GW25-e3573
Rb1 Protects Endothelial Cells from Hydrogen Peroxide-Induced Cell Senescence: Involvement of Caveolin-1
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Objectives: Endothelial senescence has been proposed to be involved in endothelial dysfunction and atherogenesis. This study investigates the effects of ginsenoside Rb1, a major constituent of ginseng, on H2O2-induced endothelial senescence. And here we have investigated the expression and production of caveolin-1, a protein that has been shown previously to be upregulated in stress-induced premature senescence.

Methods: Primary human umbilical vein endothelial cells (HUVECs) senescence was induced by H2O2 as judged by senescence-associated β-galactosidase activity (SA-β-gal). Caveolin-1 mRNA expression was analyzed by real-time PCR. Caveolin-1 protein expression was determined by Western blot and laser scanning confocal microscopy.

Results: Treatment of HUVECs with 60μM H2O2 induced premature senescence. Pretreatment of HUVECs with Rb1 was found to reverse endothelial senescence, as witnessed by a significant decrease of senescence cell numbers (approximately 2-fold reduction). Rb1 could markedly decrease Caveolin-1 mRNA expression compared to cells treated with H2O2 alone. Meanwhile, Caveolin-1 protein expression decreased in the 20μM Rb1-pre-treated cells compared to that in cells treated with H2O2 alone. By laser scanning confocal microscopy, we also found that Rb1 can effectively decrease Caveolin-1 protein expression.

Conclusions: Our report demonstrates that Rb1 can exert reversal effects on H2O2-induced cellular senescence through modulating Caveolin-1 expression.

GW25-e4120
IGF-1 Inhibits Apoptosis of Vascular Smooth Muscle Cells Through PI3Akt Pathway
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Objectives: Apoptosis of vascular smooth muscle cells (VSMCs) has recently been identified as an important process in a variety of human vascular diseases, including atherosclerosis. Apopt-1, a novel gene identified in cultured atherosclerotic smooth muscle cells of ApoE-deficient mouse and is known to induce apoptosis in several cells, including VSMC. Insulin-like growth factor (IGF-1) and platelet-derived growth factor (PDGF) are well characterized survival factors for VSMC. However, the interaction between the pro-apoptotic protein Apopt-1 and survival factors IGF-1 and PDGF on mediation of apoptosis in VSMC are poorly understood.

Methods: Immunochemistry; For immunocytochemistry analysis, cells were seeded onto glass coverslips. After transiently transfected with the vector encoding IGF-I, the membrane was incubated with secondary antibody (anti-rabbit IgG-HRP 10mM DTT and a caspase-9-specific monoclonal antibody). Caveolin-1 mRNA expression was analyzed by real time PCR. Caveolin-1 protein expression was determined by Western blot and laser scanning confocal microscopy.

Results: Our report demonstrates that IGF-1 can exert reversal effects on H2O2-induced cellular senescence through modulating Caveolin-1 expression.

Conclusions: Our report demonstrates that IGF-1 can exert reversal effects on H2O2-induced cellular senescence through modulating Caveolin-1 expression.

GW25-e4157
Embodiment of Therapeutic Principle of TCM in “Arrhythmia Emergency Dealing with General Principles”
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Objectives: As an important part of the basic theory of TCM, the therapeutic principle of TCM guide the clinical treatment of TCM in different stages and different levels. To guide the clinical treatment of arrhythmia of integrated traditional Chinese and western medicine, grasp of the clinical application and significance better, and has reference value for the clinical treatment of arrhythmia emergency dealing with general principles.

Methods: This article analysis the arrhythmia emergency dealing with general principles by the therapeutic principle of TCM.

Results: In the “Arrhythmia emergency dealing with general principles”, the first, identify and correct the hemodynamic disorder reflect the “specimen emergency” in the therapeutic principle of TCM; The second, correct basic diseases and compensate for tissue damages. The third, measure the benefit and risk reflect the “as the goal of smooth, neutral thinking” in the therapeutic principle of TCM; The fourth, balance the treatment and prevention reflect the “preventive treatment of diseases” in the therapeutic principle of TCM; The fifth, in the treatment of arrhythmia itself, slow ventricular rate to stable condition reflect the “homotherapy for hypertheropy” in the therapeutic principle of TCM; The sixth, the drug application principle in acute phase of arrhythmia reflect the “treating diseases in accordance with the patient’s constitution and treatment by differentiation of syndromes” in the therapeutic principle of TCM.

Conclusions: This article analysis the arrhythmia emergency dealing with general principles by the therapeutic principle of TCM, which can grasp of the clinical application and significance better, and has reference value for the clinical treatment of arrhythmia of integrated traditional Chinese and western medicine.

GW25-e4203
The effect of angiotensin II receptor type 1 autantibodies on fetal rats’ cardiac hypertrophy and underlying mechanisms
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Objectives: The local renin angiotensin system (RAS) is an independent risk factor which can promote the fetal myocardial hypertrophy. AT1A, the autobody of the angiotensin II (Ang II) type 1 receptor (AT1R), was exist in preclamptic women and might be a new pathological factor that induced fetal myocardial hypertrophy. However, the specific mechanism is still unclear. This study aims to investigate which may contribute to stabilize atherosclerotic plaque in patients with atherosclerosis.

GW25-e4137
The Effects of H2O2 on the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel Current and its Mechanisms in Neonatal Rat Cardiomyocytes
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Objectives: To identify the effects of exogenous hydrogen peroxide on the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel (HCN) current and its mechanisms in Neonatal Rat Ventricle Cardiomyocytes (NRVM).

Methods: NRVM from 1- to 3-day-old Wistar rats were prepared by collagenase digestion, and incubated in 37°C, 95%CO2 for patch-clamp recording. HCN channel protein expression was detected by western-blotting analysis.

Results: Our data showed that exposure (~20 min) of NRVM to H2O2 (100 μmol/ L) markedly increased Ii density (4.7±0.6 pA/pF vs. 11.7±1.1 pA/pF) along increased conductance (Gmax: 48.7±5.6 pS/pF vs. 192.6±6.1 pS/pF), a shift in activation voltage (V1/2) to positive potentials (-81.2±1.6 mV vs. -64.7±2.0 mV) and increase of rate of activation (τact) (523.4±24.7 ms vs. 337.5±24.9 ms). Moreover, stimulation by H2O2 was largely inhibited by the non-specific tyrosine kinase blocker genistein (1μmol/L) and the c-Src-specific inhibitor PP2 (10 μmol/L). Augmented tyrosine phosphorylation of HCN2 channels with H2O2 treatment by detected the H2O2 Western blot using the phosphotyrosine specific antibody 4G10. Furthermore, the augmented Ii current was inhibited by pre-treatment with Trx receptor inhibitor (Auranofin 10μmol/L, 13-cis-retinoic acid 1μmol/L). On the other hand, Ii current of NRVMs was also increased by treated with non-specific PTP inhibitors, phenylarsine oxide (PAO 1μmol/L) or Na-orthovanadate (Na3VO4 10μmol/L).

Conclusions: These data suggest that the c-Src family of tyrosine kinase mediate the augmentation of Ii density by oxidant agent H2O2 via a redox mechanism involving the Trx system.

GW25-e4158
The Effects of H2O2 on the Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel Current and its Mechanisms in Neonatal Rat Cardiomyocytes
Liu Gang, Zheng Mingqi
Dept. of Cardiology, The First Hospital of Hebei Medical University

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Conclusions: These data suggest that the c-Src family of tyrosine kinase mediate the augmentation of Ii density by oxidant agent H2O2 via a redox mechanism involving the Trx system.
whether or not cardiac local RAS abnormality caused by AT1-AA contributes to fetal cardiac hypertension.

**Methods:** To establish AT1-AA-positive pregnant rat model, AT1-AA was purified from preeclamptic patients and the pregnant rats was given a tail vein injection of AT1-AA on 13, 15 days of gestation. The myocardial cell morphology changes were detected by HE staining. The fetal rat heart heavy body weight/body weight ratio were detected by radioimmunoassay and immunohistochemical techniques, respectively. The ATIR expressions in culture myocardial cells were determined by western blot.

**Results:** The titer of the serum AT1-AA were significantly higher (0.28±0.096 vs 0.112 ±0.058, P<0.01 vs control group) and the systolic blood pressure was increased in AT1-AA positive pregnant rats (136±11mmHg vs. 101±4mmHg, P<0.05, vs. control group). Left ventricular wall was thickened and diameter of cardiomyocytes was increased in the AT1-AA positive fetal heart at Med pregnancy (18 days), while the high expression of ATIR (P<0.05) and significantly increased local Ang II (1320±25pg/ml vs 498±124 pg/ml, P<0.05, vs. control group) were detected in fetal myocardial tissue. Additionally, the expression of ATIR in cultured myocardial cell membrane of fetal rat was increased after incubated with ATI-1 AA for 48 hours (P<0.05).

**Conclusions:** AT1-AA can induce fetal rats’ left ventricular hypertrophy and excessive activation of cardiac local RAS may be one of important mechanisms. This study may provide a new strategy and targets for the prevention and treatment of congenital cardiovascular diseases.

**GW25-e4226**

**Vascular Damage Induced by Autoantibodies Against Angiotensin II Type 1 Receptor in Pregnant Rats**

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**Objectives:** Preeclampsia is a kind of serious pathologic complication during pregnancy. Recent studies have demonstrated that autoantibodies against angiotensin II type 1 receptor (AT1-AA) existed in preeclampsic patients. In some patients the AT1-AA appears during pregnancy, while some are congenital positive whose AT1-AA appeared before pregnancy. As the pathogenesis of preeclampsia is based on systemic vascular damage and increased vascular tension, the roles AT1-AA played in the development of vascular damage in the two types of preeclampsia remain unclear. The present study was designed to determine whether AT1-AA causes vascular damage during pregnancy and the difference between the two types of AT1-AA-positive pregnant rats.

**Methods:** The model of AT1-AA congenital positive pregnant rats were derived from AT1-AA-positive rats actively immunized with the epitope of the second extracellular loop of angiotensin II type 1 receptor (AT1IR), which is the binding epitope of endogenous activating autoantibodies against ATIR from patients with preeclampsia. Another type is made by passively immunizing the pregnant rats with AT1-AA which were generated and purified from AT1-AA actively immunized rats. The titers of AT1-AA were determined by ELISA. Animals were euthanized on day 18 of pregnancy. Endothelin-1 (ET-1) in the sera of rats was determined and vascular cellular adhesion molecule 1 (VCAM-1) expression in the third branch of mesenteric arteries endothelium was assessed using confocal microscopy. The function of resistant arteries was detected in isolated third branch of mesenteric arteries by microvascular ring technique. The expression of vascular smooth muscle α-actin (SM α-actin) was detected by immunohistochemistry.

**Results:** The content of ET-1 and vascular endothelial VCAM-1 level were both increased in two types of AT1-AA positive pregnant rats than those of the vehicle group. In addition, mesenteric arteries endothelium-dependent vasodilatation was attenuated in both models, while endothelium-independent vasodilatation and the expression of SM α-actin were decreased in only AT1-AA congenital positive pregnant rats rather than in the passive immunized pregnant rats.

**Conclusions:** Our study demonstrated that AT1-AA contributed to vascular dysfunction in pregnant rats, while AT1-AA would lead more severe damage in congenital positive pregnant rats of AT1-AA appearing before pregnancy than that during pregnancy.

**GW25-e4292**

**Systematic analysis of the clinical and biochemical characteristics of maternally inherited hypertension in Chinese Han families associated with mitochondrial genome mutations**

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**Objectives:** Hypertension is a very common cardiac vascular disease. Previous studies showed that mitochondrial DNA mutations may be associated with cardiovascular disease, including hypertension.

**Methods:** In this study we first did segregation analysis and systematically evaluated the whole mitochondrial DNA on 9 maternal inherited hypertension families and the clinical, genetic and molecular characterization of 73 maternal members from these Chinese Han families and 216 healthy controls. In the maternal members, there’re 12 members had CHD, 6 with cerebrovascular disease, 5 with diabetes, 9 with hyperlipidemia, 3 with renal disease.

**Results:** The laboratory test showed that the potassium, sodium level of the maternal members were higher than that of the control group (P<0.01), with no difference in FBS, TC, triglyceride, LDL-cholesterol and creatinine (P=0.05). While the HDL-cholesterol level of the maternal members was lower than that of the control group (P=0.04). Sequence analysis revealed a total of 172 base changes, including 17 insertions, 4 deletions, 88 single nucleotide polymorphisms, 4 base changes in the transfer RNA (tRNA) genes, 4 in the transfer RNA (tRNA) genes, and 22 amino acid substitutions, with the remainder involving the noncoding regions or synonymous changes. We identified 7 amino acid changes presented in the 9 maternal inherited hypertension families, including 8 mutations in the ATPases 6, 3 in the Cyt b. More interesting, tRNA^Ser^ (525N) tRNA^Glu^ was identified absent in the controls and 1% in 2704 mtDNAs, with potential functional significance.

**Conclusions:** This study showed that mtDNA may contribute to the pathogenesis of hypertension in these Chinese Han families due to their structure and function. In the near future, more mtDNA mutation could be candidate genes for hypertension.

**GW25-e4333**

**High-resolution Analysis of DNA Copy Number Alterations in Chinese Patients with Isolated Secundum Atrial Septal Defect**

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**Objectives:** Secundum atrial septal defect (ASD) is the third most common congenital heart malformation and occurs as an isolated defect or as a feature of more complex syndromes. We provide here the submicroscopic imbalances of isolated congenital heart disease (CHD) with a focus on the secundum ASD phenotype, which has not been previously described in detail. We hypothesized that the cases with secundum ASD have specific spectrum of chromosomal imbalance, which might help identify new disease-related loci or genes for ASD.

**Methods:** A total of 116 Chinese patients with isolated secundum ASD and 340 ethnically matched controls were prospectively screened using whole-genome array comparative genomic hybridization (array-CGH).

**Results:** A genome-wide survey of 116 isolated secundum ASD patients identified 6 de novo copy number variants (CNVs) that were absent or extremely rare (< 0.1%) in 340 controls. In one of these genomic imbalances (3q21.2), genes known to be connected with heart development were implicated (PLXNA1). Furthermore, recurrent CNVs were also identified at 16q23.1 and 9q22.33.

**Conclusions:** Although their causal relationship with secundum ASD remains to be established, this CNV profile provides a spectrum of genomic imbalances in this ASD spectrum, and subsequently improves the CNV phenotype correlations. Additionally, these findings have potential implications for the genetic counseling of those patients with isolated secundum ASD.

**GW25-e4410**

**The Effects of Lysophosphatidylcholine on action potentials of cardiomyocytes and its mechanisms**

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**Objectives:** To explore the effects of LPC on action potentials of cardiomyocytes and its mechanisms.

**Methods:** Neonatal rat cardiomyocytes from 1 to 3-days Wistar rats were prepared, and the specific Human cardiac T-type calcium channel α1 subunits, Cav3.1 and Cav3.2, were stably expressed in HEK293 cells (HEK293-Cav3.1 and HEK293-Cav3.2). They were incubated for 24h for T-type Ca2+ current recording.

**Results:** LPC (10 mM) or 12-myristate 13-acetate (PMA, 1 mM) markedly accelerated the spontaneous beating rates. I_{Ca,L} was significantly increased by LPC in neonatal cardiomyocytes, which was inhibited by specific Cav3.2 channel blocker, Ni2+ (50 mM). Meanwhile, Ni2+ completely blocked the effect of LPC on automaticity in spontaneous beating cardiomyocytes.

**Conclusions:** LPC augmented Cav3.2 channel current to increase the automaticity, which may play a role in triggering arrhythmias in pathophysiological conditions of the heart.

**GW25-e4421**

**Angiotensin II upregulates the HCN Channel in Neonatal Rat Cardiomyocytes via a redox mechanism**

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