

**Methods** 40 male BABL/c mice were divided into two groups, the DCM group and the control. The DCM group mice were peritoneal injected Coxsackievirus B3 (CVB3) monthly. After 180 days, all mice were sacrificed and IL-17 mRNA of splenocytes were measured by RT-PCR.

**Results** In the DCM mice, the heart weight was higher, and the ventricular wall was thinner than the control, and fibrosis in hearts were observed. IL-17 mRNA of splenocytes in DCM mice could be detected and the controls' were zero ( $0.15 \pm 0.04$  vs  $0.00 \pm 0.00$ ,  $p < 0.01$ ).

**Conclusion** We successfully built murine DCM model by monthly peritoneal injection of CVB3 for 180 days in the DCM group. In the DCM mice, the heart weight was higher, and the ventricular wall was thinner than the control, and fibrosis in heart was observed. The mRNA levels of IL-17 were promoted in Coxsackievirus induced DCM mice. This result suggested that IL 17 which secreted by Th17 subset could be detected in DCM mice, it seems that the Th17 cells might differentiated in DCM mice.

### e0152 ENDOGENOUS $\kappa$ OPIOID PEPTIDE MEDIATES THE CARDIOPROTECTION INDUCED BY ISCHAEMIC POSTCONDITIONING

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**Aim** Postconditioning is brief cycles of reperfusion and ischaemia during the early phase of reperfusion following a prolonged ischaemic insult. Opioids are well-known endogenous triggers of preconditioning. Because postconditioning shares the protective pathways with preconditioning, G protein-coupled receptor activation may serve as an essential mechanism that triggers protection of postconditioning. Receptor binding studies showed that  $\kappa$  opioid receptor ( $\kappa$ -OR) is a predominant opioid receptor in heart. Therefore, we determined whether endogenous agonist of  $\kappa$ -OR, dynorphin, triggers postconditioning, especially reduces apoptosis of I/R myocardium and to identify its underlying mechanism.

**Methods** Besides the vehicle, the other SD rats underwent a 30 min left anterior descending occlusion followed by 120 min of reperfusion with or without a postconditioning stimulus (three cycles of 10 s reperfusion and 10 s reocclusion) initiated at the onset of reperfusion. The selective  $\kappa$  opioid receptor antagonist nor-binaltorphimine (Nor-BNI, 2 mg/kg, intravenously), administered 5 min before the reperfusion. The blood plasma was analysed spectrophotometrically for determination of CK and LDH levels. Myocardial apoptosis was quantitatively analysed by detection of TUNEL with an apoptosis detection kit. Six fields from the perinfarct zone were analysed and the number of TUNEL positive cardiomyocytes was counted on 400 high power fields. Immunoreactive Dynorphin were measured by an antigen competitive ELISA.

**Results** CK (U/L) and LDH (U/L) were significantly higher in I/R group than those in the control ( $3401 \pm 251$  vs  $689 \pm 76$ ,  $2329 \pm 216$  vs  $753 \pm 97$ ,  $p < 0.01$ ). Postconditioning significantly reduced the release of CK and LDH from I/R myocardium ( $2026 \pm 268$  vs  $3401 \pm 251$ ,  $1543 \pm 169$  vs  $2329 \pm 216$ ,  $p < 0.01$ ). These reduction were abolished by nor-BNI ( $p < 0.01$ ). Regional myocardial I/R resulted in a significant increase in cardiomyocyte apoptosis ( $18.7 \pm 2.5$  vs  $1 \pm 0.25$ ,  $p < 0.01$ ). Postconditioning exerted a significant anti-apoptotic effect ( $10.4 \pm 1.3$  vs  $18.7 \pm 2.5$ ,  $p < 0.01$ ). This protective effect was attenuated by pretreatment with Nor-BNI ( $p > 0.05$ ). Immunoreactive dynorphin content (pg/ml) in serum significantly increased after postconditioning ( $78.5 \pm 12$  vs  $37.3 \pm 6.5$ ,  $p < 0.01$ ). Increased dynorphin did not reduced by  $\kappa$  opioid receptor antagonist Nor-BNI ( $p > 0.05$ ).

**Conclusions** We find that cardiac protection and anti-apoptotic effect of postconditioning is mediated by activating  $\kappa$  opioid

receptor. And cardiac protective and anti-apoptosis effect of postconditioning is mediated by enhanced dynorphin express in rats. Recently, clinical use of postconditioning as a treatment for cardiovascular disease has been an increasing attention, and opioid receptor triggers postconditioning, so the study of the relationship between  $\kappa$  opioid receptor and ischaemia reperfusion injury (IRI) may provide a new insight for the curing of IRI.

### e0153 EFFECT OF PLATELET MICROPARTILES ON THE EXPRESSION OF CELL ADHESION MOLECULE IN ENDOTHELIAL CELL

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**Objective** To examine the expressions of cell adhesion molecule (E-selectin, VCAM-1, ICAM-1) in HUVECs (CRL-1730), which is affected by platelet microparticles (PMPs). To investigate the effects of platelet microparticles in coronary heart disease.

**Methods** 1. PMPs was extracted from anticoagulated blood with sodium citrate. The purity of PMPs was measured by flow cytometry. 2. The prepared PMPs and CRL-1730 cell were co-cultured. The first part was divided into five groups based on the concentration of PMPs. The concentration were: 0, 10  $\mu$ g/ml, 30  $\mu$ g/ml, 50  $\mu$ g/ml, 100  $\mu$ g/ml, each group contained four wells. Cells in wells were collected after 4 h. The second part was divided into three groups based on the time of co-cultivation: 2 h, 4 h, 24 h, and the concentration of PMPs that were added into each wells was 50  $\mu$ g/ml. Each group contained four wells. Cells in wells were collected after 2 h, 4 h, 24 h respectively. 3. The RNA of cells was extracted. Semi-quantitative reverse transcription-PCR (SQRT-PCR) was used to detect the relative expression of E-selectin, ICAM-1 and VCAM-1 respectively.

**Results** In this study, we found that cultured HUVEC (CRL-1730) expressed E-selectin, ICAM-1 and VCAM-1 mRNA in basic states. The expressed levels of E-selectin, ICAM-1 and VCAM-1 were increased when HUVEC (CRL-1730) were interfered by PMPs of certain concentration ( $p < 0.05$ ). But the PMPs stimulated HUVECs (CRL-1730) at different times, the expressions of E-selectin, ICAM-1 and VCAM-1 were of no difference ( $p > 0.05$ ).

**Conclusions** 1. The high purity of PMPs were successfully prepared in the study. 2. The PMPs may increase the expressions of E-selectin, ICAM-1 and VCAM-1 on HUVEC (CRL-1730). It may explain a possible mechanism of PMPs in coronary heart disease.

### e0154 EXPRESSION OF TUMOUR NECROSIS FACTOR- $\alpha$ CONVERTING ENZYME AND TUMOUR NECROSIS FACTOR- $\alpha$ IN RATS WITH ALCOHOLIC CARDIOMYOPATHY

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**Purpose** Chronic excessive consumption of alcohol causes ventricular remodelling and eventually leads to alcoholic cardiomyopathy (ACM). Tumour necrosis factor- $\alpha$  converting enzyme (TACE) has been identified to cleave membrane-bound tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) to soluble TNF- $\alpha$ , which has crucial roles in ventricular remodelling. This study aimed to investigate the expression of TACE and TNF- $\alpha$ , and their impacts on ventricular remodelling in rats with ACM.

**Methods** 50 healthy male Wistar rats were randomly divided into a control group (n=20) and an ACM group (n=30). Animals in the ACM group were given 10% alcohol ad libitum as the drinking

water and 60% alcohol (5 ml/kg once per day) by intragastric administration in the first week; 10% alcohol ad libitum as the drinking water and 60% alcohol (10 ml/kg twice per day) by intragastric administration in the second week; 20% alcohol ad libitum as the drinking water and 60% alcohol (15 ml/kg twice per day) by intragastric administration from week 3 to week 16; and 30% alcohol ad libitum as the drinking water and 60% alcohol (15 ml/kg twice per day) by intragastric administration from week 17 to month 6. Animals in the control group received purified drinking water in the same regimen with alcohol treatment. Before and 6 months after initiating the study, left ventricular end diastolic diameter (LVEDD), left ventricular ejection fraction (LVEF), and fractional shortening (FS) were assessed by echocardiography. Six months after the study started, histopathology and ultrastructure of myocardium were examined with light and electron microscopy; mRNA expression of TACE was evaluated by real-time PCR; and protein expression of TACE and TNF- $\alpha$  was analysed using immunohistochemistry and western blot, respectively.

**Results** Following 6 months of alcohol feeding, LVEF and FS were reduced ( $p < 0.05$  for all), while LVEDD was augmented in the ACM group ( $p < 0.05$ ), as compared with the control group. Severe changes in cardiac structure were also seen in the ACM group. The mRNA and protein expression of TACE and the protein expression of TNF- $\alpha$  were up-regulated in the ACM group in comparison with the control group ( $p < 0.05$  for all). In both groups, the protein expression of TACE positively correlated with that of TNF- $\alpha$  ( $p < 0.01$ ) and LVEDD, whereas it negatively correlated with LVEF ( $p < 0.05$ ).

**Conclusions** TACE is over-expressed in the ventricle of ACM rats, and may involve in the process of ventricular remodelling via cleaving TNF- $\alpha$ . Therefore, TACE may represent a new therapeutic target in the prevention and treatment of ventricular remodelling in ACM.

#### e0155 SMOKING TREATMENT INCREASE SERUM AGES LEVEL AND HAVE EFFECTS ON EXPRESSION OF ICAM-1 IN VASCULAR ENDOTHELIAL CELLS OF RAT

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**Objective** To investigate whether smoking can increase serum advanced glycosylation end products (AGEs) level and have effects on expression of intercellular cell adhesion molecule-1 (ICAM-1) in vascular endothelial cells of rat.

**Methods** Male SD rats ( $n=138$ ) were randomly assigned to five groups according to duration of smoking treatment: 2-week group, 4-week group, 6-week group, 8-week group, smoking cessation group. The rats of following groups, that is 2w, 4w, 6w and 8w groups were further randomly divided into five subgroups according to intervening condition: control subgroup, smoking treatment for 1 h/per day subgroup, smoking treatment for 0.5 h/per day subgroup, aminoguanidine hydrochloride subgroup, puerarin subgroup; the rats of smoking cessation group were further randomly divided into three subgroups according to duration of smoking cessation: smoking cessation 2 weeks subgroup, smoking cessation 4 weeks subgroup and smoking cessation 6 weeks subgroup. The rats of 2w, 4w, 6w and 8w groups were sacrificed after smoking treatment for 2, 4, 6 and 8 weeks respectively; the rats of smoking cessation group were sacrificed after smoking treatment for 8 weeks and then cease-smoking for 2, 4, and 6 weeks respectively. Serum AGEs levels of each rat were assayed by fluorescent method. ICAM-1 mRNA and protein of vascular endothelial cells were determined by semiquantitative RT-PCR (Reverse transcription PCR) and immunohistochemistry.

**Results** Serum AGEs levels of all SM1 subgroups rats were increased after smoking treatment for 2 weeks ( $p < 0.01$ ), and reached peak at 4 weeks ( $p < 0.001$ ), then declined at 6 weeks and 8 weeks, but did not recover back to normal level; the increasing trend was depressed by aminoguanidine hydrochloride and puerarin. Levels of serum AGEs declined in smoking cessation rats, and were significantly lower at 4 weeks than those before smoking cessation ( $p < 0.001$ ). With the increased duration of smoking, ICAM-1 mRNA and protein of vascular endothelial cells were up-regulated, both aminoguanidine hydrochloride and puerarin depress the up-regulation. The expression of ICAM-1 mRNA and protein of vascular endothelial cells also declined after smoking cessation, and they were significantly lower in rats of smoking cessation of 4 weeks subgroup than those before smoking cessation ( $p < 0.05$ ).

**Conclusions** Smoking treatment increase serum AGEs level in rat. Cigarette-induced AGEs play roles in the augmented expression of ICAM-1 in vascular endothelial cells of rat with smoking treatment. Aminoguanidine hydrochloride, puerarin and smoking cessation contribute to the decrease of serum AGEs level and the expression of ICAM-1 in vascular endothelial cells of rat.

#### e0156 UROTENSIN II PROMOTES MONOCYTE CHEMOATTRACTANT PROTEIN-1 EXPRESSION IN AORTIC ADVENTITIAL FIBROBLASTS OF RAT

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**Background** Recent studies reported that vascular adventitial fibroblasts (AFs) are involved in the development of vascular inflammatory diseases, such as atherosclerosis. Urotensin II (UII), a potent vasoconstrictive peptide, could stimulate phenotype differentiation and proliferation of the AFs. The goal of this study was to investigate the effect of UII on the expression of monocyte chemoattractant protein-1 (MCP-1) in rat aortic AFs, and to study the signal transduction pathways of it.

**Methods** Growth-arrested AFs were incubated in serum-free medium with UII ( $10^{-10}$ – $10^{-7}$  mol/l). In order to explore the mechanism of UII effect, the cells were pretreated with some inhibitors of signal transduction pathways for 30 m, and then incubated with UII ( $10^{-8}$  mol/l) for 3 h to 24 h. The MCP-1 mRNA and protein expression induced by UII were evaluated by the reverse transcriptase PCR and Western Blotting, respectively. The MCP-1 secretion from the cells was determined by ELISA.

**Results** UII could upregulate MCP-1 expression significantly. The MCP-1 mRNA expression increased after 1 h ( $p < 0.05$ ) of UII ( $10^{-8}$  mol/l) treatment and reached a peak at 3 h ( $p < 0.01$ ). It then declined from 6 to 24 h, and there are no significantly differences from 0 h group. UII dose-dependently induced MCP-1 mRNA expression, with maximal effect at a concentration of  $10^{-8}$  mol/l at 3 h ( $p < 0.01$ ). The MCP-1 mRNA expression was increased by 70.10%, 109.65%, 189.73% and 122.99% in  $10^{-10}$  mol/l,  $10^{-9}$  mol/l,  $10^{-8}$  mol/l and  $10^{-7}$  mol/l group, respectively, as compared with the control group (without UII stimulation), and the upregulation was significant ( $p < 0.01$  in all groups). The effect of UII was inhibited significantly by the UII receptor antagonist SB710411 ( $10^{-6}$  mol/l), Rho protein kinase inhibitor Y27632 ( $10^{-5}$  mol/l), protein kinase C inhibitor H7 ( $10^{-5}$  mol/l), mitogen-activated protein kinase inhibitor PD98059 ( $10^{-5}$  mol/l), calcineurin inhibitor Cyclosporine A ( $10^{-5}$  mol/l) and  $Ca^{2+}$  channel blocker nifedipine ( $10^{-5}$  mol/l), ( $p < 0.01$  in all groups). In addition, UII also induced protein expression and secretion of MCP-1 in the cells, both in