persistent nitroglycerin treatment but terminated at least 4 h before ischaemia combined with intraperitoneal injection of ethanol (0.5 g/kg). Cardiac mitochondria was isolated after reperfusion. Three parameters were measured: (1) serum CK activity before ischaemia and after reperfusion; (2) after incubation with fluorescent probe JC-1, $\Delta\psi$ of isolated mitochondrial was evaluated by fluorometer and fluorescent microscope; (3) ALDH2 activity was determined spectrophotometrically by monitoring the reductive reaction of NAD^+ to NADH at 340 nm.

Results (1) Ethanol treatment caused a 27% increase in ALDH2 activity relative to control (2.74 ± 0.37 and 2.15 ± 0.46 , $p=0.044$); Treatment with persistent nitroglycerin decreased ALDH2 activity by 27% compared with ethanol treatment (2.00 ± 0.47 and 2.74 ± 0.37 , $p=0.024$). There was no statistical difference in ALDH2 activity between GTN-ON and control group (2.00 ± 0.47 and 2.15 ± 0.46 , $p=0.642$). (2) $\Delta\psi$ in cardiac myocytes in ethanol group and GTN-OFF group were 2.8 and 2.4 times higher than that in control respectively. $\Delta\psi$ in cardiac myocytes in GTN-ON group was reduced by 51% (119.33 ± 10.21 and 242.20 ± 52.18 , $p=0.006$) and 42% (119.33 ± 10.21 and 204.00 ± 86.17 , $p=0.045$) than that in ethanol and GTN-OFF group respectively. (3) Cardiac damage was reduced by 39% (0.37 ± 0.20 and 0.61 ± 0.12 , $p=0.029$) and 41% (0.36 ± 0.10 and 0.61 ± 0.12 , $p=0.031$) in ethanol group and GTN-OFF group compared with that in control.

Conclusions Acute activation of ALDH2 reduced myocardial ischaemia/reperfusion injury possibly by preserving $\Delta\psi$.

e0029 SURVIVAL OF TRANSPLANTED CELLS IN INFARCTED MYOCARDIUM AND EFFECT OF INDUCED NITRIC OXIDE SYNTHASE INHIBITOR

doi:10.1136/hrt.2010.208967.29

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Objective Cell transplantation was supposed to be a therapeutic strategy of myocardial infarction, but the problem was that most of the transplanted cells died at the early stage of transplantation. We hypothesised that over-activation of induced nitric oxide synthase (iNOS) may play an important role in the death of transplanted cells. In this experiment, we investigated the effect of iNOS selective inhibitor on the survival of transplanted bone marrow stem cells (BMSCs).

Methods BMSCs were isolated from the male SD rats. Female SD rats were subjected to myocardial infarction by ligation of left anterior descending artery. BMSCs at 1.2×10^6 were injected into the central zone of the infarct region at 1 h after ligation. BMSCs were monitored by labelled BrdU or Hoechst33342. A Sry gene sequence of Y chromosome in male rats was analysed by real-time PCR for the quantification of the survived BMSCs in host myocardium.

Results The cultured BMSCs showed a homogenous pattern with CD44+/CD34-. Accumulation of BMSCs labelled with Hoechst33342 or BrdU was noted in infarct area of the host heart. Myocardial infarction induced a significant iNOS expression with its peak expression on day 3 and lasting for 14 days. The survival rates of engrafted BMSCs by real-time PCR were 7.88%, 7.82% and 8.73% at 1 week, 3 weeks and 6 weeks after transplantation respectively. iNOS selective inhibitor 1400 W contributed to 32% increase in the survival rate of the transplanted BMSCs. Migration of transplanted BMSCs to the margin area of infarct region was observed in Hoechst33342 or BrdU labelled BMSCs.

Conclusion 1 week after transplantation, the survival rate of BMSCs was as low as around 8% and there was no progressive decrease from 3 to 6 weeks post transplantation. iNOS selective inhibitor 1400 W may result in 32% increase in the survival rate of transplanted BMSCs.

e0030 MESENCHYMAL STEM CELLS TRANSFECTED WITH HCN4 GENES BY LENTIV TO CREATE CARDIAC PACEMAKERS

doi:10.1136/hrt.2010.208967.30

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Objective The study was to test proof-of-principle if genetically engineered mesenchymal stem cells (MSCs) transfected with HCN4 genes can be modified to be cardiac pacemaker cells.

Methods 1. MSCs of rabbit were isolated from the posterior iliac crest of rabbit and were used from passages 2 to 4. 2. The self-inactivating HIV1-based lentiviral vector (LentiV) was used as transgene delivery, which was constructed with plasmid hHCN4/pcDNA3. 3. Total RNA was extracted from control MSCs and those transfected with hHCN4, and RT-PCR was performed. 4. Membrane proteins were extracted from control MSCs and those transfected with hHCN4. Western blot analysis was performed. 5. Whole-cell patch clamp was used to study membrane currents. After the I_f was recorded, cells were superfused with extracellular solution containing 4 mM caesium chloride and the currents were measured accordingly. 6. MSCs transfected with either GFP alone or GFP-hHCN4 were cocultured with neonatal rabbit ventricular myocytes. The coculture beating rate of cardiac myocytes was measured after 3 days of coculture.

Results 1. In addition to expressing characteristic hHCN4 protein, mHCN4-transfected hMSCs also express an anticipated high level of hHCN4 gene by RT-PCR and Western blot analysis. 2. I_f was elicited using hyperpolarizing steps in 10-mV increments from -40 mV to -140 mV, and it was significantly inhibited by 4 mM caesium chloride. 3. The coculture beating rate of cardiac myocytes was 87 ± 11 bpm when MSCs were transfected with control plasmid (expressing only GFP) and 149 ± 14 bpm when MSCs were expressing both GFP+hHCN4 ($p<0.05$).

Conclusion The MSC expressing hHCN4 is a demonstration of feasibility of preparing MSC-based biological pacemaker cells. The study provides a platform for further investigation of the possibility of modifying cardiac excitability with genetically engineered MSCs transfected with HCN4 gene by LentiV. MSCs transfected with hHCN4 genes by LentiV are potentially capable of actively pacing ventricular cardiac myocytes and can potentially be modified to be cardiac pacemaker cells.

e0031 CHARACTER OF PACEMAKER CURRENT IN MESENCHYMAL STEM CELLS TRANSFECTED WITH HCN4 GENES

doi:10.1136/hrt.2010.208967.31

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Objective To test if the pacemaker current can be elicited from mesenchymal stem cells (MSCs) transfected with HCN4 genes by LentiV.

Methods 1. MSCs of rabbit from the posterior iliac crest of rabbit were cultured at 37°C in a humidified atmosphere of 5% CO₂. 2. The self-inactivating HIV-based lentiviral vector (LentiV) was used as transgene delivery, which was constructed with plasmid hHCN4/pcDNA3. 3. Total RNA was extracted from control MSCs and those transfected with hHCN4, and RT-PCR was performed. 4. Whole-cell patch clamp was used to study membrane currents. I_f were elicited in the whole cell configuration by holding cells at -40 mV for 50 ms followed by 10 -mV steps (2 s) to -130 mV and returned to -40 mV (50 ms) after each step. After the I_f was recorded, cells were superfused with extracellular solution containing 4 mM caesium chloride and the currents were measured accordingly.

Results 1. In addition to expressing characteristic hHCN4 protein, mHCN4-transfected hMSCs also express an anticipated high level of hHCN4 gene by RT-PCR and Western blot analysis. And immunofluorescence image is shown for GFP. Control MSCs were negative. 2. With the use of the whole cell configuration of the patch-clamp technique, I_f was elicited using hyperpolarizing steps in 10-mV increments from -40 mV to -140 mV from a holding potential of -40 mV and was voltage-dependent. The threshold of voltage for activation of I_f is around -80 mV. Remarkably, all hHCN4 positive cells exhibit a large caesium-sensitive I_f and it was significantly inhibited by 4 mM caesium chloride.

Conclusions The pacemaker current of I_f can be elicited from the mesenchymal stem cells transfected with HCN4 genes by LentiV. The genetically engineered MSC expressing hHCN4 is a demonstration of feasibility of preparing MSC-based biological pacemaker cells.

e0032 ROLE OF ATRIAL SUBSTRATE REMODELLING IN INDUCIBILITY OF ATRIAL FIBRILLATION AFTER EPICARDIAL GANGLIONIC PLEXI ABLATION

doi:10.1136/hrt.2010.208967.32

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Objective Investigating the long-term effect of ganglionated plexi (GP) ablation on atrial fibrillation (AF) after GP ablation.

Methods 13 dogs were randomly divided into sham-operated group and GP ablation group. All animals underwent a right thoracotomy at the 4th intercostal space. Induced AF and atrial effective refractory period (AERP) were measured by burst rapid pacing at right atrium. After anterior right GP and inferior right GP ablation, AF and AERP were measured again in the GP ablation group. The animals were allowed to recover for 8 weeks, after which, AF and AERP was measured again. The levels of atrial natriuretic peptide (ANP), TNF- α and interleukin (IL)-6 in blood and atrial tissues were examined. Immunocytochemical staining of cardiac nerves was performed in tissues from the dogs.

Results AF was easily induced in the GP ablation group after 8 weeks while AF was not observed in the sham-operated group, and immediately after GP ablation. AERP and dispersion of AERP (dAERP) were increased after GP ablation, while AERP recovered after 8 weeks. Compared with sham-operated group, the levels of ANP, TNF- α and IL-6 in the right atrium increased significantly 8 weeks after GP ablation (204.6 ± 31.2 vs 299.1 ± 32.5 ; 1.3 ± 0.5 vs 4.7 ± 0.7 ; 0.9 ± 0.3 vs 1.8 ± 0.5 ; $p < 0.05$). In GP ablation group, the density of GAP43-positive, TH-positive and ChAT-positive nerves in the right atrium was 821 ± 752 , 481 ± 627 and 629 ± 644 per mm^2 , respectively, which was significantly ($p < 0.01$) lower than the nerve density in sham-operated tissues (2590 ± 841 , 1752 ± 605 and 3147 ± 886 per mm^2 , respectively).

Conclusion Atrial substrate remodelling after GP ablation may be the mechanism of induced AF.

e0033 MECHANISMS OF LOSARTAN ATTENUATES VENTRICULAR REMODELLING AND CARDIAC FUNCTION IN AORTIC BANDED RATS WITH CHRONIC HEART FAILURE

doi:10.1136/hrt.2010.208967.33

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Objective To investigate the regulation of Losartan (Los) on ventricular remodelling and cardiac functioning chronic heart failure rats induced by aortic banded.

Methods Sprague-Dawley (SD) rats underwent abdominal aorta coarctation to induce CHF, confirmed by ultrasound cardiograph and Catheterisation, or sham operation, followed by 8 weeks treatment with Losartan (20 mg/kg per day, orally) or vehicle (drinking water). Plasma norepinephrine (NE) was measured by ELISA, and plasma and tissue angiotensin II (Ang II) levels were measured using RIA. Cardiomyocyte apoptosis was examined by agarose gel electrophoresis and TUNEL's method. The mRNA levels of Bax and Bcl-2 were determined by RT-PCR and the protein expression of phosphorylated and total Akt (t-Akt and p-Akt) were assessed by Western blot.

Results Losartan-treated CHF rats had lower left ventricular end-diastolic pressure (LVEDP) [COA+Los: (11.47 ± 5.06) mm Hg vs COA+Vehicle: (21.18 ± 6.56) mm Hg, $p < 0.01$], higher left ventricular ejection fraction (LVEF) [COA+Los: (63.28 ± 4.32)% vs COA+Vehicle: (43.27 ± 4.25)%, $p < 0.05$] and lower left ventricle/body weight ratios [COA+Los: (2.41 ± 0.15) mg/g vs COA+Vehicle: (3.12 ± 0.18) mg/g, $p < 0.05$], lower plasma NE [COA+Los: (682.24 ± 64.66) pg/ml vs COA+Vehicle: (908.24 ± 75.10) pg/ml, $p < 0.05$] and myocardium Ang II [COA+Los: (60.15 ± 6.22) pg/mgprot vs COA+Vehicle: (92.31 ± 8.31) pg/mgprot, $p < 0.05$], but higher plasma Ang II [COA+Los: (629.68 ± 85.71) pg/ml vs COA+Vehicle: (475.67 ± 51.12) pg/ml, $p < 0.05$] than vehicle-treated CHF rats. Losartan-treated HF rats had no obviously "DNA ladder" which was the character of apoptosis, and the apoptosis index was also reduced [COA+Los: (5.49 ± 0.45)% vs COA+Vehicle: (9.62 ± 0.51)%, $p < 0.05$] with a lower express of Bax/Bcl-2 gene [COA+Los: (109.58 ± 7.53)% vs COA+Vehicle: (136.76 ± 8.82)%, $p < 0.05$] and a higher protein expression of p-Akt [COA+Los: (70.80 ± 6.40)% vs COA+Vehicle: (50.30 ± 4.80)%, $p < 0.05$].

Conclusion Losartan might inhibit cardiomyocyte apoptosis and improve cardiac function in aortic banded rats by blocking Ang II to bind AT₁-R and promoting the activation of Akt.

Conclusion Los attenuated ventricular remodelling and improve cardiac function in aortic banded rats by blocking angiotensin II to bind AT₁-R and reducing angiotensin II, aldosterone and inflammatory cytokine in myocardium.

e0034 INHIBITION OF ATORVASTATIN ON THE AUTOPHAGY OF VASCULAR ENDOTHELIAL CELLS

doi:10.1136/hrt.2010.208967.34

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Objective To explore the mechanism of atorvastatin's protection on vascular endothelial cells, we conducted the research of impact of atorvastatin on vascular endothelial cells autophagy in different times.

Methods We used the Hank's to replace the normal medium to induce autophagy of vascular endothelial cells. In pre-inducement and induction procedure, the cells were incubated with normal medium which includes atorvastatin or not and Hank's, respectively. Experimental cells were randomly divided into four groups: control group (Group I), the group of pre-inducement with atorvastatin (Group II), the group of inducement with atorvastatin (Group III) and the group of pre-inducement and inducement both with atorvastatin (Group IV). Transmission electron microscope (TEM) was used to detect autophagy. The RT-PCR was employed to detect the autophagy-specific markers' (Beclin 1 and Map1lc3) expression in each group of cells.

Results Compared with the group I, target genes (Beclin 1 and Map1lc3) of the group III and the group IV had a significant decreased expression ($p < 0.01$). The expression of the group II were all lower than the group I ($p > 0.05$). The group IV was better than the group III ($p > 0.05$). Via TEM detection, the cells showed typical