse levels, to observe the protection of NAC in atherosclerosis during the course of apoptosis; in serological testing, the choice of ELISA method was determined to assess the plasma MMP-9 in mice with ox-LDL levels; artery atherosclerotic plaques in the general morphological study using haematoxylin - eosin (HE) staining method, histological specimens of atherosclerotic lesions in general shape.

Result In the general model of atherosclerosis, the visual plaque morphology: NAC experimental group of mice the distribution of atherosclerotic plaque area less than high-fat diet group; HE stained specimens, NAC group foam cells were markedly reduced plaque in the volume of foam cells and plaque lipid core size significantly decreased compared with the control group; by in situ fluorescence staining method, determination of arterial intima in the expression of MMP-9 positive cells, NAC group of positive cells (6.00±2.45) than the control group (20.08±5.17) (p<0.05), statistically significant; measured the number of apoptotic cells, NAC group number of apoptotic cells (7.80±1.07%) than the control group (9.22±0.84%) (p<0.05), statistically significant; NAC group plasma ox-LDL levels (9.89±1.33 µg/dl) than the control group (15.84±2.60 µg/dl) (p<0.05), NAC group plasma MMP-9 levels (8.50±1.58 ng/ml) than the control group (12.44±2.19 ng/ml) (p<0.05), were statistically significant.

Conclusion NAC can reduce the number of atherosclerotic plaque, plaque distribution area, reducing the number of foam cells, reducing the volume of foam cells in plaques, plaque lipid core volume; NAC can reduce the arterial endometrial expression of MMP-9 in the number of positive cells; NAC can reduce atherosclerotic plaques in the number of apoptotic cells; NAC can reduce plasma ox-LDL and MMP-9 levels. This experiment further confirmed the NAC's anti-atherosclerosis effect of NAC as an anti-atherosclerosis drugs used clinically to provide an experimental basis.

e0215 **ENHANCED EXTERNAL COUNTERPULSATION PROTECTS VASCULAR ENDOTHELIAL CELLS FROM APOPTOSIS IN HYPERCHOLESTEROLEMIC PIGS**

doi:10.1136/hrt.2010.208967.215

Yan Xiong. *Division of Emergency Medicine, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China*

Objective Evidences have proved that Enhanced external counterpulsation (EECP) improves endothelial dysfunction and repairs

intimal damage by increasing vascular endothelial shear stress. Based on the assumption that unbalanced apoptosis of vascular endothelial cells (VECs) may have played a pivotal role in the pathogenesis of atherosclerotic lesions, we hypothesised that long-term EECP protects VECs from apoptosis in hypercholesteronemic pigs.

Methods 18 male domestic pigs were randomly assigned to three groups: one normal control group with a normal diet (Normal, n=6) and two hypercholesterolemic groups (HC, n=12) fed with atherosclerosis-inducing cholesterol-rich chow diet, one of which received enhanced external counterpulsation (HC, HC+EECP, n=6, respectively). Pigs in the HC+EECP group were treated with EECP for 2 h every other day for a total of 36 h. In the end of the study, the animals were sacrificed, and the thoracic and abdominal aortas harvested. The thoracic aortas were sampled for both scanning and transmission electronic microscopy whereas the abdominal aortas were stained in Sudan-III of fatty streak for macroscopic evaluation. Meanwhile, vascular endothelial cells (VECs) were isolated from the thoracic aorta by the use of collagenase. TUNEL method was used to detect the apoptotic index of VECs. At the same time, the abdominal aortas were collected for histopathological studies.

Results Fatty streaks or plaques, positively stained by Sudan dye, were hardly found in the normal group but clearly observable in the HC group. And among the cholesterol-diet animals, atherosclerotic lesions were much less severe in the EECP group than in the HC group. Scanning electronic microscopic analysis revealed that aortic VECs were irregularly arrayed, markedly desquamated, and shrank into smaller size, which indicated apoptotic events resulting in remarkable damage of endothelium in HC group. In contrast, the VECs in HC+EECP group were arrayed in a relatively streamline fashion, less desquamated and shrank, and manifested comparatively mild endothelial damage. Transmission electronic microscopic examination of aortas in HC group showed desquamated VECs loosely attached to the matrix along with foam cells, which indicated intimal damage. Apoptotic VECs at early, middle, late stage and even apoptotic bodies were visible on intimal surface. Nonetheless, these changes were relatively mild in EECP-treated animals. Meanwhile, the apoptotic index in the HC+EECP group was significantly lower than that of the HC group, but still higher than that of the Normal group ((177±12)%, (237±23)%, (127±36)%, respectively, p<0.05).

Conclusions EECP alleviates hypercholesterolaemia-induced atherosclerotic damage to the vascular intima and endothelium, and protects vascular endothelial cells from apoptosis, thereby delaying the progression of early atherosclerotic lesions. The therapeutic benefit of EECP in terms of endothelial protection may be attributed to the inhibition of VEC apoptosis.

e0216 **THE EXPRESSION AND ROLE OF INTERLEUKIN-23 IN MICE VIRUS MYOCARDITIS**

doi:10.1136/hrt.2010.208967.216

Fan Yang, Weifeng Wu, Yuluan Yan, Qing Kong, Yu Pang. *The First Affiliated Hospital of Guangxi Medical University, Nanning, China*

Objective To explore the expression of IL-23 and its role in acute viral myocarditis mice induced by coxsackie virus B3 (CVB3).

Methods To establish the model of VMC, Balb/c male mice were peritoneally injected (IP) with Coxsackievirus B3, mice peritoneally injected with PBS were taken as the controls. On 0, 1, 2, 3, 4 and 6W after IP, haematoxylin-eosin was used to assess pathological changes. The level of IL-23 mRNA in mice myocardic tissue was determined by RT-PCR. The expression of IL-23 protein in blood plasma was evaluated by EILSA.

Results Comparing with the controls, the expression of IL-23 mRNA were steady high from 1W after IP, and maintaining a higher trend until 6W. All the results at different time were higher than

those of controls, $p < 0.05$. The expression of IL-23 protein were also higher than those of controls, which in concordance with the changes of mRNA.

Conclusions Our data show that local significantly increased levels of IL-23p19mRNA in myocardium and IL-23 may play a role in the pathogenesis of mice virus myocarditis.

e0217 **STUDY ON THE PROTECTIVE EFFECT OF THE MIXTURE OF SHENGMAI PULVIS AND DANSHEN DECOCTION ON THE MYOCARDIUM OF TYPE 2 DIABETIC CARDIOMYOPATHY IN RATS MODELS**

doi:10.1136/hrt.2010.208967.217

¹Niqing, ²Wangjie, ²Zhao An-bin, ²Yu Bin, ²Wang Min, ²Huang Chun-rong, ²Li En-qing.
¹Guang'anmen Hospital of China Academy of Chinese Medical Science, Beijing, China;
²School of Medicine, Jinan University, China

Objective To study the effect of the Mixture of Shengmai Pulvis and Danshen Decoction in protecting rats of the type 2 diabetic cardiomyopathy (DCM) model.

Methods 42 SD rat models of DCM, established by combination of insulin resistance by a high-fat diet with intraperitoneal injection of high dose streptozotocin (50 mg/kg), were evaluated in the damage of the myocardium by ECG at the twelfth week after modelling, and the serum are analysed for blood glucose (GLU), cholesterol and triglyceride (TG); the content of the left cardiac ventricle myocardial collagen was quantified by Masson staining test; the level of myocardial cell apoptosis was tested with Tunel apoptosis kit; the damage extent of the myocardial subcellular structure was observed by electron microscopy; the expression levels of cardiac TSP-1, TGF- β 1 and TRB-3 proteins were detected by immunohistochemistry, the changes of the expression levels of the cardiac TSP-1, A-TGF β 1 and L-TGF- β 1 protein were detected by Western blotting; and the changes of the mRNA expression levels of TSP-1 and TRB-3 were detected by real-time quantitative PCR.

Results Compared with the control group, the rat blood glucose, cholesterol, triglycerides were significantly decreased; the myocardial tissue was less damaged and the collagen fibre content was reduced in the group of the Mixture of Shengmai Pulvis and Danshen Decoction; The myocardial sub-cellular structural damage in electron microscopy was to a lesser extent, the expression levels of the myocardial TSP-1, TGF- β 1 and TRB-3 by immunohistochemical detection and the average expression levels of the myocardial TSP-1, A-TGF β 1 and L-TGF- β 1 by Western blotting were decreased; and the expression levels of TSP-1mRNA and TRB-3 mRNA by PCR detection were decreased than those of the control group.

Conclusion The Mixture of Shengmai Pulvis and Danshen Decoction can inhibit through multiple pathways the process of myocardial fibrosis in the rat myocardium of diabetic cardiomyopathy, and significantly delay the formation course of diabetic cardiomyopathy in hyperglycemia rats.

e0218 **BIOLOGICAL CHARACTERISTICS RESEARCH OF STENT COATING WITH ZEDORAY CONSTITUENTS IN A PORCINE MODEL**

doi:10.1136/hrt.2010.208967.218

Fu-Hai Zhao, Da-Zhuo Shi, Jian-Gang Liu, Pei-Li Wang, Da-Wu Zhang, Lei Zhang, Jian-Peng Du. Cardiovascular Center of Xiyuan Hospital Affiliated to China Academy of Chinese Medical Sciences, Beijing, China

Background In-stent restenosis is caused by neointimal hyperplasia, which involves abnormal growth of vascular smooth muscle cells

(VSMC). Zedoray constituents is known to inhibit smooth muscle cell hyperplasia and migration, while inhibit ADP induced platelet aggregation.

Objective To evaluate the biological characteristics of stents coating with zedoray constituents in porcine coronary model.

Methods Bare metal stents (BMS, n=36), Sirolimus eluting stents (SES, n=36) and Zedoray eluting stents (ZES, n=36) were implanted in the proximal segment of three different epicardial coronary arteries in 36 swines randomly. Coronary angiography, optical coherence tomography (OCT) and histomorphologic analysis were performed at 30 days and 90 days after the procedure.

Results The 30 day (n=24) OCT examination showed ZES arm has larger lumen diameter (LD), acceptable mean lumen stenosis of area (MSA) compared with BMS (LD: ZES 1.9 ± 0.51 mm, SES 1.85 ± 0.41 mm, BMS 1.1 ± 0.3 mm, $p < 0.05$; MSA%: ZES 21.7 ± 19.3 , SES 23.2 ± 18.9 BMS 41.7 ± 21.3 , $p < 0.001$). By histomorphologic analysis, similar injury scores were observed at the three arms ($p > 0.05$). However, significant inflammation score reduction was seen in ZES group (ZES: 0.65 ± 0.54 , SES: 1.03 ± 0.44 , BMS: 0.94 ± 0.75 , $p < 0.001$) compared to other two groups at 30 day, no differences in three groups at 90 day. Either at 30 day or 90 day, by qualitative analysis, well developed endothelium was seen in ZES arm, while impaired endothelium was observed with part of stent strut naked at vessel lumen at SES arm.

Conclusion Zedoray eluting stents can reduce neointimal hyperplasia with good endothelia coverage in porcine coronary model.

e0219 **STUDY ON THE ROLE OF CD4+CD25+TREG ON ATHEROSCLEROSIS IN APOE-/- MICE**

doi:10.1136/hrt.2010.208967.219

Xuemei Li, Yujie Li, Dongdan Zheng, Hao Tang, Mingxiang Wen. The First Affiliated Hospital, SUN Yat-sen University, Guangzhou, China

Objective To investigate the mechanism of CD4⁺CD25⁺treg in atherosclerosis in ApoE^{-/-} mice.

Methods 28 weeks-old male ApoE^{-/-} mice were randomly divided into two groups feeding high-fat diet (AH) or normal diet (AN). Ten C57BL/6J male mice feeding normal-diet (BN). After 12 weeks, the whole aorta from the root to crotch of iliac artery was separated and the whole blood were centrifugated to get the serum. Paraffin sections of aorta were stained with H&E and morphometric parameters were measured using Image Pro Plus 6.0 system. Immunohistochemical stain was performed to verify the expression of the Foxp3⁺ and CD25⁺ cells in atherosclerotic tissue (%). The ratio of CD4⁺CD25⁺/CD4⁺ in spleen was calculated by Flow Cytometry. The serum concentration of TGF- β 1, IL-10 were detected by ELISA. SPSS13.0 was used to analyse the data. All values were expressed as Mean \pm SD. Independent-Samples T Test was applied to compare two samples of quantitative data after testing their normality. $p < 0.05$ was considered statistically significant.

Results There were advanced atherosclerotic plaques in ApoE^{-/-} mice, but in C57BL/6J. The thickness of intima (μ m), plaque area (μ m²), plaque/lumen ratio in AN group were significantly higher than in BN group (12.24 ± 1.34 vs 7.33 ± 2.23 , 600265.4 ± 263876.25 vs 0 , 15.93 ± 5.45 vs 0 , all $p < 0.01$). So did in AH group than that in AN group (all $p < 0.05$). The percentage of splenic CD4⁺CD25⁺treg (%) and the serum concentration of TGF- β 1, IL-10(pg/ml) in AN group were significantly decreased than in BN group (9.4 ± 4.00 vs 13.8 ± 3.97 , 116.05 ± 32.27 vs 191.27 ± 95.27 , 41.83 ± 16.15 vs 61.84 ± 23.05 , all $p < 0.05$). These in AH group significantly reduced than in AN group (5.8 ± 1.51 vs 9.4 ± 4.00 , 83.97 ± 33.45 vs 116.05 ± 32.27 , 27.50 ± 11.54 vs 41.83 ± 16.15 , all $p < 0.05$). There were no Foxp3⁺ and CD25⁺ cells in plaques-free intima in BN group. The expression of Foxp3, CD25⁺ cells were significantly decreased in AH group than in AN group (1.28 ± 1.20 vs 3.04 ± 1.92 , 2.00 ± 1.39 vs 3.98 ± 1.67 , all $p < 0.05$).