Methods 226 male drinkers were enrolled in this study with a standard polysomnography. ALDH₂ genotypes were detected by PCR-restriction fragment length polymorphism (PCR-RFLP).

Results (1) We detected three kinds of genotypes and two kinds of allele of ALDH₂ gene G1951A polymorphism. The frequency of ALDH₂ genotype GG, GA, AA were 0.854, 0.137, 0.009 and allele G and A were 0.923, 0.077 respectively in 226 male drinkers.

The genotypic and allelic frequencies were in Hardy–Weinberg equilibrium ($=0.3606$, $p=0.5482$). (2) The frequency of ALDH₂ genotype GG/(GA+AA) (0.855/0.145) and allele G/A (0.925/0.075) in OSAHS patients had no statistical differences comparing with the control group ($p>0.05$). Analysing the data stratified with BMI, there was also no significant differences of the frequency of ALDH₂ genotype and allele between OSAHS group and the control group either in normoweight or overweight subjects ($p>0.05$). (3) There were no any differences in AHI, the lowest SaO₂ and the longest apnoea duration between subjects with the two genotypes (GG and (GA+AA)) either in OSAHS patients or the control group.

Conclusion ALDH₂ gene G1951A polymorphism was found in male drinkers, it had no any association with OSAHS.

e0026 ASSOCIATION BETWEEN THE M235T, T174M POLYMORPHISM OF THE ANGIOTENSINOGEN GENE AND LEFT VENTRICULAR HYPERTROPHY IN ESSENTIAL HYPERTENSION IN KAZAKANS

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Objective To investigate whether the M235T, T174M polymorphisms of the angiotensinogen gene were associated with left ventricular hypertrophy (LVH) in Xinjiang Kazakans with essential hypertension.

Methods 86 patients with essential hypertension and left ventricular hypertrophy and 95 patients with essential hypertension and non-left ventricular hypertrophy. Left ventricular hypertrophy was evaluated by the electrocardiography voltage criteria and the Romhilt-Estespoint-score system. The AGT gene M235T, T174M polymorphisms were amplified by PCR and analysed by RFLP.

Results (1) The genotype distributions of the M235T and T174M in both groups were in agreement with Hardy–Weinberg equilibrium. (2) The frequencies of the AGT genotypes and alleles were not significantly different between LVH and NLVH with hypertension. (3) When analysing the frequencies of genotypes and alleles of M235T according to gender ($p>0.05$), no significant differences were found between LVH and NLVH. However, we found a significant difference in frequencies of genotypes and alleles of T174M in NLVH group according to gender.

Conclusion There are no associations between M235T genotype and T174M genotype with the presence of LVH in this study.

e0027 GROWTH DIFFERENTIATION FACTOR 15 INDUCE THE PROLIFERATION OF CARDIAC FIBROBLASTS IN A DOSE DEPENDENT MANNER

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Objective Growth differentiation factor 15(GDF-15) is a member of Transforming growth factor- β superfamily which is one of the most important profibrotic protein released during Inflammation. It has

been suggested that GDF-15 seems to be related to the remodelling processes by anti-hypertrophic in cardiomyocytes. Cardiac fibrosis is one part of the important pathology mechanisms during remodelling. It was still unknown whether GDF-15 could influence remodelling by modulating cardiac fibroblast proliferation and extracellular matrix (ECM) metabolism. Therefore, our study aims to investigate the expression and effects of GDF-15 in the fibroblasts.

Methods Primary myocardial fibroblasts were isolated and cultured from neonatal rats and divided into six groups subjected to different conditions: 10^{-6} mol/l Endothelin-1 (ET-1), 10^{-5} mol/l norepinephrine (NE), 3 ng/ml TGF- β 1, 5 pg/ml rhGDF-15, 150 pg/ml rhGDF-15 and no stimuli. The expression of GDF-15 mRNA was detected by Realtime-PCR and the expression of GDF-15 protein was measured by Enzyme-linked Immunosorbent Assay (ELISA). The proliferation of fibroblast induced by rhGDF-15 was measured by 3-(4, 5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) and Flow cytometry; the migration of fibroblast was shown by Scarification test. The level of phosphor-ERK was determined.

Results GDF-15 is upregulated in ET-1, NE and transforming growth factor (TGF) β 1 group; the GDF-15 mRNA is respectively 1.21 ± 0.03 , 1.84 ± 0.09 , 1.95 ± 0.39 folds of control group (respectively $p<0.05$, $p<0.05$, $p<0.01$); and protein in the supernatant is respectively 3.27 ± 0.81 pg/ml, 3.55 ± 0.20 pg/ml and 3.75 ± 0.70 pg/ml, vs 0.41 ± 0.17 pg/ml of control group (respectively $p<0.05$, $p<0.001$, $p<0.01$). rhGDF-15 (150 pg/ml) increased the OD value as compared to control group (0.56 ± 0.03 vs 0.46 ± 0.02 , $p<0.05$), but 5 pg/ml rhGDF-15 did not (0.43 ± 0.01 vs 0.46 ± 0.02 , $p>0.05$). rhGDF-15 (150 pg/ml) could also raise the cell in S phase nearly three times more than control group ($9.62\pm1.17\%$ vs $3.80\pm0.78\%$, $p<0.01$). The scarification test showed that rhGDF-15 could enhance the migration of fibroblast. The ERK was activated after treatment with rhGDF-15.

Conclusion GDF-15 is upregulated by ET-1, norepinephrine and TGF- β 1 in myocardial fibroblasts. rhGDF-15 (150 pg/ml) could enhance the proliferation and migration of fibroblast, which may participate in the progression of myocardial fibrosis and thus was associated with ERK activation. Abbreviations: Growth differentiation factor 15(GDF-15), Extra cellular matrix (ECM), Endothelin-1(ET-1), norepinephrine (NE), Enzyme-linked Immunosorbent Assay (ELISA), 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT), Transforming growth factor (TGF), Extracellular Signal-Regulated Kinase (ERK).

e0028 THE EFFECT OF ACUTE ACTIVATION OF ALDH2 ON MYOCARDIAL ISCHAEMIA/REPERFUSION INJURY IN RAT

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Background Our previous study combined several animal experiments conducted recently showed aldehyde dehydrogenase-2 (ALDH₂) was correlated with myocardial ischaemia/infarction injury. But whether ALDH₂ is beneficial was controversial. We speculated the way in which the ALDH₂ activity was changed may be chiefly responsible for the controversy.

Objective To investigate the effect of acute activation of ALDH₂ on myocardial ischaemia/reperfusion injury in rat.

Methods 20 male Sprague-Dawley rats were divided into five groups: sham group ($n=3$); control group ($n=4$): occlusion of the left anterior descending coronary artery (30 min) followed by reperfusion (15 min); ethanol group ($n=5$): intraperitoneal injection of ethanol (0.5 g/kg) 60 min prior to ischaemia; GTN-ON group ($n=3$): 18 h persistent nitroglycerin treatment (0.1 mg/h, delivered by a patch) combined with intraperitoneal injection of ethanol (0.5 g/kg) 60 min prior to ischaemia; GTN-OFF group ($n=5$):

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

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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

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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

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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.