## e0136 ULTRASTRUCTURAL CHANGES AND EXPRESSION OF ENOS IN MYOCARDIAL CAPILLARY ENDOTHELIAL CELLS IN RATS WITH HYPERTENSION AND DIABETES MELLITUS

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**Introduction** The percentage of angina and cardiac dysfunction and cardiovascular events increased significantly in hypertensive diabetes mellitus compared with essential hypertension and type 2 diabetes mellitus alone. Hypertension and diabetes are often associated with cardiac microvascular abnormalities. The major cardiac microcirculation alterations are the capillary rarefaction and endothelial cell functional impairment, which may be in the development of coronary insufficiency. We presume that the more serious injury in cardiac microvascular structure and function followed by hypertensive diabetes mellitus.
**Aims** The purpose of the present study is to investigate ultrastructural changes and expression of eNOS in rat myocardial capillary endothelial cells as a response to hypertension with diabetes mellitus.
**Methods** The rat model of hypertension with diabetes mellitus (SHDM) and the rat model of diabetes mellitus (DM) alone were induced by an intraperitoneal injection of streptozotocin combined with high fat diet in spontaneously hypertensive rats (SHR) and SD rats, respectively. The groups were as follows: SD, DM, SHR, and SHDM. After 16 weeks, ultrastructure of the myocardial capillaries was examined by transmission electron microscopy and the expression of endothelial nitric oxide synthase (eNOS) was detected by immunohistochemistry.
**Results** Ultrastructural alterations of myocardial capillaries in SHR, DM and SHDM were oedematous endothelial cell, endothelial protrusions into lumen and narrowed /or irregular lumen respectively. These alterations indicate that cell injury was more significant in the myocardium of DM and SHDM. Thickening of basal lamina in myocardial capillaries with SHR was less considerable compared with SD (31.3±4.2 nm vs 28.6±3.6 nm, $p>0.05$), while thickening was a prominent feature of DM (46.6±5.3) nm and SHDM (51.5±4.6) nm (compared with SD or SHR, $p<0.01$). The expression of eNOS in myocardial tissue was significantly lower in SHDM than in SHR or DM.
**Conclusions** These results suggest that a combination of hypertension and diabetes mellitus enhanced the damage of myocardial capillaries endothelial cell ultrastructure and function more than either hypertension or diabetes mellitus alone.

## e0137 ASSOCIATION BETWEEN LOCAL AND SYSTEMIC LEVELS OF INTERLEUKIN-1β AND INTERLEUKIN-10 IN CORONARY ARTERY DISEASE AND ITS CLINICAL RELEVANCE

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**Objective** To explore whether the systemic level of pro-inflammatory factor interleukin-1β (IL-1β) and anti-inflammatory factor IL-10 can reliably reflect their local levels at atherosclerosis lesions in patients with coronary artery disease (CAD).
**Method** 18 consecutive patients with stable angina pectoris (SAP), 21 consecutive patients with unstable angina pectoris/non-ST-segment elevation myocardial infarction (UA/NSTEMI), 30 consecutive patients with ST-segment elevation myocardial infarction (STEMI) and 30 health patients as control group. Systemic samples were obtained from aorta root in all patients (n=99), local samples from distal of the coronary lesion in patients with CAD (n=69), and samples from coronary sinus of 15 patients with STEMI.
**Results** No significant difference at systemic level of IL-1β among the four groups ($p=0.251$), increased the systemic level of IL-10 in STEMI group than the control group ($p=0.001$), and no difference between the local and the systemic level in SAP group ($p=0.864$, 0.545). In the UA/STEMI group, the local level of IL-10 was increased compared with systemic level ($p=0.043$), but no difference of IL-1β was found ($p=0.264$). The local levels of IL-1β and IL-10 were both increased compared with systemic level in the STEMI group ($p=0.003$, 0.029 respectively). The level of IL-1β in coronary sinus tended to be decreased compared with culprit lesion ($p=0.062$), but the IL-10 showed no great changes ($p=0.743$).
**Conclusions** The systemic level of pro-inflammatory marker IL-1β and anti-inflammatory maker IL-10 could not complete reliablyreflect the local changesin patients with CAD. These findings may promote our understanding regarding pathogenesis of CAD and implications of findings from previous clinical observations.

## e0138 AT1 RECEPTOR BLOCKER VALSARTAN ATTENUATES LEFT VENTRICULAR REMODELLING AND FAILURE IN A RAT MODEL OF ADRIAMYCIN-INDUCED DILATED CARDIOMYOPATHY BY UPREGULATING THE EXPRESSION OF ANGIOTENSIN-CONVERTING ENZYME 2 AND ANGIOTENSIN-(1−7)

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**Introduction** Adriamycin (ADR) is an effective antineoplastic agent whose use has been limited by its cardiotoxic side effects. Angiotensin-converting enzyme 2 (ACE2) has emerged as a novel regulator of cardiac function by converting angiotensin II into angiotensin-(1−7). Studies suggest that ACE2 and Ang-(1−7) may play a critical role in the regulation of cardiac function. This study is to investigate the effect of AT1 receptor blocker valsartan on the expression of ACE2 and Ang (1−7) in a rat model of adriamycin-induced dilated cardiomyopathy.
**Methods** Weight-matched Adult male Wistar rats were randomly divided into three groups as follows: 1. the ADR group, in which 2.5 mg/kg of ADR was weekly injected via a tail vein for 10 weeks (n=25); 2) concomitant AT1 receptor blocker valsartan and ADR, in which valsartan was administered by daily gavage at a dose of 30 mg?kg-1?day-1(n=10); 3. the control group (n=10). Haemodynamics and echocardiographic measurements were obtained at 12 weeks after treatment. The plasma concentrations of Ang II and angiotensin (1−7) were determined by immunoradiometric assay. The expression of ACE2 in left ventricular myocardium was investigated by Immunohistochemistry. The expression of collagen in LV were investigated with Van Gieson staining techniques.
**Results** LV cavity dilatation was significantly attenuated in ADR-induced dilated cardiomyopathy rats given valsartan. Valsartan partially normalised LV contractile function, which was significantly reduced in ADR rat. The plasma concentrations of Ang II were higher in the ADR group than the control group ($p<0.01$), which was further increased by valsartan ($p<0.01$). The plasma concentrations of ang (1-7) was higher in the ADR group than the control group, valsartan treatment further increased the plasma concentrations of ang (1-7) by 1.5-fold ($p<0.01$). The expression of ACE2 was increased in the ADR group, and was further significantly augmented in valsartan treated rats. Myocardial fibrosis occurred significantly in ADR group, which was partly reversed by valsartan treatment ($p<0.01$).

**Conclusion** Pretreatment with the AT1 receptor blocker valsartan can attenuate left ventricular remodelling and failure in a rat model of adriamycin-induced dilated cardiomyopathy by upregulating the expression of angiotensin-converting enzyme 2 and angiotensin-(1–7).

## e0139 THE CARDIOMYOGENIC POTENTIAL OF CARDIAC STEM CELLS IN AN IN VITRO COCULTURE SYSTEM

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Features so far documented in reliably isolated and manipulated cardiac stem cell (CSCs) resident within the cardiac tissue suggest that innovative treatments to repair damaged myocardium could have promising clinical applications. However, the capability of resident cardiac stem cell (CSCs) to differentiated into newly formed cardiomyocyte is still controversial. In this study, the cardiomyogenic potential of c-kit+ CSCs isolated from normal adult mouse hearts was evaluated in an in vitro co-culture system.
**Methods** Magnetic activated cell sorting (MACS) was used to prepare c-kit+ cells from the hearts of ACT-EGFP/MHC-nLAC double transgenic mice. These animals exhibit widespread enhanced green fluorescent protein (EGFP) expression and cardiomyocyte-restricted nuclear b-galactosidase activity, thus permitting simultaneous tracking of cell survival and differentiation. The c-kit+ cells were cocultured with feeder layer neonatal rat cardiomyocytes (NRCMs) for 7 days. Confocal and fluorescence microscopes were used to quantify the differentiation rate of c-kit+ cells in the immunostained cocultures.
**Results** A subset of the c-kit+ cells underwent cardiomyogenic differentiation when cocultured with NRCMs (0.15% out of all 70, 747 EGFP+ cells screened), but not when cultured alone or when cocultured with mouse fibroblasts (0.00% of the EGFP+ cells screened). The newly formed cardiomyocytes were EGFP+, nuclear b-galactosidase+ and a-actinin+ with clear sarcomere structure, indicating mature functional properties.
**Conclusion** Normal adult c-kit+ CSCs are able to differentiate into functional cardiomyocytes, but it is a rear event at least in the in vitro coculture system.

## e0140 ENDOPLASMIC RETICULUM STRESS INDUCED-APOPTOTIC MODEL BY TUNICAMYCIN IN CULTURED NEONATAL RAT CARDIOMYOCYTES

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**Objective** To establish endoplasmic reticulum stress induced-apoptotic model by tunicamycin in cultured neonatal rat cardiomyocytes.
**Methods** Neonatal rat cardiomyocytes in primary culture were exposed to tunicamycin of different concentrations. MTT assay and flow cytometry analysis were applied to measure cardiomyocyte viability. Western blot was used to examine the expression levels of GRP78 and CHOP.
**Results** Cell viability was time and concentration-dependently decreased. The treatment of tunicamycin produced 42.8±5.8% of apoptotic population in cardiomyocytes. The levels of GRP78 and CHOP significantly upregulated at 6 h. After tunicamycin treatment for 24 h, the upregulation of GRP78 and CHOP reached the maxium.
**Conclusion** We successfully constructed tunicamycin-induced apoptotic model in cultured neonatal rat cardiomyocytes. The optimal concentration and time of tunicamycin treatment was 100 ng/ml, 72 h, respectively.

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## e0141 THE EFFECTS AND MECHANISMS BETWEEN DIDS AND EDRV ON ACUTE ISCHAEMIA-REPERFUSION INJURY MYOCARDIUM

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**Aim** To investigate the effects and Mechanisms of chloride channel inhibitor, 4, 4′- Diisothiocyanostilbene-2, 2′- disulfonic acid (DIDS) and free radical scavenger, Edaravone (EDRV) on myocardial ischaemia/reperfusion injury (I/RI)in vivo.
**Methods** Male Sprague-Dawley rats, subjected to 30 min of myocardial ischaemia and 4 h of reperfusion, were divided into five groups: sham group, I/RI group, DIDS group, EDRV group and DIDS+EDRV group. Left ventricular systolic pressure (LVSP), The maximal first derivative of developed pressure (±dp/dtmax), Myocardial infarction size, serum creatine kinase (CK) activity, lactate dehydrogenase (LDH) activity, superoxide dismutase (SOD) activity, malondialdehyde (MDA) concentration, myocardial apoptotic index, reactive oxygen species (ROS) were detected.
**Results** There were no statistical difference in heart rate in each animal suffering myocardial ischaemia/reperfusion compared with sham group ($p>0.05$, n=8). LVSP and $\pm dp/dt_{max}$ were decreased during the period of myocardial ischaemia except sham but there was no statistical difference ($p>0.05$, n=8), However, following reperfusion, the data showed that DIDS and EDRV significantly improved myocardial function in I/RI rats (n=8, $p<0.05$) and DIDS +EDRV combined administration had a much stronger cardioprotective effect than DIDS or EDRV did alone (n=8, $p<0.05$); the levels of activity of serum creatine kinase (CK) (n=8), lactate dehydrogenase (LDH) (n=8), myocardial infarction size (n=8), myocardial apoptotic index (n=6)showed that there were no statistical difference was observed between DIDS and EDRV groups, DIDS+EDRV treatment further decreased compared with DIDS or EDRV treatment alone ($p<0.05$) for the above-mentioned results; and the levels of the concentration of malondialdehyd (MDA), superoxide dismutase (SOD), reactive oxygen species (ROS), $O^{2-}$ and OH·showed that DIDS reduces free radical weaker than EDRV ($p<0.05$, n=8), and DIDS+EDRV combined administration had a stronger cardioprotective effect than DIDS or EDRV did alone and combined administration possesses synergies action ($p<0.05$, n=8).
**Conclusion** 1. DIDS and EDRV protect myocardium from MI/R injury via improving cardiac function, reducing infarct size and suppressing cardiomyocyte apoptosis; 2. The mechanisms of cardioprotective effects of DIDS and EDRV were involved in inhibition of ROS activity. The protective effect of combined administration can be further enhanced, suggesting DIDS protects ischaemia/reperfusion injury myocardium via other distinctive mechanisms except above.

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