S6.1

Ginsenoside-Rd induces cholesterol efflux from macrophage-derived foam cells

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Aim: Ginsenoside-Rd, a purified component from panax notoginseng saponins, has been described to reduce atherosclerotic lesion formation, yet the mechanism is not fully understood. This study is designed to investigate the potential role of Ginsenoside-Rd in modulating cholesterol deposition and to explore its underlying mechanisms in macrophages. **Methods:** The murine macrophage-derived foam cells induced by 25 μg/mL oxidized low-density lipoprotein (oxLDL) in RAW264.7 cells for 48 h. Ginsenoside-Rd 10, 20 μmol/L was added during oxLDL incubation respectively. Oil Red O staining and lipid content analysis were used to evaluation lipid accumulation. Uptake of lipid and cholesterol efflux was assayed by DiI-labeled Ox-LDL and [^{3}H]-labeled cholesterol. Immunohistochemistry and Real Time PCR were employed to investigate the protein and mRNA expression of ATP-binding membrane cassette transport protein A1 (ABCA1), ABCG1, and scavenger receptor-BI (SR-BI). **Results:** Ginsenoside-Rd treatment markedly suppressed oxLDL-mediated lipid accumulation in a concentration-dependent manner, which was due to an increase in cholesterol efflux but not the uptake of oxLDL. In addition, no change was observed in cholesterol efflux to lipid-free apoAI, but cholesterol efflux to HDL was significantly increased by Ginsenoside-Rd treatment. Consistly, ginsenoside-Rd enhanced the mRNA and protein expression of ABCG1 but did not alter the mRNA and protein expression of ABCA1 and SR-BI. **Conclusion:** These data suggest that ginsenoside-Rd abrogates the formation of foam cells by enhancing ABCG1-dependent cholesterol efflux, which elucidate a precise mechanism involved in the prevention of atherogenesis by ginsenoside-Rd.

Keywords: ginsenoside-Rd; foam cells; cholesterol efflux; ATP-binding membrane cassette transport protein A1; ATP-binding membrane cassette transport protein G1; scavenger receptor-BI

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S6.2

Cigarette smoke particles increase the contraction and expression of endothelin B receptor in rat coronary artery

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Aim: Cigarette smoking is a risk factor for coronary artery diseases and endothelin system plays a key role in the pathogenesis. The study was to examine a hypothesis that dimethylsulphoxideipid soluble smoke particles (DSP) up-regulates endothelin type B (ET_B) receptors in coronary artery. **Methods:** The isolated rat coronary artery ring segments were organ cultured for 24 h. The contractile response of the artery was recorded by myograph. The expression of mRNA and protein of the ET_B receptors was studied using quantitative real-time PCR and immunohistochemistry. **Results:** ET_B receptor agonist, sarafotoxin 6c induced a weak contraction in fresh coronary artery. After culture the contraction curve mediated by ET_B receptor was shifted towards the left with an increased E_{max}. DSP 0.2 and 0.4 μl/mL further shifted the concentration-contractile curves towards the left with further increased E_{max}. The culture increased ET_B receptor mRNA and protein levels from fresh arteries, which was further enhanced by DSP. PD98059 (ERK1/2 inhibitor), wedelolactone (NF-kappaB inhibitor), actinomycin D or cycloheximide significantly inhibited the DSP-enhanced contraction and expression of mRNA and protein of ET_B receptor. However, p38 inhibitor further increased DSP-enhanced contraction and protein expression of ET_B receptor. **Conclusion:** DSP up-regulated ET_B receptors in rat coronary artery *via* ERK1/2, and NF-κB pathway.

Keywords: cigarette smoke; coronary artery; endothelin B receptor; ERK; NF-κB; rats

S6.3

Tropomyocin receptor kinase B amends central cardiovascular regulatory dysfunction induced by mevinphos intoxication model of brain stem death

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Aim: This presentation investigates the role of tropomyocin receptor kinase B (TrkB), which is the receptor for brain-derived neurotrophic factor (BDNF), and its downstream signaling pathways in rostral ventrolateral medulla (RVLM) during experimental brain stem. **Methods:** Our previously established experimental model of brain stem death that employed microinjection of the organophosphate insecticide mevinphos bilaterally into RVLM of Sprague-Dawley rats was used, in conjunction with cardiovascular, pharmacological and biochemical evaluations. **Results:** A significant increase in TrkB protein, phosphorylation of TrkB at Tyr516 ($pTrkB^{Y516}$), Shc at Tyr317 ($pShc^{Y317}$) or extracellular signal-regulated kinase (ERK) at Thr202/Tyr204, or Ras activity in RVLM occurred preferentially during the pro-life phase of experimental brain stem death. Microinjection bilaterally into RVLM of a specific TrkB inhibitor, K252a, antagonized those increases. Pretreatment with anti-$pShc^{Y317}$ antiserum, Src homology 3 binding peptide (Grb2/SOS inhibitor), farnesylthioacetic acid (Ras inhibitor), manumycin A (Ras inhibitor) or GW5074 (Raf-1 inhibitor) blunted the preferential augmentation of Ras activity or ERK phosphorylation in RVLM and blocked the upregulated nitric oxide synthase I (NOS I)/protein kinase G (PKG) signaling, the pro-life cascade that sustains central cardiovascular regulation during experimental brain stem death. **Conclusion:** We conclude that activation of TrkB by BDNF, followed by recruitment of Shc/Grb2/SOS adaptor proteins, leading to activation of Ras/Raf-1/ERK signaling pathway plays a crucial role in ameliorating central cardiovascular regulatory dysfunction via upregulation of NOS I/PKG signaling cascade in RVLM in brain stem death.

Keywords: organophosphate; brain stem death; central cardiovascular regulation; TrkB; Shc/Grb2/SOS adaptor protein

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S6.4

The effect of muscone on calcium activity in cardial myocytes in rats

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Aim: Muscone is the main active ingredient in ambrette musk which is used to treat angina induced by the reduction of energy and oxygen. The present study aims to investigate the effects of muscone on Ca^{2+} activity by examining L-type Ca^{2+} channel (LTCC) and intracellular calcium ($[Ca^{2+}]_i$) in cardiomyocyte in rats, and explore the mechanisms underlying cardio protective effects of ambrette musk. **Methods:** Single cardiomyocyte was isolated enzymatically from male Wistar rats. $[Ca^{2+}]_i$ was monitored by confocal microscope through loading Fluo-3AM, and the cell current was recorded by the patch-clamp technique. **Results:** An increase of $[Ca^{2+}]_i$ was observed when the concentration of muscone increased from 5, 15 to 25 μg/mL. However, it started to decrease at 50 μg/mL. Muscone decreased the current density of LTCC ($I_{Ca.L}$) (pA/pF) under different concentrations. It inhibited LTCC dose-dependently from 5 to 50 μg/mL and even maintained the $[Ca^{2+}]_i$ in higher concentration level for some reason. **Conclusion:** Muscone can decrease the $I_{Ca.L}$ (pA/pF) in a dose-dependent manner. The inhibition of LTCC may be a possible mechanism via which angina and other myocardial vascular diseases can be treated by ambrette musk, a traditional Chinese medicine in which muscone is one of the important active components. This may be a new finding that gives us a better insight into the mechanisms of clinical efficacy from ambrette musk.

Keywords: muscone; $[Ca^{2+}]_i$; L-type calcium channel (LTCC)

S6.5

Neuroprotective activity of Shengnaokang Preparation against cerebral ischemia/reperfusion-induced injury in rats

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Aim: Shengnaokang preparation (SP), consisting of fifteen Chinese herbal medicines, has been used for the treatment of cardiovascular and cerebrovascular related diseases with the effects of activating blood flow and dissolving stasis. In present study, the effect of pre-treatment with SP on cerebral ischemia/reperfusion (I/R) induced injury was investigated and the action mechanism was explored. **Methods:** Focal cerebral I/R were induced in rats by transient middle cerebral artery occlusion for 2 h followed by reperfusion for 24 h. Different dose of SP was administered intragastrically to rats once a day for 7 d before operation and 24 h after the initiation of reperfusion. **Results:** Pre-treatment with SP reduced the neurological deficit scores, the cerebral infarction volume, and malondialdehyde content markedly, up-regulated superoxide dismutase and glutathione peroxidase levels, and down-regulated inducible nitric oxide synthase and total nitric oxide synthase levels in serum. And meanwhile SP infusion protected neuron from death, reduced the number of caspase-3 positive cells significantly. **Conclusion:**

Pre-treatment with SP is able to ameliorate neurological dysfunction effectively caused by I/R which is associated with the anti-apoptosis anti-oxidation and neuroprotective activities.

Keywords: Shengnaokang Prescription; focal cerebral ischemia/reperfusion injury; anti-oxidation; anti-apoptosis; neuroprotection

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S6.6

Frequence-dependent effect of isoliensinine on monophasic action potential in guinea pig myocardium

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Aim: Reverse frequence-dependent prolongation of action potential duration (APD) decreases the efficacy of antiarrhythmic drugs which act via prolongation of the APD. Isoliensinine (IL), a bisbenzylisoquinline alkaloid extracted from seed embryo of *Nelumbo nucifera* Gaertn, has antiarrhythmic effect via prolongation of APD. Thus the objective of this research was to investigate the frequence-dependent effect of IL on APD *in vivo*. **Methods:** Guinea pig was assisted breathing with ventilator after anesthetized, thoracic cavity was opened to expose heart by surgery. Heart pacing-rate was controlled by electrical stimulator set for cycle length between impulses from 250 to 80 ms step 10 ms. The recordings of monophasic action potentials (MAP) were used *in vivo* to observe APD changes at different stimulation frequencies, effective refractive period (ERP) was recorded simultaneously. The duration of MAP to 90% repolarization (MAP_{90}) were analyzed. Animals were randomly divided into three groups: normal saline, amiodarone and IL group, drugs were administered intravenously. **Results:** IL 2.5 mg/kg obviously prolongated APD of guinea pig myocardium, and counteract the shortening of APD induced by accelerated heart rate. IL could significantly prolongated ERP from 91.67±4.08 ms to 111.67±4.08 ms. The efficacy of IL was better than amiodarone. **Conclusion:** IL can prolongate APD, and the efficacy is not reverse frequence-dependent, suggesting IL has significant antiarrhythmic effect especially at fast rhythm of the heart.

Keywords: isoliensinine; monophasic action potential; frequence-dependent; effective refractive period

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S6.7

Modulation by corilagin on LOX-1 in ox-LDL-injured HUVEC

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Aim: Oxidized low-density lipoprotein (ox-LDL) is a main etiologic factor in atherogenesis, and antioxidants are accepted as effective treatment of atherosclerosis. In this study, the protective effects of corilagin on the oxidative damage of human umbilical vein endothelial cells (HUVECs) caused by ox-LDL and its mechanism were investigated. **Methods:** HUVECs were incubated with different doses of corilagin (12.5, 25, 50, and 100 μmol/L), and injured by 50 mg/L of ox-LDL. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the absorbance (A_{570}) of HUVECs. The protein expression of lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) was assayed by immunofluorescence and LOX-1 mRNA expression by RT-PCR method, respectively. **Results:** Corilagin reduced cytotoxicity caused by ox-LDL in a dose-dependent manner and down-regulated the expression of LOX-1 protein and mRNA. **Conclusion:** The results suggested that corilagin has protective effects on HUVECs due to its down-regulating the expression of LOX-1 protein and mRNA which might be the mechanism for treating atherosclerosis.

Keywords: atherosclerosis; corilagin; HUVEC; ox-LDL; LOX-1

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S6.8

A83-01, a TGFβRI inhibitor, can proliferate adult cardiac progenitor cells and improve cardiac contractility of myocardial infarcted mice

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Aim: A population of cardiomyoblast expressing GFP under the regulation of Nkx2.5-enhancer (Nkx2.5-GFP^+ cell) was identified in postnatal mice and could be expanded by A83-01 (A83, a TGFβRI inhibitor). The present study aimed to clarify whether A83 could regulate Nkx2.5-GFP^+ cells in adult mice *in vivo* and alleviate cardiac dysfunction after myocardial infarction (MI). **Methods and results:** Nkx2.5 enhancer-eGFP transgenic reporter mice were used to label Nkx2.5$^+$ cardiomyoblast with GFP that were counted and sorted by flow cytometry. Inducible Nkx2.5-enhancer-tTA-cre/loxP-mtdTomato-loxP-mGFP (Tgck/mTmG) transgenic reporter mice were used to map the differentiation of the renewed cardiac cells *in vivo* and were administered with doxycycline till 1 week before A83 treatment. Cardiac function was examined via analyzing pressure-volume relations. A83 (10 mg/kg, ip, QD, 7 d) could significantly increase cardiac Nkx2.5-GFP^+ cell number in adult mice in parallel with the increase of renewed cardiomyocytes in A83-treated Tgck/mTmG mice. In post-MI mice, cardiac Nkx2.5-GFP^+ cells were slightly increased 7 d after MI and the phenomenon was further enhanced in A83-treated group. Cardiac function was evaluated two weeks later after MI. The properties of cardiac contractility, including ventricular elastance and preload recruitable stroke work, were significantly improved in A83-injected mice in parallel with the increase of more renewed cardiomyocytes expressed Ki67 antigen. **Conclusion:** A83 could ameliorate cardiac dysfunction in post-MI adult mice via expanding cardiac Nkx2.5$^+$ cardiomyoblast to compensate the lost myocardium.

Keywords: Nkx2.5; TGFβ; ALK5; A83-01

S6.9

Advance of studies on anti-atherosclerosis mechanism of *Polygonum multiflorum*

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The traditional Chinese medicine *Polygonum multiflorum* is the dry root tuber of the polygonum plant *Polygonum multiflorum* Thunb. The main active components of included stilbeneglycoside, anthraquinone, polyose, *etc*. These components had strong anti-atherosclerosis effect, so it is the valuable traditional Chinese medicine to study and develop for preventing and treating atherosclerosis. Recent studies showed that the anti-atherosclerosis mechanism of *Polygonum multiflorum* was closely related to regulation effect of blood lipid, protection of vascular endothelial cells, stabilization of atherosclerotic plaques, anti-inflammatory effect, inhibitory effect on vascular smooth muscle cells proliferation and antioxidant effect. And other effects of *Polygonum multiflorum* may be involved in anti-atherosclerosis mechanism, which need to be explored.

Keywords: *Polygonum multiflorum*; atherosclerosis; vascular endothelial cells; vascular smooth muscle cells

S6.10

Neuroprotection by sildenafil: neuronal networks potentiation in acute experimental stroke

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Aim: Sildenafil, a phosphodiesterase type 5 inhibitor, has been found to produce functional recovery in ischemic rats by increasing the cGMP level and triggering neurogenesis. The aim of this study was to investigate further sildenafil mechanisms. **Methods:** Male Sprague-Dawley rats underwent middle cerebral artery occlusion and reperfusion, followed by intraperitoneal or intravenous treatment with sildenafil starting 2 h later. Behavorial tests were performed on d 1 or d 7 after reperfusion while cerebral infarction, edema, Nissl staining, Fluoro-Jade B staining and electron microscopy studies were carried out 24 h post-stroke. The cGMP-dependent Nogo-66 receptor (Nogo-R) pathway, synaptophysin, PSD-95/ neuronal nitric oxide synthases (nNOS), brain-derived neurotrophic factor (BDNF)/

tropomyosin-related kinase B (TrkB) and nerve growth factor (NGF)/tropomyosin-related kinase A (TrkA) were measured. **Results:** Sildenafil enhanced neurological recovery, and inhibited infarction, even following delayed administration 4 h after stroke onset. Furthermore, sildenafil reduced the loss of neurons and modulated the expressions of the cGMP-dependent Nogo-R pathway. Moreover, sildenafil protected the structure of synapses and mediated the expressions of synaptophysin, PSD-95/nNOS, BDNF/TrkB and NGF/TrkA. **Conclusion:** Sildenafil produces significant neuroprotective effects on injured neurons in acute stroke, and these are mediated by the cGMP-dependent Nogo-R pathway, NGF/TrkA and BDNF/TrkB.
Keywords: neuronal network; neuroprotection; sildenafil; stroke

S6.11

BMECG promotes neurogenesis and maintains neural stem cells after cerebral infarction

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Aim: The neural stem cells (NSCs), play a crucial role in stroke treatment, which can be regulated by a few of traditional Chinese medicines. The study investigated the promotion effect of Mongolian Medicine "BaiMai Powder" effective compounds group (BMECG) on the proliferation of NSCs. **Methods:** We were cultured NSCs which is isolated from new born rat cerebral cortical *in vitro* and divided into 6 groups which were exposed to oxygen glucose deprivation/reoxgenation (OGD/R). The CFSE immunofluorescence staining was employed to identify the proliferation of NSCs by flow cytometry. Furthermore, the bilateral common carotid arteries (BCCAs) was established on Kunming mice, and all groups were ig for 7 d, respectively. We observed neurobehavioral changes and rota-rod treadmill test. After that, the brain of mice detected by immunohistochemistry with labeling of Nestin and pathological observation at 7 d after BCCAs. **Results:** We found that, BMECG can protect NSCs from OGD/R injury by stimulating NSCs proliferation. Compared with the model group, BMECG significantly increase the time of staying in the rod (P<0.01) and the number of Nestin positive cell in cerebral cortex in BMECG group (P<0.01). **Conclusion:** These results further support the hypothesis that neuroprotective effect of BMECG may be related to the ability of stimulating self-renewal of NSCs which was proved for the first time, which provide a new insight and strategy of anti-neuropathy of stroke.
Keywords: "BaiMai Powder"; effective compounds group; neural stem cells; proliferation; stroke
Acknowledgements: This research was supported by the National Natural Science Foundation of China (No 81173657).

S6.12

Effects of scutellarin on isolated coronary and cerebral artery from SD and SHR rats

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Aim: Here we investigated the vascular activity of scutellarin (SCU) in basilar artery (BA) and coronary artery (CA) rings isolated from Sprague-Dawley (SD) rats and spontaneous hypertension rats (SHR). **Methods:** Isometic force was monitored to obtain cumulative-concentration-response curves by wire myography. **Results:** In isolated CA/BA rings from SD and SHR rats, following contractions stimulation by the thromboxane receptor agonist U46619, SCU (10–1000 μmol/L) dose-dependently induced relaxation; pre-incubation with 1–300 μmol/L SCU significantly suppressed the contractile response to U46619. In artery rings from SD rats, SCU-induced relaxation was significantly blocked by the PKG inhibitor Rp-8-Br-cGMPS; reduced by the NOS inhibitor *L*-NAME; and slightly limited by the ATP-dependent K^+ blocker glibenclamide, the Ca^{2+}-dependent K^+ channel blocker iberiotoxin, and the L-Ca^{2+} channel opener Bay K8644. SCU decreased pH in CA/BA rings; however, similar pH decreases induced by HCl produced a different pH-response profile, indicating that pH lowering did not cause SCU-induced vasodilation. **Conclusion:** These results demonstrate that SCU causes relaxation and suppresses contraction in isolated CA/BA from SD and SHR rats while NOS, ion channels and PKG pathway might be involved.
Keywords: scutellarin; rat; coronary artery; brain basilar artery
Acknowledgements: This study was supported by grants from the National Natural Science Foundation of China (No 30960450 and 8117311) and Yunnan Provincial Science and Technology Department (No 2011FA022, 2012BC012, 2008CD054, and 2008ZC111M).

S6.13

Hypoxic constriction of porcine coronary artery: role of MYPT1

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Aim: Hypoxic vasoconstriction has been found depending on endothelium-derived NO (EDNO), but the underlying mechanism remains unclear. The present study was to determine whether or not calcium-sensitization via activation of the regulatory subunit of myosin light chain phosphotase (MYPT1) is involved. **Methods:** Experiments were performed on isolated porcine coronary artery (outside diameter: ~5 mm). Endothelium was removed mechanically. Isometric tension of vessel rings was measured in organ chamber technique with a gas mixture containing 95% O_2-5% CO_2 for normoxia and 95% N_2-5% CO_2 for hypoxia. The phosphorylation of MYPT1 and myosin light chain (MLC) under normoxia and hypoxia conditions were analyzed by Western blotting. **Results:** Hypoxia induced constriction of porcine coronary artery pretreated with U46619 (30–300 nmol/L) or high potassium (30–60 mmol/L). It was abolished by endothelium removal, inhibition of eNOS with nitro-L-arginine, and inhibition of soluble guanylyl cyclase (sGC) with ODQ. The hypoxic effect also occurred in endothelium denuded artery treated with exogenous NO donor but not with cell permeable cGMP analog 8-Br-cGMP. The hypoxic vasoconstriction was not affected by inhibitors of myosin light chain kinase (ML-7), PKC (GF109203X), and actin polymerization (Cytochalasin B). It was significantly inhibited by Y27632, an inhibitor of Rho kinase. Western blotting showed that, under hypoxic condition, there was an enhanced phosphorylation of MYPT1 at Ser-853 and MLC. **Conclusion:** The present study demonstrates that hypoxia augmented contraction of porcine coronary arteries in a manner dependent on NO and sGC. An increased calcium-sensitization resulted from Rho kinase-mediated phosphorylation of MYPT1 and MLC may be involved.
Keywords: hypoxic vasoconstriction; Rho kinase; MYPT1
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S6.14

Effects of sodium ferulate on the left ventricular hypertrophy of rat induced by aorta coarctation

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Aim: To investigate the effects of sodium ferulate on myocardial hypertrophy induced by constricting abdominal aorta in rats *in vivo*. **Methods:** Male Spragye-Dawley (SD) rats were randomly divided into the sham, model and low-, mid-, and high- dose sodium ferulate (20, 40, 80 mg/kg per day) groups. Except for sham group, myocardial hypertrophy model of the rats was induced by constricting abdominal aorta and were administered from the next day after surgery once daily for consecutive 25 d. Changes of cardiac hypertrophy indexes and hemodynamic parameters of rats were observed. Left ventricular myocardial fiber diameter (MD) was determined. Electron microscopy was used to observe the ultrastructure of myocardial tissue. Real Time PCR was used to determine the mRNA expression of atrial natriuretic factor (ANF). Angiotensin II (Ang II) and endothelin-1(ET-1) content in plasma and cardiac tissues were determined by radioimmunoassay (RIA). **Results:** Compared with the sham group, the left ventricular hypertrophy index (LVHI), the left ventricular weight/right ventricular weight (LVW/RVW), and MD were remarkably increased, the expression of ANF mRNA was obviously elevated. Sodium ferulate could remarkably decrease LVHI, tend to or significantly decrease LVW/RVW, improve the indicators of systolic and diastolic function, and down-regulate the expression of ANF mRNA, reduce the elevated Ang II and ET-1 content in plasma and cardiac tissues of rat and improve the systolic and diastolic functions of left ventricle. **Conclusion:** Sodium ferulate is effective in attenuating in the left ventricular hypertrophy induced by constricting abdominal aorta and protecting cardiac function.
Keywords: sodium ferulate; left ventricular hypertrophy; coarctation of abdominal aorta; angiotensin II; endothelin-1
Acknowledgements: This work was supported by a Brainstorm Project on Social Development by Department of Science and Technology of Guizhou Province, China (No [2011]3036).

S6.15

Thymoquinone causes endothelium-dependent augmentation depending on activation of soluble guanylylcyclase in porcine coronary arteries

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Aim: Experiments were designed to determine the effects ofthymoquinone, an alkaloid with vasodilator properties, in coronary arteries. **Methods:** Rings, with or without endothelium, of porcine coronary arteries were suspended in conventional organ chambers for isometric tension recording. Certain rings were incubated with inhibitors of nitric oxide (NO) synthase (*L*-N^G-nitroarginine methyl ester, *L*-NAME) or soluble guanylylcyclase(1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, ODQ). They were contracted with prostaglandin $F_{2\alpha}$ and exposed to increasing concentrations of thymoquinone. **Results:** Thymoquinone caused a sustained further increase of tension in rings with endothelium. This augmentation was prevented by endothelium-removal, *L*-NAME and ODQ. Incubation with the NO-donor detaNONOatein *L*-NAME-treated rings restored and even increased the contractile response to thymoquinone. In contrast, incubation with 8-bromo cyclic GMPof ODQ-treated preparations did not restore the contraction under thymoquinone. **Conclusion:** These findings demonstrate that thymoquinone causes an endothelium-dependent augmentation similar to that seen in hypoxia (Chan *et al*., Am J Physiol: H2313, 2011). This facilitation also requires endothelium-derived NO and activation of soluble guanylylcyclase, but not the presence of cyclic GMP.

Keywords: 8-Bromo cyclic GMP; detaNONOate; hypoxia; nitric oxide; prostaglandin $F_{2\alpha}$

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S6.16

Sustained delivery of rapamycin for the treatment of atherosclerosis in ApoE knockout mice

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Aim: Rapamycin (RAP) can inhibit the growth and migration of T cells and smooth muscle cells which play important role in maintaining the plaque stability. This study is to test the potential of RAP and its sustained release in inhibiting progression and enhancing stability of atherosclerotic plaques in $ApoE^{-/-}$ mice. **Methods:** RAP nanoparticles (RNPs) can be produced through a simple oil-in-water emulsion technique. $ApoE^{-/-}$ mice were fed 0.25% cholesterol for totally three months. In the last two months, the animals were treated with RAP (group A), RNPs (1 and 3 mg/kg, groups B and C, respectively) and vehicle (group D), no drugs (group E). Blood was withdrawn for plasma cytokine determination. Brachiocephalic arteries were cut and fixed for histology and immunohistochemistry analysis. **Results:** Oil Red O staining revealed that the plaque formation in the brachiocephalic artery significantly decreased in group A than group E, while more than group B and C. Immunohistochemistry analysis demonstrated more positive staining of smooth muscle cells (SMCs) in group A than group E, but less than groups B and C. Plasma cytokine determination indicated significant reduced levels of inflammatory cytokines in groups A, B, and C than those in group E. **Conclusion:** RAP can effectively inhibit the growth of plaque and stabilize the rupture-prone plaque in $ApoE^{-/-}$ mice. RNPs can substantially enhance the therapeutic effect of RAP.

Keywords: anti-inflammatory; stability; plaque

S6.17

Oxysophoridine protects against focal cerebral ischemic injury via inhibits oxidative stress and apoptosis

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Aim: Our previous study demonstrated that oxysophoridine (OSR) had protective effects on cerebral neurons damage *in vitro*. In this study, we further investigated whether OSR can reduce ischemic cerebral injury *in vivo* and its possible mechanism. **Methods:** After administration with OSR, mice were subjected to make the model of middle cerebral artery occlusion (MCAO). Then neurological scores and infarct volume were estimated. Morphological examination was performed. Oxidative stress levels were assessed by measurement of MDA, SOD and GSH-Px levels. The expression of caspase-3, Bax and Bcl-2 were investigated by immunohistochemistry and Western-blot analysis. **Results:** OSR reduced neurological deficit scores, infarct volume and relieved neuronal morphological damage. OSR also markedly decreased MDA content, and increased SOD, GSH-Px activities. Apoptotic neurons were lower in OSR treatment groups. OSR (250 mg/kg) significantly down-regulated caspase-3, Bax expression and increased Bcl-2 expression. **Conclusion:** These findings indicated that OSR has a protective effect on focal cerebral ischemic injury, the mechanism may be related to attenuating oxidative stress and apoptosis.

Keywords: OSR; cerebral ischemic injury; oxidative stress; neuronal apoptosis

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S6.18

NAD (P) H oxidase is involved in the mechanism of Tanshinol protecting against ischemia/reperfusion injury in Langendorff-perfused rat hearts

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Aim: The present study was to investigate the effects of Tanshinol on ischemia/reperfusion (I/R) injury in Langendorff-perfused rat hearts and its underlying mechanisms. **Methods:** The isolated rat hearts were perfused in the Langendorff mode and were subjected to either continuous perfusion for 90 min or 30 min for balance, global ischemia and reperfusion, respectively. Left ventricular developed pressure (LVDP), coronary flow (CF), SOD activity and MDA content in myocardial homogenate, Bcl-2 and Bax, NAD (P) H oxidase subunits of Nox4 and $p47^{phox}$ protein expression of myocardial were measured by Western blot. **Results:** Tanshinol could antagonize the decrease of LVDP and CF induced by I/R, and could protect SOD, and inhibit biomembrane lipid peroxidation. Meanwhile, Tanshinol treatment could markedly increase the expressions of Bcl-2 and decrease the expressions of Bax. In addition, Tanshinol treatment could significantly inhibit the I/R-induced increase of Nox4 and $p47^{phox}$ protein expression in myocardial cells. **Conclusion:** Tanshinol exhibits significant protective effect against myocardial I/R injury in isolated rat heart, which is related to antagonizing the increasing of Nox4 and $p47^{phox}$ protein expression, alleviating oxidative stress and inhibiting apoptosis.

Keywords: Tanshinol; Langendorff; hemodynamic; lipid peroxidation; apoptosis; NAD (P) H

S6.19

Coptisine protects rat heart against myocardial ischemia/reperfusion injury by suppressing myocardial apoptosis and inflammation

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Aim: Coptisine is an isoquinoline alkaloid isolated from *Coptidis Rhizoma*. The present study investigated the potential effect of coptisine on myocardial ischemia and reperfusion (I/R) damage in rats and the underlying mechanisms. **Methods and Results:** Electrocardiogram examination showed that the administration of coptisine 10 min before ischemia significantly decreased I/R-induced arrhythmia after 30 min ischemia followed by 3 h reperfusion. Echocardiography was performed before ischemia and 24 h post-I/R, separately. The M-mode records showed that the reductions of ejection fraction (EF) and fractional shortening (FS) were attenuated in coptisine-treated rats compared with the I/R rats. Evans Blue/TTC staining showed that coptisine notably reduced infarct size. On the other hand, TUNEL assay demonstrated that coptisine suppressed myocardial apoptosis, which may be related to the up-regulation of Bcl-2 protein and inhibition of caspase-3 activation. Coptisine also attenuated the proinflammatory cytokines including IL-1β, IL-6, and TNF-α in heart tissue. Western blot and immunohistochemical

analysis showed that coptisine markedly reduced Rho, Rho-kinase 1 (ROCK1), and ROCK2 expression and attenuated the phosphorylation of myosin phosphatase targeting subunit-1 (MYPT-1), a downstream of ROCK. **Conclusion:** Coptisine exerts pronounced cardioprotection in rats subjected to myocardial I/R likely through suppressing myocardial apoptosis and inflammation by inhibiting the Rho/ROCK pathway.

Keywords: coptisine; ischemia/reperfusion (I/R); apoptosis; inflammation; Rho-kinase (ROCK)

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S6.20

Influence of the focal ischemia reperfusion on a novel estrogen receptor alpha 36 and correlated estrogenic signaling pathway in hippocampus

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Aim: Previous studies showed that ER-α36, a novel variant of estrogen receptor alpha (ER-α) was widely expressed in breast cancer. It mediated the membrane initiated estrogenic signaling pathway which was involved in cell proliferation and apoptosis but the mechanism of ER-α36 in brain was still unclear. Tamoxifen (TAM) is one of the selective estrogen receptor modulators (SERMs) which was mainly applied in the treatment of breast cancer. In this study, we detected the expression of ER-α36 in brain throughout the focal ischemia reperfusion which mimic the pathematology of stroke *in vivo* to explore the function of ER-α36 and MAPK/ERK and PI3K/Akt. **Methods:** MAPK and Akt phosphorylation level after focal cerebral ischemia-reperfusion injury was explored via Western blot. We also explored the function of ER-α36 in the neuroprotective effects of TAM with ovariectomized rats on stroke using immunohistochemistry and Nissl staining. **Results:** Compared with the sham group, declining endogenous estrogen strengthened the damage degree of cerebral ischemia injury and reduced the expression of both ER-α66 and ER-α36 in CA1 of hippocampus. Declining endogenous estrogen inhibited the activation of MAPK/ERK and PI3K/Akt signal pathway in hippocampus. Furthermore, the expression of ER-α36 in hippocampus was increased after treated with TAM. **Conclusion:** ER-α36 may mediate the neuroprotective actions of estrogen after stroke. And the neuroprotective effect was associated with the estrogenic signaling proteins which mediated by ER-α36. ER-α36 was involved in the neuroprotection effect of TAM.

Keywords: MAPK/ERK; ERα36; hippocampus; neuroprotection; tamoxifen

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S6.21

In vitro anti-atherogenic effects of asiaticoside on human aortic endothelial cells

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Aim: Early stage of atherosclerosis involves endothelial dysfunction even before the formation of atherosclerotic plaques. Proinflammatory cytokines such as TNF-α has been shown to play an important role in this early event. *Centella asiatica* is a perennial herb and commonly used in traditional medicine to treat ulcer and arthritis. Asiaticoside, a major triterpenoid saponin component of *C asiatica* has been reported to possess wound healing, anti-oxidant and anti-inflammatory activities *in vivo*. The main objective of this study was to evaluate the anti-atherogenic effects of asiaticoside in human aortic endothelial cells (HAEC) especially in terms of early stage of atherosclerosis which involves mainly endothelial hyperpermeability/dysfunction induced by TNF-α. **Methods:** Toxicity effect of asiaticoside on HAEC was examined by using MTT assay. HAEC were treated with asiaticoside at doses ranged from 200 μmol/L to 3.125 μmol/L up to 24 h. Next, FITC-dextran permeability assay was used to determine the effect of asiaticoside on TNF-α induced increased permeability in HAEC. Treatment of HAEC with asiaticoside alone for 30 min (50, 25, 12.5, 6.25, and 3.125 μmol/L) without inducer was performed to ensure that the compound alone did not cause any changes to basal permeability. Then, HAEC were pretreated with asiaticoside for 30 min (50, 25, 12.5, 6.25, and 3.125 μmol/L) followed by stimulation of increased permeability with TNF-α for 6 h. **Results:** Asiaticoside at the doses ranged from 200 μmol/L to 3.125 μmol/L did not cause significant cell death to HAEC even up to 24 h. Asiaticoside alone treated for 30 min at the doses of 50–3.125 μmol/L also did not markedly change the basal permeability of HAEC. Therefore, pretreatment time of 30 min for asiaticoside was chosen. Furthermore, treatment with 10 ng/mL of TNF-α for 6 h significantly increased permeability of HAEC as compared to normal cells. Pretreatment with asiaticoside for 30 min dramatically decreased the TNF-α increased permeability at all doses tested. **Conclusion:** Asiaticoside could inhibit increased endothelial permeability induced by TNF-α. Therefore, it might be a potential anti-atherogenic agent by suppressing endothelial dysfunction in atherosclerosis. Further studies are needed to elucidate the underlying mechanisms of action for asiaticoside.

Keywords: atherosclerosis; HAEC; asiaticoside; endothelial dysfunction; permeability

S6.22

Impact of sigma-1 receptor in cardiovascular and neurodegenerative diseases

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We recently found very high expression of sigma-1 receptor (sigma-1R), an orphan receptor, in rat heart tissue and defined the cardiac sigma-1R as a direct target for selective serotonin reuptake inhibitor, fluvoxamine and selective sigma-1R agonist, SA4503 in eliciting cardioprotection in both pressure overload-induced and transverse aortic constriction (TAC)-induced heart failures in rodents. The sigma-1R localizes to the mitochondria-associated endoplasmic reticulum (ER) membrane (MAM) and interacts with IP_3 receptor, thereby promoting Ca^{2+} transport into mitochondria from the ER (Hayashi and Su, Cell 2007; 131: 596). We first documented down-regulation of sigma-1R following heart failure, thereby causing impairments of mitochondrial Ca^{2+} transport and ATP production (BBA 2013; 1830: 3082). Stimulation of sigma-1R by fluvoxamine or SA4503 completely rescues the down-regulated sigma-1R expression and mitochondrial ATP production in both pressure overload- and TAC-induced heart failures. Recently, a E102Q mutation in sigma-1R (sigma-$1R^{E102Q}$) was discovered in familial amyotrophic lateral sclerosis (ALS) patients. We found that sigma-$1R^{E102Q}$ over-expression in neuro2A cells promoted its dissociation from ER membranes and its cytoplasmic aggregation, and in turn impaired mitochondrial ATP production. Under ER stress conditions, sigma-$1R^{E102Q}$ over-expression caused aberrant extra-nuclear localization of the TAR DNA-binding protein-43 (TDP-43) and mitochondrial degradation. Taken together, sigma-1R is a novel therapeutic target for cardiovascular and neurodegenerative diseases.

S6.23

Study on cardioprotective duration of noninvasive delayed limb ischemic preconditioning against myocardial ischemia-reperfusion injury in rats

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Aim: To test the difference of duration between different strategies of noninvasive delayed limb ischemic preconditioning (NDLIP) for cardioprotection against I/R injury. **Methods:** Male Wistar rats were randomized into the following groups: control groups, consecutive NDLIP groups. The control groups, including the sham operation group, the ischemia-reperfusion (I/R) group, the myocardial ischemic preconditioning (MIPC) group, the femoral artery ischemic preconditioning (FAIP) group; NDLIP groups, including 1st d after 1-d NDLIP, 3rd d after 1-d NDLIP, 5th d after 1-d NDLIP, 1st d after 3-d NDLIP, 3rd d after 3-d NDLIP, 5th d after 3-d NDLIP, 1st d after 7-d NDLIP, 3rd d after 7-d NDLIP, 5th d after 7-d NDLIP groups. Left ventricular function, incidence of ventricular arrhythmia, and ST-segment were measured during I/R. Myocardial infarct size, heart fatty acid binding protein (H-FABP), and glycogen phosphorylase BB (GPBB) were determined at the end of experiment. **Results:** Compared to the I/R group, the MIPC, FAIP, 1st d after 3-d NDLIP, 1st d after 7-d NDLIP groups showed amelioration of ventricular arrhythmia, improved left ventricular function, lower ST-segment elevation, reduced myocardial infarct size, decreased H-FABP and GPBB activity. **Conclusion:** Our results suggest that 3 and 7 d NDLIP can provide the similar degree of cardioprotection.

Keywords: limb ischemic preconditioning; myocardial ischemia-reperfusion; cardioprotection

S6.24

The study on enhancing the cardioprotective effects of noninvasive delayed limb ischemic preconditioning against myocardial ischemia-reperfusion injury in rats

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Aim: To test the difference between different strategies of noninvasive delayed limb ischemic preconditioning (NDLIP) for cardioprotection against I/R injury. **Methods:** Male Wistar rats were randomized into the following groups: control groups, consecutive NDLIP groups. The control groups, including the sham operation group, the ischemia-reperfusion (I/R) group, the myocardial ischemic preconditioning (MIPC) group, the femoral artery ischemic preconditioning (FAIP) group; the consecutive NDLIP groups, including continuously using for 1, 3, 5 and 7 d groups. Each group was tested on the first day after NDLIP. Left ventricular function, onset and duration of ventricular arrhythmia, and ST-segment were measured during I/R. Myocardial infarct size, heart fatty acid binding protein (H-FABP), and glycogen phosphorylase BB (GPBB) were determined at the end of experiment. **Results:** Compared to the I/R group, the MIPC, FAIP, continuous NDLIP for 3, 5 and 7 d groups showed amelioration of ventricular arrhythmia, improved left ventricular function, lower ST-segment elevation, reduced myocardial infarct size, decreased H-FABP, and GPBB activity. **Conclusion:** Our results suggest that continuous NDLIP for 3 d provides effective cardioprotection against I/R injury, and the cardioprotection can not be enhanced by extending NDLIP.

Keywords: limb ischemic preconditioning; myocardial ischemia-reperfusion; myocardial infarction; arrhythmia; myocardial protection; cardiac function

S6.25

Study on lasting (long-term) cardioprotective effects of multiple courses noninvasive delayed limb ischemic preconditioning against myocardial ischemia-reperfusion injury in rats

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Aim: To test the difference between multiple courses of noninvasive delayed limb ischemic preconditioning (NDLIP) for lasting (long-term) cardioprotection and provide the optional strategy for clinical application. **Methods:** Male Wistar rats were randomized into the following groups: control groups, consecutive NDLIP groups. the control groups, including the sham operation group, the ischemia-reperfusion (I/R) group, the myocardial ischemic preconditioning (MIPC) group, the femoral artery ischemic preconditioning (FAIP) group; NDLIP groups, including 1-d NDLIP +1-d interval(INTV) group, 1-d NDLIP +2-d INTV group, 3-d NDLIP +3-d INTV group, 3-d NDLIP +5-d INTV group. Left ventricular function, incidence of ventricular arrhythmia, and ST-segment were measured during I/R. Myocardial infarct size, heart fatty acid binding protein(H-FABP), and glycogen phosphorylase BB (GPBB) were determined at the end of experiment. **Results:** Compared to the I/R group, the MIPC, FAIP, 1-d NDLIP +1-d INTV group showed amelioration of ventricular arrhythmia, improved left ventricular function, lower ST-segment elevation, reduced myocardial infarct size, decreased H-FABP, and GPBB activity. **Conclusion:** Our results suggest that multiple courses of 1-d NDLIP +1-d INTV regimen may provide lasting cardioprotection and may be the optional strategy for clinical application.

Keywords: limb ischemic preconditioning; myocardial ischemia-reperfusion; cardioprotection

S6.26

The acute effects of nonylphenol, an environmental estrogen, on the contractility of rat heart in a non-monotonic manner via its influence on L-type Ca^{2+} channel currents

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Aim: In this study, we focused on the acute effects of NP on myocardial contractility to determine the rapid non-genomic cardiac actions of NP in isolated rat heart and investigate the mechanisms of action by determining the effects of NP on L-type Ca^{2+} current in rat ventricular myocytes. **Methods:** The hearts were then taken out and mounted on Langendorff apparatus for perfusion with a modified Krebs-Henseleit solution (KHs). Hemodynamic parameters including the heart rate (HR), the left ventricular pressure (LVP), the maximal values of the first derivative of LVP ($+LVdp/dt_{max}$) and the rate pressure product (RPP) were assessed. Single cardiac myocyte were isolated from rat and L-type Ca^{2+} current was recorded with whole-cell patch-clamp technique. **Results:** The lower concentrations (10^{-12}–10^{-10} mol/L) of NP increased cardiac contraction and the higher concentrations (10^{-8}–10^{-6} mol/L) of NP decreased cardiac contraction. In electrophysiological experiments, lower concentrations (10^{-12}–10^{-10} mol/L) NP activate or enhance L-type Ca^{2+} current and higher concentrations (10^{-8}–10^{-6} mol/L) NP significantly inhibited $I_{Ca\text{-}L}$. **Conclusion:** Nonylphenol induced rapid effects on the contractility in rat heart in a non-monotonic manner via its influence on L-type Ca^{2+} channel currents.

Keywords: nonylphenol; xenoestrogen; L-type Ca^{2+} channel; cardiac myocytes

S6.27

Inhibitory effect of ginsenoside Re on neointimal hyperplasia of carotid artery in balloon-injured rats

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Aim: To examine the effects of ginsenoside Re (Re) on neointimal hyperplasia of carotid artery in balloon-injured rats and explore relevant mechanisms. **Methods:** Balloon-injured carotidartery models was established. Animals were then injected (ip) with normal saline or Re in the model, sham operation (injected with distilled water), and Ginsenoside Re-low groups (Re 6 mg/kg/d), Ginsenoside Re-moderate groups (Re 12 mg/kg/d), ginsenoside Re-high groups (Re 24 mg/kg/d), respectively. Injured carotid arteries were taken after 14 d in order to observe neointimal hyperplasia with light microscope via HE staining and measure the various membranous area by Q-win image processing system. The expression of mitogen-activated protein kinase phosphatase-1 (MKP-1) mRNA levels were analyzed by real-time RT PCR. The expression of phosphorylation extracellular signal-regulated kinase1/2 (pERK1/2) and MKP-1 were examined by immunohistochemistry and analyzed with Image-Pro Plus. **Results:** Lumens of carotid artery were clearly narrow after balloon-injury (P<0.01). Compared with model groups, the stenosis of lumen in groups of high and moderate dosages of Re were decreased remarkably (P<0.05). Besides, NIA, NIA/MA of carotid artery were decreased as well (P<0.05), the expression of MKP-1 mRNA level and protein pERK1/2 was increased but the expression of pERK1/2 was reduced (P<0.05). **Conclusion:** Re can significantly inhibit balloon-injury induced neointimal hyperplasia and the anti-proliferative effect of Re appears to be due to lowering expression of pERK1/2 and raising expression of MKP-1.

Keywords: Re; balloon-injury; pERK1/2; MKP-1

S6.28

Myocardial expression of Foxp3 in cardiac hypertrophy induced by isoprenaline and effect of triptolide in mice

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Aim: Inflammation and immune have been demonstrated to be involved in the pathophysiological process of cardiac hypertrophy and fibrosis, and forkhead/winged helix box transcription factor P3 (Foxp3) has a wide range of implications for inflammation and immune response, as well as transcriptional regulation of various fetal genes. The present study was designed to explore the myocardial expression of Foxp3, its relation with cardiac hypertrophy and the effects of triptolide on that in mice. **Methods:** Sixty male mice were divided randomly into six groups, *ie*, control, model, vehicle, and triptolide (10, 30, and 90 μg/kg) groups. Cardiac hypertrophy was induced by administration of isoprenaline (5 mg/kg, sc, once daily) for 14 d. Triptolide was given intraperitoneally once daily from the second day. Myocardial expressions of TGF-β1, NFATc3, CD4, and Foxp3 were determined with immunohistochemistry and Western blotting techniques, respectively. Serum brain natriuretic peptide (BNP) concentration was measured using ELISA method. **Results:** The expression of Foxp3 was reduced significantly in myocardial interstitium of model mice by comparison of controls. Triptolide ameliorated myocardial injury, decreased cardiac hypertrophy indexes markedly. At the dosage of 30 or 90 μg/kg, triptolide elevated the expressions of α-MHC, Foxp3 and CD4, and decreased the expressions of TGF-β1, NFATc3, as well as β-MHC significantly (P<0.05 or 0.01). Simultaneously, it obviously decreased the serum BNP content. **Conclusion:** Triptolide effectively ameliorate cardiac hypertrophy through elevating the expression of CD4 and Foxp3, decreasing the

expression of TGF-β and NFATc3, and balancing the disproportion of regulatory T cells.

Keywords: forkhead transcription factor P3; cardiac hypertrophy; triptolide; inflammation; immune

S6.29

Neuroprotection of total flavones of rhododendra in cerebral ischemia/reperfusion and in neuronal cultures

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Aim: Total flavones of rhododendra (TFR) is a powerful antioxidant which is isolated from the traditional Chinese herb rhododendra. However, little is known regarding TFR's protective mechanism in ischemia/reperfusion (I/R) injury. TFR may have a beneficial role for neurons in cerebral ischemia/reperfusion caused by accident. We therefore observed the protective effects of TFR on neurons injured by ischemia/reperfusion *in vitro* and *in vivo* and explored the underlying mechanisms. Ischemia and reperfusion was induced by cerebral four-vessel occlusion. **Methods:** The rats were divided into 6 groups at random: sham group, model group and TFR preventive treatment group. The levels of protein expression and phospho-JNK1/2 were detected by Western blotting. On the other hand, rat hippocampal neurons in primary culture were studied during the different periods of oxygen-glucose deprivation and reperfusion with oxygen and glucose. Cell viability was determined by methyl thiazoleterazolium (MTT) assay. The activity of lactate dehydrogenase (LDH) leaking from neurons and the contents of nitric oxide (NO) and malondialdehyde (MDA) were detected by spectrophotometry. The concentration of intracellular free calcium ($[Ca^{2+}]_i$) was monitored by fluorescence spectrophotometer with the Ca^{2+} sensitive fluorescent probe Fluo-3. **Results:** Pretreatment of model rat with TFR (10, 30, and 100 mg/kg, ig) significantly decreased MDA level. TFR (30 and 60 mg/kg) inhibited the activation of JNK1/2 in ischemia reperfusion. Treatment with TFR (final concentration 10, 20, 40, 80, and 160 mg/L) during ischemia/reperfusion-mimetic incubation *in vitro* concentration-dependently attenuated neuronal damage with characteristics of increasing neuronal viability, decreasing LDH and NO release, and blunting elevation of intracellular calcium concentration. **Conclusion:** TFR has remarkable protective effects against global cerebral ischemic reperfusion injury in rats. The mechanisms that TFR protect against cerebral ischemia and reperfusion injury include inhibiting the activation of JNK1/2 and calcium overload.

Keywords: total flavones of rhododendra; hippocampal neurons; ischemia/reperfusion; nitric oxide; $[Ca^{2+}]_i$; JNK1/2

S6.30

Propofol inhibits currents of both human *ether-a-go-go*–related gene and its non-sense mutation, Q738X, in HEK293 cells

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Aim: QT interval prolongation can result from intake of drugs known to affect the rapidly activating delayed rectifier K^+ channel (I_{Kr}) encoded by human *ether-a-go-go*-related gene (hERG). We aimed to investigate the pathophysiological consequences of a non-sense mutation, Q738X, in hERG and the effects of propofol on reconstituted wild type (WT) and Q738X hERG currents. **Methods:** The hERG current was recorded by the whole cell patch clamp technique. The express level and subcellular localization of hERG proteins was studied by Western blot and double immunofluorescence staining. **Results:** Q738X alone did not generate hERG current and Q738X mutation did not cause dominant negative suppression of WT-hERG current. The lack of dominant negative effect by Q738X was due to failure of mutant subunits to coassemble with WT subunits. Propofol dose-dependently inhibited both WT-hERG and WT/Q738X-hERG currents, but did not affect the disruption of hERG protein trafficking. The IC_{50} for WT-hERG and WT/Q738X-hERG currents with propofol were 60.95±6.47 μmol/L and 14.17±2.81 μmol/L, respectively. **Conclusion:** Q738X mutation causes hERG channel dysfunction by disruption of tetrameric assembly of hERG channels. Propofol might induce QT prolongation via a direct inhibition of current through the hERG channel, especially in the presence of other triggering factors such as genetic variants in hERG.

Keywords: propofol; QT interval; hERG gene; Q738X mutation; hERG protein trafficking

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S6.31

Protective effects of luteolin-7-O-glucoside from Dracocephalum Rupestre Hance on doxorubicin-treated H9c2 cardiomyocytes through PTEN/Akt pathway

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Aim: Previous research has shown that luteolin-7-O-glucoside pretreatment had a significant protective effect against doxorubicin-induced cytotoxicity. It could reduce cardiomyocyte apoptosis, intracellular calcium overload, leakage of creatine kinase and lactate dehydrogenase, and reactive oxygen species generation induced by doxorubicin. But the mechanisms are unclear. In this study, the effects and mechanisms of luteolin-7-O-glucoside on H9c2 cells were investigated. **Methods:** The cardiomyocyte morphology of each group was detected; total protein concentrations were determined with BCA kit; the expression of PTEN, p-PTEN, Akt and p-Akt were investigated by Western blotting. **Results:** Compared with normal group, the cells of DOX group rounded, cell shape is irregular, cell debris increased significantly, while the cell morphological damage of groups treated with luteolin-7-O-glucosid 24 h is reduced. In DOX group, the expression of p-PTEN and p-Akt were lower than normal group. In the groups treated with luteolin-7-O-glucosid (20 μmol/L), the expressions of p-PTEN and p-Akt protein were up-regulated. The expression of Akt was similar and the expression of PTEN was increased. **Conclusion:** Luteolin-7-O-glucoside could protect cardiomyocytes from doxorubicin-induced cytotoxicity through PTEN/Akt pathway.

Keywords: luteolin-7-O-glucoside; doxorubicin; PTEN; Akt

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S6.32

Effects of taurine-magnesium coordination compound on abnormal inward rectifier potassium channel current induced by hypoxia-reoxygenation in cardiomyocytes of rats

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Aim: To investigate the anti-arrhythmic mechanism of taurine magnesium coordination compound (TMCC) on abnormal inward rectifier potassium current (I_{K1}) induced by hypoxia-reoxygenation in cardiomyocytes of rat. **Methods:** Enzymatic dissociation which through Langendorff retrograde aortic perfusion was used to get single rat ventricular myocytes. In voltage clamp mode, whole-cell patch clamp was used to record I_{K1} in normal cardiomyocytes and single rat ventricular cardiomyocytes of arrhythmia induced by hypoxia-reoxygenation. **Results:** TMCC had no effect on I_{K1} from normal cardiomyocytes. Compared to the control group, the inward current peak of I_{K1} in the hypoxia-reoxygenation model was reduced significantly (-13.05±1.431 pA/pF *vs* -6.94±0.59 pA/pF, $P<0.01$), the inward current I–V curve of I_{K1} was shifted upward. Compared with hypoxia/reoxygenation group, TMCC (100, 200, and 400 μmol/L) restored the inward current peak of I_{K1} to (-7.21±0.79) pA/pF, (-7.28±0.22) pA/pF, (-10.96±0.78) pA/pF ($n=6$, $P<0.01$), and 24.24 μmol/L amiodarone restored it to (-8.80±0.97) pA/pF, and shifted the I–V curve downward. Besides, compared to the control group, the outward current peak of I_{K1} in the hypoxia-reoxygenation model was reduced significantly (2.43±0.32 pA/pF *vs* 1.31±0.28 pA/pF, $P<0.01$), the outward current I–V curve of I_{K1} was shifted downward. Compared with hypoxia/ reoxygenation group, TMCC (100, 200, and 400 μmol/L) restored the outward current peak to (0.90±0.14) pA/pF, (1.84±0.46) pA/pF, (2.36±0.40) pA/pF, and 24.24 μmol/L amiodarone restored it to (2.16±0.69) pA/pF, shifted the I–V curve upward. **Conclusion:** TMCC has no effect on the I_{K1} in normal cardiomyocytes. However, TMCC can restore the inward current and the outward current of I_{K1}, which is reduced by hypoxia-reoxygenation. It suggests that the effect may be one of the mechanisms of anti-hypoxia-reoxygenation-induced arrhythmias of TMCC.

Keywords: taurine magnesium coordination compound (TMCC); arrhythmia; ventricular cardiomyocytes; inward rectifier potassium channel; whole-cell patch clamp technique

S6.33

Cardioprotective effect of selective sigma-1 receptor agonist SA4503 in transverse aortic constriction mice

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Aim: We previously reported that a SSRI, fluvoxamine with high affinity for sigma-1 receptor, ameliorates transverse aortic constriction (TAC)-induced heart failure (Am J Physiol HCP, 2010; 299: H1535–1545). We here investigated cardioprotective effects of SA4503, selective sigma-1 receptor agonist, in TAC-induced heart failure model mice. **Methods:** Male ICR mice were subjected to TAC. SA4503 (0.1, 0.3, and 1.0 mg/kg) and NE100, selective sigma-1 receptor antagonist (1.0 mg/kg), were administered orally once a day for 4 weeks starting from 2 weeks after TAC. Cultured cardiomyocytes were treated with SA4503 (0.1–1 μmol/L) after hypertrophy induced by angiotensin II (Ang II). **Results:** Chronic SA4503 administration significantly attenuated myocardial hypertrophy and improved the impaired fractional shortening in heart function. SA4503 also restored decreased sigma-1 receptor expression following TAC. Interestingly, ATP contents in the left ventricle significantly decreased 6 weeks after TAC and it was significantly restored by chronic SA4503 administration. Sigma-1 receptor stimulation with SA4503 also significantly inhibited Ang II-induced cardiomyocyte hypertrophy in culture. **Conclusion:** Selective sigma-1 receptor agonist, SA4503 ameliorates cardiac hypertrophy and heart failure by restoring mitochondrial ATP production, thereby eliciting anti-hypertrophic action via cardiac sigma-1 receptor stimulation.

Keywords: heart failure; cardiac hypertrophy; Sigma-1 receptor; SA4503

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S6.34

Inhibitiory effect of evodiamine on angiotensin II- induced proliferation in rat vascular smooth muscle cells

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Aim: To investigate the effects of evodiamine (Evo), a component of *Evodiarutaecarpa* (*Juss*) Benth, on vascular smooth muscle cells (VSMC) proliferation induced by angiotensin II (Ang II) and explore the potential mechanisms. **Methods:** In the cultured VSMC, the effect of evodiamine on the proliferation of VSMC was observed by MTT assay and activity of nitric oxide synthetase (NOS), content of nitric oxide (NO) in the supernatant liquid of culture cells were measured. The flow cytometry was used to analyze the cell cycle. The expressions of extracellular signal-regulated kinase-1 (ERK-1), proliferating cell nuclear antigen (PCNA), c-myc mRNA and mitogen-activated protein kinase phosphatase-1 (MKP-1) protein were assessed using RT-PCR and Western blotting, respectively. **Results:** AngII significantly induced VSMC proliferation. Evodiamine 0.1 and 1 μmol/L could significantly attenuated AngII-induced VSMC proliferation, decreased Ang II - enhanced MTT intensity of VSMC, increased the NOS activity and NO production in the supernatant liquid of culture cells, decreased the S phase cells and increased the cell percentage in G_0/G_1 phase, up-regulated the eNOS mRNA expression, suppressed Ang II-induced over-expressions of c-myc, PCNA and ERK-1 mRNA .At the same time, MKP-1 protein expression was up-regulated. **Conclusion:** Evodiamine is effective against Ang-II induced VSMC proliferation. The mechanisms may be related to endogenous NO production and release, down-regulation of the expression of ERK-1 mRNA and up- regulation of the expression of MKP protein.

Keywords: evodiamine; vascular smooth muscle cells; proliferation; ERK-1; MKP-1

S6.35

T7, a novel histone deacetylase inhibitor, suppresses vascular endothelial growth factor receptor signaling and angiogenesis

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Aim: Cancer remains a major cause of mortality around the world. The metastatic spread of tumor cells is associated with resistance to conventional therapy and is the leading cause of death for cancer patients. Tumor cells produce a range of growth factors that promote tumor metastasis by stimulating angiogenesis, which is one of the major routes for tumor invasion and metastasis. It thus represents a rational target for therapeutic intervention. Hydroxanates and its derivatives have attracted considerable attention, due to their wide spectrum of activities including anti-tumor activities. **Methods:** In an effort to develop novel inhibitors to suppress angiogenesis and tumor growth, we selected T7, a novel aliphatic hydroxamate, and characterized its anti-angiogenic activities. **Results:** T7 concentration-dependently inhibited vascular endothelial growth factor (VEGF)-induced proliferation, migration and endothelial tube formation in human umbilical endothelial cells (HUVECs). T7 also attenuated VEGF-induced microvessel sprouting from aortic rings *ex vivo* and markedly reduced HCT116 colorectal cancer cells-induced angiogenesis *in vivo*. In addition, T7 inhibited the phosphorylation of VEGFR2, Akt and ERK in HUVECs exposed to VEGF. **Conclusion:** This study provides evidence that T7 modulates vascular endothelial cell remodeling and leads to the inhibition of tumor angiogenesis. These results also support the role of T7 as a potential drug candidate and warrant the clinical development in the treatment of cancer.

Keywords: angiogenesis; hydroxamate; HUVEC; VEGF

S6.36

Moxifloxacin: cardiovascular effects in conscious, jacketed cynomolgus monkeys following oral (Nasogastric intubation) administration

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Aim: Moxifloxacin is a fluoroquinolone with a broad spectrum of antibacterial activity. The aim of this study was to investigate the cardiovascular effects of moxifloxacin, administered to conscious, jacketed cynomolgus monkeys as single oral doses. This was a validation study performed to assess the new DSI Jacketed External Telemetry (JET) system recently installed at CDSER. **Methods:** There were 6 animals (3 males and 3 females) in two groups. Animals of the same gender were group-housed in the same cage. Animals received 4 doses of vehicle control (3 females) or vehicle control followed by moxifloxacin (3 males) with single ascending doses at 15, 45, and 135 mg/kg. All animals had at least 3 to 4 dose-free days between each dose level. The JET system (Data Science International, USA), including two receivers and 6 devices, was used for continuous ECG data and body temperature (surface) collection for approximately 1 h before, until 24 h after each dose. **Results:** Administration of moxifloxacin to group-housed conscious male primates was associated with a dose-dependent prolongation of QTc Interval (increase of 9% and 16% *vs* time-matched vehicle data after 45 and 135 mg/kg, respectively). In female monkeys, administered multiple doses of vehicle, QTc was comparable across consecutive dosing days. The results demonstrate the usefulness of the JET system in conscious monkeys for evaluating the cardiovascular effects of drugs under normal physiological conditions.

Keywords: moxifloxacin; cardiovascular effects; Jacketed External Telemetry; QTc interval

S6.37

Modulation effect of berberine on adenosine-5'-monophosphate kinase activity in ischemia reperfusion heart injury in rat

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Aim: Berberine exhibits numerous pharmacological effects, the mechanism for its protective effects against cardiac ischemia-reperfusion injury is investigated. **Methods:** Male Wistar rats were treated with berberine (100 mg/kg per day, ig) for 14 d and controls rats were treated with water. Hearts were isolated *in vitro* and perfused in the Langendorff mode and subjected to 30 min of global ischemia followed by 30 min of reperfusion and hemodynamic data were examined. In a separate set of experiments, hearts were subjected *in vivo* to left anterior descending coronary artery ligation for 30 min followed by 120 min reperfusion and hemodynamic data, type and duration of arrhythmias, and myocardial infarct size were determined. AMP-activated protein kinase (AMPK) level, ADP/ATP and AMP/ATP ratios were examined in non-ischemic areas and risk areas of the heart. **Results:** Subsequent to ischemia-reperfusion injury, left ventricular developed pressure, left ventricular end diastolic pressure and maximum rate of intraventricular pressure contractility and relaxation were significantly improved in the berberine treatment groups compared to controls. Berberine treatment decreased infarct size and diminished the duration and incidence of arrhythmias compared to controls. Berberine treatment significantly decreased AMPK protein concentration, and the ratio of ADP/ATP and AMP/ATP in the myocardial risk areas. In contrast, berberine treatment significantly increased AMPK protein concentration, and the ratio of ADP/ATP and AMP/ATP in the non-ischemia areas compared to controls. **Conclusion:** These findings suggest that berberine may exert its cardioprotective effect on ischemia-reperfusion injury via regulation of AMPK activity in both non-ischemic areas and risk areas of the heart.

Keywords: berberine; ischemia-reperfusion; left ventricular function; AMPK

S6.38

Potential applications of disintegrins in arterial thrombosis, cancers and integrin-related diseases

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Viperidae snake venom proteins affect blood coagulation and platelet function in a complex manner. Among them, the most efficacious anti-platelet constituents are Arg/Lys-Gly-Asp containing disintegrins (polypeptides with 47–84 amino acid residues), the specific antagonists of platelet alphaIIbbeta3 (*ie*, glycoprotein IIb/IIIa).

Integrins are a superfamily of noncovalently associated alpha, beta-heterodimeric cell adhesion molecules, mediate cell-cell and cell-extracellular matrices interaction, and play important roles in platelet aggregation, inflammatory reactions, tissue remodeling, angiogenesis and tumor metastasis. In this report, we briefly describe how disintegrins were discovered and developed into clinical drugs (*ie*, Aggrastat and Integrilin) for preventing restenosis of coronary arteries in myocardial-infarcted patients undergoing percutaneous coronary intervention.

Since disintegrins have been found to bind alphaVbeta3, alpha5beta1 or alpha4beta1 which are expressed on vascular endothelial cells, phagocytes and tumor cells, their potential applications in integrin-related diseases, such as angiogenesis, tumor metastasis and septic inflammatory syndromes are explored. For example, a genetic derivative of disintegrin, rhodostomin (Rn), possessing a much higher affinity toward alphaVbeta3 with a greatly reduced affinity to platelet alphaIIbbeta3, has been found to be efficacious *in vivo* in inhibiting tumor growth in those highly alphaVbeta3-expressing tumor cells such as glioma, melanoma and renal carcinoma through suppressing angiogenesis, and osteoponetin- or vitronectin-dependent tumor survival and migration. On the other hand, we demonstrated that the wild-type Rn interferes with the activation of monocytes/macrophages triggered by lipopolysaccharide (LPS) and Pam3CSK4, through interrupting the crosstalk between alphaVbeta3 integrin and toll-like receptors (including TLR4 and TLR2), suggesting that the protective function of Rn against both Gram-negative and –positive bacteria activated phagocytes may attribute to its anti-TLRs activation and the subsequent antiinflammatory activity.

Keywords: disintegrin; thrombosis; angiogenesis; cancer; sepsis

S6.39

Protective effect of baicalein against myocardial ischemia-reperfusion injury in rats

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Aim: To investigate protective effect of baicalein on myocardial ischemia-reperfusion injury in rats. **Methods:** The myocardial ischemia-reperfusion injury model was established in rats by ligating left anterior descending coronary artery. The contents of Na^+-K^+-ATPase, Ca^{2+}-ATPase, lactic acid (LD), lactate dehydrogenase (LDH), and creatine kinase (CK) in myocardium were determined by chromatometry. Degree of myocardiolysis was observed by microscope. Expression of Bcl-2 and Bax proteins in myocardium were determined by immunohistochemistry, and the ratio of Bcl-2/Bax proteins was calculated. **Results:** Experimental results *in vivo* showed that baicalein significantly increased the contents of Na^+-K^+-ATPase,Ca^{2+}-ATPase, LDH and CK; reduced the contents of lactic acid in myocardium. In addition, baicalein also reduced the expression of Bax proteins in myocardium and increased the ratio of Bcl-2/Bax proteins. **Conclusion:** Baicalein can exerts protective effect on myocardial ischemia-reperfusion injury in rats, the mechanisms were likely due to reducing LDH and CK release from myocardial cells, improving energy metabolism and inhibiting the apoptosis of myocardial cells.

Keywords: baicalein; myocardial ischemia; reperfusion injury

S6.40

Vascular oxidative stress against nitric oxide bioavailability

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Vascular inflammation is associated with oxidative stress which is one of key initiators of the development of vascular dysfunction in hypertension and diabetes. Identifying new regulators and intracellular signaling pathways involved in either production or scavenging of reactive oxygen species may help to reveal novel therapeutic targets in drug discovery. Our recent studies indicate that bone morphogenic protein 4 (BMP4) is likely to act as a common initiator for endothelial cell dysfunction involving up-regulation of COX-2 in hypertension and diabetes; targeting BMP4 instead of COX-2 could avoid COX-2 inhibition-related side effects when aiming to reduce vascular events. Most recently, we have shown that the activation of vitamin D receptor curtails the activity of the AT1 receptor-oxidative stress axis in arteries from hypertensive rats and human, which could become a novel strategy to ameliorate hypertension-associated vascular dysfunction. Beneficial molecules such as adiponectin derived from adipose tissues are effective as paracrine factors in improving endothelial function partly via reducing oxidative stress in the vascular wall as we indicated in our previous publication that adipose tissue could be an important intervention target for newly developed PPARγ agonists in the alleviation of diabetic vasculopathy. Searching for novel modulators of oxidative stress-mediated cellular activities may facilitate to develop effective and hopefully safer therapeutics against vasculopathy in hypertension and diabetes.

S6.41

Gas molecules as regulators in cardiovascular pharmacology

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Nitric oxide (NO) is an important paracrine mediator of vascular and platelet function and is produced in the vasculature by the enzyme endothelial NOS (eNOS). We demonstrated in human platelets that the polymerization state of β-actin crucially regulated the activation state of eNOS, and hence NO formation, through altering its binding of heat shock protein 90. And association of globular actin with eNOS plays an essential and necessary role in agonist-induced eNOS activation, through enabling its phosphorylation by Akt at serine residue 1177 in HUVEC.

Aspirin activates basal platelet NOS acutely, but not chronically. By contrast, both short- and long-term aspirin treatment inhibits platelet β-adrenergic NOS activation. This indicates that aspirin exerts divergent effects on basal and β-AR-stimulated platelet NOS activity. Further study suggests that aspirin acetylates eNOS acutely in platelets, and this causes an increase in its activity as well as a decrease in its phosphorylation.

Atherosclerosis is associated with reduced vascular hydrogen sulfide (H_2S) biosynthesis. GYY4137, a slow-releasing H_2S compound, may effectively mimic the time course of H_2S releasing *in vivo*. In our study, GYY4137 inhibits lipid accumulation induced by ox-LDL in RAW 264.7 cells, decreases vascular inflammation and oxidative stress, improves endothelial function and reduces atherosclerotic plaque formation in apo$E^{-/-}$ mice.

S6.42

Effects of increased blood pressure variability on platelet adhesion and aggregation *in vivo* and *in vitro*

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Aim: Increased Blood pressure variability (BPV) plays a significant role in thrombotic target organ damage in hypertension, and platelet activation takes part in thrombosis. This study was designed to investigate the effect of increased BPV on platelet adhesion and aggregation. **Methods:** Male Sprague-Dawley rats underwent sinoaortic denevated (SAD) or sham operation. At 8 weeks after SAD or sham operation, platelet aggregation induced by ADP and collagen was measured. Platelet adhesion to collagen was evaluated with a perfusion chamber. The expression of P-selectinon on platelet surface was determined with flow cytometry. Moreover, an increased BPV model *in vitro* was established when pressure was kept constant with increased pressure variability in a channel. Platelet adhesion to collagen was also determined. **Results:** Compared with sham-operated rats, platelet aggregation induced by ADP and collagen were higher in SAD rats. Additionally, platelet adhesion to collagen and expression of platelet surface P-selectin increased significantly in SAD rats and in *in vitro* test. Moreover, platelet adhesion was positively related to pressure variability without relation to pressure *in vitro*. **Conclusion:** Increased BPV may enhance platelet activation.

Keywords: blood pressure; blood pressure variability; sinoaortic denervation; platelet activation
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S6.43

Inhibition of angiogenesis by HDAC inhibitor ZYJ-34c *in vitro*

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Aim: A tetrahydroisoquinoline-based hydroxamic acid derivative (ZYJ-34c) as histone deacetylase inhibitor, inhibited the growth of human breast carcinoma (MDA-MB-231) xenograft, a mouse hepatoma-22 (H22) pulmonary metastasis and the growth of a human colon tumor (HCT116) xenograft. Angiogenesis is critical to tumor growth and is also a potential target for cancer therapy. In this study, the inhibition of angiogenesis by ZYJ-34c *in vitro* was investigated. **Methods:** The human umbilical vascular endothelial cells (HUVECs) proliferation was assayed by MTT methods. Anti-angiogenesis activity of ZYJ-34c was evaluated by Matrigel HUVECs tube formation assays and rat aortic ring assay *in vitro*. Suberoylanilide hydroxamic acid (SAHA) was used as positive control. **Results:** The MTT assay indicated that ZYJ-34c suppressed HUVECs proliferation with an lower IC_{50} value (1.12±0.21 μmol/L) compared to SAHA IC_{50} value (3.52±0.65 μmol/L). Anti-angiogenesis activity assays showed that the tube formation of HUVECs and sprouting of endothelial tubules from rat aortic rings was reduced in a concentration-dependent fashion. **Conclusion:** Our results indicate that ZYJ-34c showed anti-angiogenic activity *in vitro*, and that angiogenesis inhibition may be one mechanism of anti-cancer effect.

Keywords: histone deacetylase inhibitor; ZYJ-34c; anti-angiogenesis
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S6.44

Effect of protein tyrosine phosphatase inhibitor in the regulation of atrial ANP release

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Aim: It was reported that phosphoprotein is involved in the regulation of ANP release. In this study, the effect of potassium bisperoxo(1,10-phenanthroline) oxovanadate [bpv(phen)], a protein tyrosine phosphatase inhibitor, in the regulation of atrial ANP release was investigated. **Methods:** Experiments have been done in beating rabbit atria allowing measurements of changes in atrial dynamics, transmural extracellular fluid translocation, cAMP efflux and ANP release. **Results:** Administration of bpv(phen) increased atrial myocytic ANP release without significant changes in atrial dynamics. Atrial cyclic AMP efflux was not significantly changed. ANP increased slowly but steadily during the presence of bpv(phen). In the presence of bpv(phen), PMA increased ANP release which was not significantly different from the response without bpv(phen). Go6976, a protein kinase C selective inhibitor, blocked bpv(phen)-induced activation of ANP release. **Conclusion:** These findings suggest that bpv(phen) increases atrial myocytic ANP release via protein kinase C signaling.

Keywords: atrial natriuretic peptide; protein kinase C; protein tyrosine phosphatase
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S6.45

Aldosterone metabolism and nonsteroidal anti-inflammatory drugs (NSAIDs): A link to increased cardiovascular risk?

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Considerable evidence exists that use of non-selective (*eg,* diclofenac) and cyclooxygenase (COX)-2 selective (*eg*, celecoxib) NSAIDs is associated with increased cardiovascular (CV) risk. Although the mechanism of the increased CV risk associated with NSAIDs is incompletely understood the prevailing view is that the inhibitory selectivity of NSAIDs towards COX-1 and COX-2 accounts for the CV toxicity. Aldosterone (ALDO) is a vasculotoxic molecule that, when present in excess, increases CV risk. This risk is independent of the effects of ALDO on renal electrolyte homeostasis and blood pressure. ALDO is metabolised to tetrahydroALDO (THA), which is excreted in urine as THA-glucuronide**.** ALDO also undergoes direct glucuronidation to form ALDO 18β-glucuronide. ALDO 18β-glucuronide (~40%) and THA-glucuronide (~60%) account for the majority of daily ALDO eliminated in urine. ALDO 18β-glucuronide is formed predominantly in the kidney, with a small component of hepatic 18β-glucuronidation. Using recombinant human UGTs (1A3, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15, 2B17) we established that UGT2B7 catalyses ALDO 18β−glucuronidation, which is inhibited by a variety of NSAIDs. Further in a cohort of rheumatoid arthritis patients chronic use of diclofenac was associated with greater arterial dysfunction in comparison to naproxen, indomethacin and ibuprofen (least arterial dysfunction) and, the association between arterial dysfunction and inhibition of ALDO 18β−glucuronidation was independent of other CV and rheumatologic factors.

Keywords: aldosterone metabolism; NSAIDs; cardiovascular risk

S6.46

Functional analysis of TNNI3K gene and the new drug development for suppressing risk of cardiogenic sudden death

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TNNI3K is a MAP kinase specifically and continuously expressed in cardiac muscle and interacted with cardiac troponin I (TNNI3, or cTnI). The aim of this study is to investigate role of TNNI3K interacting with cTnI in modification of the physiological function of cardiomyocytes and suppression of some pathological injuries and to explain the new molecular mechanism for TNNI3K-mutant in the ischemic coronary heart diseases, and hope to develop some new therapeutic strategies.

In the *in vitro* experiments, 1) Effects of PKC inhibitor (GF109203X) or PKA activator (Br-8-cAMP) on the beating frequency were investigated in P19CL6-derived culture beating cardiomyocytes. Results showed that TNNI3K-overexpression increased the contractility and beating frequency together with restrained phosphorylation of cTnI through activation of the PKA pathway but not by blockade of the PKC pathway. 2) Availability of plasma TNNI3K level was investigated using with anti-TNNI3K poly-antibodies in patients diagnosed as AMI (n=98), chronic heart failure (CHF, n=105) and acute renal failure (ARF, n=56); and in healthy volunteers (n=100). Data shown that circulating TNNI3K levels were significantly higher in AMI (P<0.001) when compared with other two groups, indicating that measurement of circulating TNNI3K level may be a novel and useful tool to diagnose AMI.

In the *in vivo* experiments, a mutated TNNI3K gene obtained from substitution of serine at 835-836 sites with alanine and was directly transfected into beating hearts using an *in vivo* gene electroporation method. Then, incidence of arrhythmic outbreak was examined in 1) control; 2) wild TNNI3K gene, and 3) TNNI3K-mutant gene introduction groups. The incidences of arrhythmias including bigeminy, tachycardia and bradycardia in the TNNI3K-mutant group were significantly higher than that in other two groups. It is expected that the elucidation of the molecular mechanisms would be connected for creation of some new anti-arrhythmic drugs.

S6.47

Role of perivascular adipose tissue in regulation of vascular tone

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Ample evidence indicates that the perivascular adipose tissue (PVAT) plays important roles in regulating vascular tone via releasing vasorelaxing and constricting mediators. Among them, a spontaneously released PVAT-derived relaxing factor (PVATRF) acting by opening the potassium channels on the smooth muscle cell has been proposed. We have demonstrated for the first time that endogenous palmitic acid methyl ester (PAME) released in the superior cervical ganglion of the rat is a potent vasodilator (Lin *et al*, PNAS 105: 19526–31, 2008). We therefore propose that PAME is the PVATRF. We have observed that

isolated aortic PVAT preparations of the normotensive Wistar Kyoto rats (WKY) spontaneously and calcium dependently release PVATRF (estimated by relaxation of bioassay aortic muscle ring) and PAME (estimated by GC/MS). Relaxation of aortic muscle rings induced by both PVATRF and PAME is mediated by opening the voltage-dependent potassium (Kv) channel. Aortic relaxations induced by PVATRF- and PAME-containing Krebs solutions are heat resistant and are equally attenuated after hexane extractions. Culture mediums of differentiated adipocytes, but not those of fibroblasts, contain significant PAME, causing vasorelaxation. On the other hand, the PVAT preparations of the spontaneously hypertensive rat (SHR) release significantly less PVATRF and PAME with an increased release of angiotensin II (Ang II). PAME-induced relaxation of the SHR aortic smooth muscle also diminishes drastically compared to that of the WKY. Both diminished PAME release and PAME-induced vasorelaxation are due to Ang II activation of AT_1 receptors and is ameliorated by losartan. These results indicate that both PAME and PVATRF exhibit similar biochemical and pharmacological characteristics, suggesting that PAME is a major PVATRF, and, together with other PVAT-derived mediators, plays noble roles in vascular tone regulation and pathogenesis of hypertension (Lee *et al*, Circulation 124: 1160–71, 2011). Our findings further suggest that the antihypertensive effect of losartan is partly attributed to increased PAME release in the PVAT and reversal of Ang II-initiated diminishment of PAME-induced vasorelaxation, offering a new strategy in managing systemic hypertension.

S6.48

Thymoquinone causes endothelium-dependent contractions depending on activation of soluble guanylyl cyclase in isolated rat arteries

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Aim: Experiments were designed to determine the endothelium-dependent and independent effects of thymoquinone in isolated rat arteries. **Methods:** Rings, with or without endothelium, of rat mesenteric arteries or aortae were suspended in conventional organ chambers for isometric tension recording. Certain rings were incubated with inhibitors of nitric oxide (NO) synthase [N_ω-nitro-*L*-arginine methyl ester (*L*-NAME)] or soluble guanylyl cyclase [1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ)]. They were contracted with phenylephrine and exposed to increasing concentrations of thymoquinone, thymol or 1,4-benzoquinone. **Results:** Thymoquinone caused a sustained further increase of tension in mesenteric arterial rings with endothelium. This augmentation was prevented by endothelium-removal, *L*-NAME and ODQ. Incubation with the NO-donor diethylenetriamine NONOate (detaNONOate) in *L*-NAME-treated rings restored the contractile response compared to thymoquinone. Similar results were obtained in isolated aortae. Endothelium-dependent, NO-dependent augmentation was obtained also with 1,4-benzoquinone but not with thymol. **Conclusion:** In rat arteries, thymoquinone causes endothelium-dependent augmentation which requires endothelium-derived NO and activation of soluble guanylyl cyclase. This effect is due to the quinone moiety of the compound.

Keywords: 1.4-benzoquinone; detaNONOate; *L*-NAME; nitric oxide; ODQ; phenylephrine, thymol

S6.49

Histone deacetylase inhibition attenuates development of hypertension through mineralocorticoid receptor acetylation

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Background: Inhibition of histone deacetylases (HDAC) results in attenuated development of hypertension in DOCA-induced hypertensive rats and spontaneously hypertensive rats. However, the molecular mechanism remains elusive. **Aim:** We hypothesized that HDAC inhibition (HDACi) attenuates transcriptional activity of mineralocorticoid receptor (MR) through its acetylation and prevents development of hypertension in DOCA-induced hypertensive rats. **Methods:** Expression of MR target genes was measured by quantitative real-time PCR (qRT-PCR). Recruitment of MR and RNA polymerase II on promoters of target genes was analyzed by chromatin immunoprecipitation (ChIP) assay. Live cell imaging was performed for visualization of nuclear translocation of MR. MR acetylation was determined by Western blot with anti-acetyl-lysine antibody after immunoprecipitation (IP) with anti-MR antibody. Transcriptional activity of MR was determined by luciferase assay. For establishment of a hyperaldosteronism animal, Sprague-Dawley rats underwent uninephrectomy and received subcutaneous injection of 40 mg/kg per week of deoxycorticosteron acetate (DOCA) as well as drinking water containing 1% NaCl. **Results:** Treatment with a HDAC class I inhibitor resulted in reduced expression of MR target genes in accordance with reduced recruitment of MR and RNA polymerase II (Pol II) on promoters of target genes. HDACi promoted MR acetylation, leading to decreased transcriptional activity of MR. Knockdown or inhibition of HDAC3 resulted in reduced expression of MR target genes induced by mineralocorticoids. **Conclusion:** These results indicate that HDACi attenuates transcriptional activity of MR through its acetylation and prevents development of hypertension in DOCA-induced hypertensive rats.

Keywords: HDAC; HDAC inhibitor; mineralocorticoid receptor; DOCA; hypertension

S6.50

DL0805 inhibits angiotensin II-induced vasoconstriction in a Ca^{2+}-dependent and Ca^{2+}-independent manner via blocking AT_1R in the rat thoracic aortic rings

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Aim: To investigate the effects of DL0805, a Rho kinase inhibitor, on the vasoconstriction activity induced by angiotensin II (Ang II) in the rat thoracic aortic rings and to explore its vasodilative mechanisms. **Methods:** The isotonic contractions of the thoracic aortic rings from SD rats were recorded, and the effects of DL0805 on the single dose and concentration cumulative response curves of Ang II were recorded. Intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) was measured with Fura2/AM in VSMCs. The phosphorylation levels of myosin light chain 2 (MLC2) and myosin phosphatase target unit 1 (MYPT1), and protein level of Rho kinase 1 (ROCK1) in the rat aortic rings were detected by Western blot. **Results:** DL0805 induced relaxation in the rat aortic rings through endothelium-dependent and -independent pathways. DL0805 (5, 25, and 50 μmol/L) shifted the concentration-response curve of Ang II to right with non-parallel manner. The response was depressed and the p$D'2$ value was 4.65±0.04. DL0805 (1 and 10 μmol/L) inhibited both Ang II-induced Ca^{2+} release from internal stores and Ca^{2+} influx. DL0805 (25 and 50 μmol/L) significantly attenuated the phosphorylation levels of MLC2 and MYPT1, and the increased protein level of ROCK1 in the aortic rings stimulated by Ang II. **Conclusion:** DL0805 inhibits the vasoconstriction induced by Ang II in a Ca^{2+}-dependent and Ca^{2+}-independent manner via blocking angiotensin type 1 receptor (AT_1R) in the rat thoracic aortic rings.

Keywords: DL0805; angiotensin II; rat thoracic aortic rings; [Ca^{2+}]$_i$; Rho kinase

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Scutellarin repairs endothelium dependent relaxation damage induced by ischemia reperfusion

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Aim: The purpose of this study was to determine the effects of SCU on vascular endothelium dependent relaxation injury induced by ischemia reperfusion (IR) and the underlying mechanisms. **Methods:** Rat myocardial and cerebral IR models, blood vessel hypoxia reoxygenation (HR) models of coronary artery (CA) and cerebral basilar artery (BA) were used. Vascular endothelium dependent relaxation was assessed by acetylcholine (ACh)-induced dilation in Wire Myograph system. **Results:** In rat myocardial and cerebral IR animal model, SCU (45 and 90 mg/kg, iv) significantly reduced ischemic area and infarct volume; SCU (45 mg/kg, iv) remarkably repaired ACh-induced relaxation; cGMP dependent protein kinase

(PKG) inhibitor Rp-8-Br-cGMPS (50 μg/kg, iv) blocked the reduction of ischemia area and infarct volume. In isolated CA/BA rings, HR impaired endothelial relaxation, but pre-incubation with SCU (100 and 500 μmol/L) repaired it; Rp-8-Br-cGMPS and Rp-8-Br-PET-cGMPS (4 μmol/L) significantly blocked it. **Conclusion:** SCU prevents against myocardial and cerebral IR-induced vascular endothelium dependent relaxation injury which is related with PKG.

Keywords: scutellarin; ischemia reperfusion; endothelial dependent relaxation

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S6.52

Effect of daidzein on the changing of vasoactive substance in myocardial hypertrophy rats

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Aim: To observe the protective effects of daidzein (DD) on rat's myocardial hypertrophy caused by isopropylarterenol (Iso) and explore the relationship of vasoactive substance. **Methods:** The rat's myocardial hypertrophy model is established through hypodermic injection which extended 10 days at back skin with Iso, 1 mg/kg per day. Once the model is formed, it will be administered various doses of DD, DMSO or NS through hypodermic injection from the second day, and the process will extend 14 days. After the last administration, the rat will be in absolute diet status for 12 h and its blood and heart are taken under anesthesia. The indexes of heart weight was calculated, the concentration of hydroxyproline in myocardium tissue was detected by alkali method, the level of angiotensin II (AngII), endothelin (ET), atrial natriuretic peptide (ANP) and 6-keto-prostaglandinF1alpha, (6-keto-PGF1alpha) in plasma were investigated with radioimmunity kits, and the content of NO in serum was estimated by nitrate reductase assay. **Results:** There is obvious difference compared Iso group with control group. Indexes of heart weight and content of hydroxyproline of Iso group are much higher than control one, level of ANP and NO are decreased while AngII and ET are decreased. However, indexes of heart weight is decreased in DD groups, furthermore, ANP, NO and 6-keto-PGF1alpha level are enhanced while AngII and ET concentration are reduced in DD groups. **Conclusion:** DD has a protection effect on rat's myocardial hypertrophy caused by Iso, and its mechanism probably results of modulating the concentration of vasoactive substance such as AngII, ANP, ET, NO and 6-keto-PGF_{1alpha}.

Keywords: daidzein; isopropylarterenol; myocardial hypertrophy; vasoactive substance

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S6.53

Effects of icariin on pulmonary arterial hypertension induced by monocrotaline in rats

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Aim: It has been reported that icariin (ICA), being similar to sildenafil which was used to treat pulmonary arterial hypertension (PAH), is a phosphodiesterase-5 (PDE-5) inhibitor. In this study, the effects of ICA on pulmonary PAH induced by monocrotaline (MCT) were observed in rats. **Methods:** Sprague-Dawley male rats were randomly divided into 6 groups: Control, Model, ICA (20, 40, and 80 $mg \cdot kg^{-1} \cdot d^{-1}$) and sildenafil (25 mg/kg), 12 rats in each group. Rats received a single subcutaneous injection of MCT (50 $mg \cdot kg^{-1}$) to induce PAH model. By the end of the second week, animals were treated with intragastric administration according to groups except rats of the control and model group were given the equal volume saline for 4 weeks. Rats were anesthetized two hours after the last administration. A right heart catheter was inserted through the right jugular vein for measurement of mean pulmonary artery pressure (mPAP) with PowerLap system. The heart was dissected, and the ratio of the right ventricle to left ventricle plus septum weight (RV/LV+ S) was calculated as right ventricular hypertrophy index (RVHI) and the PO_2 and PCO_2 of artery blood were measured by blood-gas analyzer. Precapillary pulmonary arteries histomorphologic changes and the ratio of pulmonary artery smooth muscle layer thickness to vascular diameter were analyzed by hematoxylin and eosin elastica staining of paraffin embedded rat lung tissues. **Results:** Six weeks after a single subcutaneous injection of MCT, the mPAP of animals notably increased nearly threefold, with a concomitant decline of PO_2 and increasing of PCO_2. Marked right ventricular hypertrophy was evident, and the precapillary artery smooth muscle layer thickening was histologically apparent with some animals dying during this period because of right heart failure. When administered from weeks 3–6, ICA (40–80 $mg \cdot kg^{-1} \cdot d^{-1}$) reversed significantly the development of mPAP, remodeling of vascular and right heart hypertrophy with preservation of gas exchange. **Conclusion:** ICA can alleviate rat PAH induced by MCT.

Keywords: icariin; pulmonary arterial hypertension; monocrotaline; rats

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S6.54

Inhibition of Rho-kinase ameliorates myocardial remodeling and fibrosis in pressure overload and myocardial infarction: Role of TGF-β1-TAK1

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Aim: Inhibition of Rho-kinase displays vasodilation property although its effect on cardiac remodeling in heart against pressure overload and ischemia has not been fully elucidated. The present study was designed to examine the effect of fasudil, a Rho-kinase (ROCK) inhibitor, on myocardial remodeling and underlying mechanisms in pressure overload and myocardial infarction (MI) mice. **Methods:** Pressure overload was produced by constriction of the transverse aorta (TAC) for 3 weeks. Left ventricular (LV) geometry, cardiac hypertrophy, fibrosis, and remodeling were evaluated by transthoracic echocardiography and cardiac histology. Expressions of the hypertrophic and profibrotic markers were analyzed in TAC and MI mice with or without fasudil treatment. **Results:** LV cavity dilatation and dysfunction evaluated by echocardiography were significantly suppressed in the fasudil-treated MI group compared with the MI group ($P<0.05$); however, there were no significant difference between the TAC group and the fasudil-treated TAC group. Inhibition of ROCK exhibited reduced interstitial fibrosis, which was observed both in TAC and MI mice ($P<0.05$). The beneficial effects of fasudil were closely associated with the change of the specific profibrotic gene expression and TGF-β1-TAK1 pathway. **Conclusion:** Taken together, these results indicate that Rho-kinase is substantially involved in the myocardial remodeling after TAC and MI associated with upregulation of profibrotic gene expression and TGF-β1-TAK1 pathway; further suggest that the protective effect of fasudil on heart against pathological stimuli was through inhibiting reactive fibrosis.

Keywords: Rho-kinase; TAC; MI; myocardial fibrosis; TGF-β1-TAK1

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S6.55

Ligustrazine protects cardiomyocytes from apoptosis and injury in adriamycin-induced acute heart failure mice

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Aim: Ligustrazine is a monomer extracted from the Chinese herb Chuan Qiong. In this study, we examined the protective effect of Ligustrazine on adriamycin (ADR)-induced acute heart failure (AHF) and underlying molecular mechanisms. **Methods:** Male Kunming mice were randomly divided into six groups: Control group (saline), AHF group (ADR 20 mg/kg, twice), different dose Ligustrazine group (15, 30, and 60 $mg \cdot kg^{-1} \cdot d^{-1}$) and enalapril group (10 $mg \cdot kg^{-1} \cdot d^{-1}$). All groups were administered drugs for 2 weeks, then ADR 20 $mg \cdot kg^{-1}$ was given intraperitoneally twice to establish AHF model. Cardiac enzymes including aspartate aminotransferase (AST), creatine kinase (CK) and MB isoenzyme of creatine kinase (CK-MB) were detected. Histopathological examination was performed by hematoxylin-eosin (HE) staining. Western-blot analysis was used to investigate the expression levels of Bax, Bcl-2, and TNF-α. **Results:** AST, CK, and CK-MB of left ventricle of AHF mice showed a significant increase while Ligustrazine treatment decreased their levels in myocardial tissue. Myocardial tissue of AHF mice was damaged and myocardial

fiber arranged disorderly. Myocardial cells showed vacuolar degeneration and necrosis in AHF mice. Administration of Ligustrazine obviously attenuated myocardial damage and myocardial fiber disordered arrangement. Further studies revealed that Ligustrazine decreased the expression of pro-apoptotic factor of Bax and pro-inflammatory cytokine TNF-α while increased the expression of anti-apoptotic factor of Bcl-2. **Conclusion:** Ligustrazine exerts protective effect on adriamycin-induced AHF partially by inhibition of cardiomyocytes apoptosis and inflammatory factor expression

Keywords: Ligustrazine; acute heart failure; adriamycin; cardiac enzymes; apoptosis

S6.56

Delayed myocardial protection by non-invasive limb ischemic preconditioning inhibits cardiomyocyte apoptosis and the fibrolysis system

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Aim: Our past research has shown that transient limb ischemia can induce remote late preconditioning that protects the myocardium from ischemia/reperfusion (I/R). In this study, we further tested if late preconditioning by non-invasive limb ischemia (NLIP) could offer cardioprotective effects against myocardium I/R injury. **Methods:** Sixty male Wistar rats weighing 240–260 g were randomly divided into the three groups: I/R, cardiac ischemic preconditioning (CIP), and NLIP. Myocardial infarct size, myocardial apoptosis after prolonged I/R, and the levels of the controlling genes Bcl-2 and Bax were determined at the end of the experiment. The activity of fibrolysis factors, cardiac troponin I (cTnI) and superoxide dismutase (SOD) were measured before ischemia, after ischemia, and after reperfusion. **Results:** Myocardial infarct size was significantly reduced in the CIP and NLIP groups as compared with the I/R group (P<0.01). Pretreatment with CIP and NLIP significantly decreased the number of apoptotic cells (P<0.05), and the ratio of Bcl-2/Bax was increased (P<0.05). Compared with the I/R group, CIP and NLIP antagonized the decrease in t-PA activity (P<0.05) and the increase in PAI-1 activity (P<0.05), as well as the decrease in cTnI activity (P<0.01) and increase in SOD activity (P<0.05) after reperfusion. **Conclusion:** Remote preconditioning induced by NLIP provides late cardioprotection against myocardium I/R injury.

Keywords: ischemic preconditioning; myocardial apoptosis; myocardial ischemia; fibrolysis

S6.57

Hydroxysafflor yellow A protects against methylglyoxal-induced injury in the cultured human brain microvascular endothelial cell

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Aim: Diabetic individuals gain high levels of methylglyoxal (MGO) and advanced glycation end-products (AGEs) which plays an important role in diabetic vascular complications, such as stroke. Our previous data demonstrated that hydroxysafflor yellow A (HSYA), a major active chemical component of the safflower yellow pigments, had antiglycation effect on AGEs formation *in vitro*, but there is little known about whether HSYA can protect MGO-induced injury in the cultured human brain microvascular endothelial cell (HBMEC). **Methods:** Using cultured HBMEC, cell injury was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) formation, lactate dehydrogenase (LDH) release and AnnexinV/PI staining. AGEs and caspase-3 formation were measured by Western blotting. **Results:** Incubation of MGO for 24 h concentration-dependently induced HBMEC injury, which was protected by HYSA from 10 μmol/L to 100 μmol/L. Further study illustrated the protection of HYSA was probably associated with inhibiting cell apoptosis. What's more, MGO promoted AGEs accumulation in the cultured HBMEC, which was also inhibited by HSYA. **Conclusion:** Thus, our results proved that HSYA could inhibit MGO-induced injury in the cultured HBMEC, which was associated with its antiglycation effect.

Keywords: hydroxysafflor yellow A; methylglyoxal; advanced glycation end-products; human brain microvascular endothelial cell; apoptosis

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S6.58

Osthole attenuates vascular remodeling in balloon-injured rats by inhibiting the expression of NF-κB

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Aim: To examine the effect of Osthole on vascular remodeling in balloon-injured rats and explore its possible mechanisms. **Methods:** After balloon-injured carotid artery of rats model was established, animals were randomly divided into Osthole (20 and 40 $mg \cdot kg^{-1} \cdot d^{-1}$), sham and model groups. Osthole groups were given Osthole for 14 d, while the sham and model groups received the same volume of normal saline instead. After consecutive 14 d, the neointimal hyperplasia level and the degree of vascular smooth muscle cell (VSMC) proliferation were evaluated by calculating the neointimal area (NIA) and the NIA/media area (MA) ratio (NIA/MA). The expression of PCNA and NF-κB (p65) were examined by immunohistochemistry. **Results:** Compared with model group, Osthole could significantly decrease the elevated NIA, NIA/MA percentage. In immunohistochemistry assays, it was found that Osthole could decrease the elevated expression of PCNA and NF-κB (p65). **Conclusion:** Osthole can significantly attenuate the vascular remodeling in balloon-injured rats. The mechanism may be involved in the suppressions of NF-κB .

Keywords: Osthole; ballon-injury; inflammation; NF-κB

S6.59

Telemetric ambulatory arterial stiffness index is associated with aortic stiffness determining factors

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Aim: Ambulatory arterial stiffness index (AASI) has been proposed as a new measure of arterial stiffness for predicting cardio-cerebro-vascular morbidity and mortality. In this study, the direct relationships between AASI and arterial stiffness determining factors have been investigated. **Methods:** We utilized beat-to-beat intra-aortic blood pressure (BP) telemetry to characterize AASI in Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). By determination of aortic structural components and analysis of their correlations with AASI, we provided the first direct evidence for the associations between AASI and arterial stiffness determining factors including the collagen content and the ratio of collagen/elastin. **Results:** AASI was positively correlated with pulse pressure in both WKY and SHR, less dependent on BP and BP variability than pulse pressure, and relatively stable especially the number of BP readings not less than ~36. The correlations between AASI and aortic components were comparable for various AASI values derived from BP readings not less than ~36. Not only AASI but also BP variability and pulse pressure demonstrated a direct relationship with arterial stiffness. **Conclusion:** These findings indicate AASI may become a routine measure in human arterial stiffness assessment. It is recommended to use a cluster of parameters such as AASI, BP variability and pulse pressure which can be simultaneously obtained from the routine ambulatory BP monitoring for evaluating arterial stiffness in different clinical settings.

Keywords: blood pressure telemetry; arterial stiffness; collagen; elastin; stroke

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S6.60

ROS inhibitors improve reduction of Na^+/H^+exchanger (NHE) activity by intermittent hypoxia in neonatal cardiomyocyte

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Aim: To investigate whether ROS inhibitors could improve the effect of intermittent hypoxia (IH) for 1–4 d on ability to recover from acidosis in cardiomyocyte. **Methods:** Neonatal cardiomyocytes were treated with IH (alternating 5% O_2 30 min, 20% O_2 30 min) for 1, 2, 3, and 4 d. Cells were acid loaded with NH_4Cl, and rates of pHi recoveryand acidification were measured by BCECF-AM loading. Level of protein expression was detected by Western blot. Level of ROS concentration was detected by CM-H_2DCFDA and HE. **Results:** We found that the rate of pH_i recovery from acidosis was much slower than room air (RA) group after 4 d of IH, but buffering power did not change after IH. This phenomenon was abolished by amiloride, HOE642 (HNE inhibitor) or Na^+-free NT but not DIDS (Na^+/HCO_3^- cotransporters, NBC inhibitor). We also found intracellular ROS increased significantly. Furthermore, the recovery rate has no difference between RA and

IH treated 1, 10-phenanthroline (Fenton's reaction generated OH·block), SOD (O^{2-} scavenger). **Conclusion:** These results suggest that ROS is a key mediator to inhibit HNE activity in IH. And ROS inhibitor could improve this process.
Keywords: cardiomyocyte; intermittent hypoxia; intracellular pH; ROS

S6.61

Chronic intermittent hypoxia induced changes in NMDA receptor subunits in central autonomic regions

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Aim: Chronic intermittent hypoxia (CIH) enhanced hypertension development in Sprague Dawley (SD) rats and spontaneously hypertensive rats (SHRs) that has been demonstrated in several studies. Activation of NMDA receptors in central autonomic regions (CARs) contributes to elevate the individual blood pressure via control of sympathetic outflow. CIH enhanced infarct size after 14-day CIH in SHRs. This was accompanied by an increase in myocardial HIF-1α activity, HIF-1 linking endothelin-1 (ET1) and ET-A receptor proteins. Because HIF-1α could augment the expression of NMDA receptor subunits in several experimental preparation, our aim is to investigate the involvement of the NMDA receptor subunits and HIF-1α in the cardiovascular consequences of 14-day CIH in SD rats. **Methods:** The CIH was designed by applied 30 s pure nitrogen followed by 45 s air to SD rats during the light phase (6 h) for 14 consecutive days. The expression of NMDA receptor subunits was detected by Western Blotting. **Results:** CIH treatment increases 42.2% and 67.2% over the room air treatment in the expression of NMDA receptor subunits NR1 and NR2A in rostral ventrolateral medulla (RVLM), a sympathoexcitating CAR. On the other hand, CIH only slightly changes the protein level of NR1 and NR2A (8.7% and -10.4%) in the caudal ventrolateral medulla, a sympathoinhibiting CAR. **Conclusion:** The preliminary data implies that the enhancement of the NMDA receptor subunits in the RVLM may result in pressor effect in the SD rats after CIH.
Keywords: chronic intermittent hypoxia; central autonomic regions; NMDA

S6.62

Effect of carvedilol in attenuating the acute myocardium infarction-induced myocardial fibrosis in rat

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Aim: Carvedilol, nonselective β-adrenoreceptor antagonist, was shown protective effects against acute myocardium infarction(AMI)-induced myocardial injury, however, the mechanisms underlying the anti-fibrosis effect of carvedilol has not been well known. The aim of the present study was to investigate the potential mechanism for the anti-fibrosis effect of carvedilol against AMI-induced myocardial fibrosis in rat. **Methods:** Male SD rats were randomized into the sham group, LAD surgery-AMI model group, AMI plus low dose of carvedilol treatment group (1 mg/kg per day, CAR-L), AMI plus medium dose of carvedilol treatment group (5 mg/kg per day, CAR-M) and AMI plus high dose of carvedilol treatment group (10 mg/kg per day, CAR-H). The passage 3 neonatal SD rat cardiac fibroblasts were used for hypoxia/normoxia (2 h/4 h) treatment in the presence of carvedilol (0, 1, 2 and 4 μmol/L). **Results:** Cardiac remodeling and impaired heart function were observed after 4-week LAD surgery treatment, however, and the cardiac remodeling and decreased ejection fraction (EF%) and fractional shortening (FS%) were efficiently rescued in the CAR-M and CAR-H groups. The up-regulated expression of Col1a1, Col3a1 and α-SMA at mRNA and protein level was significantly reduced in the CAR-M and CAR-H groups. The *in vitro* study showed that Col1a1, Col3a1 and α-SMA expression both at mRNA and protein level were down-regulated by carvedilol in rat cardiac fibroblasts in a dose-dependent manner. Smad3 inhibitors, SIS-3 and naringenin, could efficiently decrease Col1a1, Col3a1 and α-SMA expression in rat cardiac fibroblasts. Activated Smad3 was shown significantly reduced in carvedilol-treated rat cardiac fibroblasts. **Conclusion:** Carvedilol negatively regulates Smad3 signal pathway and inhibits extracellular matrix related Col1a1, Col3a1 and α-SMA expression, contributing to the anti-fibrosis effect of carvedilol against AMI-induced myocardial fibrosis in rat.
Keywords: carvedilol; AMI; extracellular matrix; myocardial fibrosis
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S6.63

The protective action of ketanserin against LPS-induced shock in mice is mediated by inhibiting iNOS expression via the MEK/ERK pathway

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Aim: Nitric oxide (NO) plays an important role in the pathogenesis of endotoxic shock. The present work tested the hypothesis that ketanserin could attenuate endotoxic shock by inhibiting the expression of iNOS. **Methods and Results:** The results demonstrated that ketanserin could inhibit iNOS expression in the heart, lungs, liver and kidneys and nitrate production in the serum upon endotoxic shock in mice. In RAW264.7 cells, ketanserin significantly inhibited the expression of iNOS, and decreased the production of NO, TNFα, IL-6, and reactive oxygen species (ROS) upon LPS challenge. Ketanserin also increased the level of ATP and mitochondrial membrane potential in RAW264.7 cells upon LPS exposure. LPS-induced iNOS expression was inhibited by the 5-HT_{2A} receptor antagonist ritanserin, and not the α_1 receptor antagonist prazosin. Knockdown of 5-HT_{2A} receptor by siRNA abolished the inhibitory effect of ketanserin on the expression of iNOS. These results indicated that the inhibitory effect of ketanserin on the expression of iNOS is mediated by blocking the 5-HT_{2A} receptor. Furthermore, ketanserin significantly inhibited the activation of ERK1/2 and NF-κB signal. Pretreatment of PD98059, a specific inhibitor of ERK1/2, blocked the inhibitory effect of ketanserin on the expression of iNOS and NO production, indicating a critical role of the MEK/ERK1/2 signaling pathway. **Conclusion:** Our findings indicated that inhibition of the expression of iNOS via the MEK/ERK pathway mediates the protective effects of ketanserin against LPS-induced shock in mice.
Keywords: ketanserin; endotoxic shock; inducible nitric oxide synthase; nitric oxide

S6.64

Involvement of NMDA receptors in the rostral ventrolateral medulla in acute ethanol-induced depressor responses in spontaneously hypertensive rats

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Aim: Intake of ethanol (alcohol) affects cardiovascular system. The precise mechanisms underlying ethanol regulation of cardiovascular function remains unclear. Glutamate systems especially NMDA receptors, a subtype of glutamate receptors, are important targets of ethanol. The present study was undertaken to examine the role of NMDA receptors in the rostral ventrolateral medulla (RVLM), a central sympathetic regions that controls sympathetic outflow and hence cardiovascular function, in ethanol cardiovascular effects. **Methods:** Glutamate levels were measured by HPLC-ECD method from samples collected by microdialysis probes inserted unilaterally into the RVLM. The blood pressure responses were measured in urethane anesthetized spontaneously hypertensive rats (SHR) and Wistar-Kyoto normotensive rats (WKY). Ethanol was applied by intraperitoneal injection (IP). **Results:** IP higher dose of ethanol (3.2 g/kg) caused a significant increase in the levels of glutamate in the RVLM following the administration in SHR but not in the WKY. IP ethanol (3.2 g/kg) caused a significant decrease in blood pressure in SHR and WKY. Interestingly, the depressor responses were more obvious in SHR than those in WKY. Bilateral microinjection of NMDA receptor antagonists into the RVLM 5 min after administration of ethanol significantly reduced ethanol-induced depressor responses in SHR but not in WKY. **Conclusion:** The results showed that glutamate activation of NMDA receptors in the RVLM may participate in acute ethanol cardiovascular effects.
Keywords: alcohol; sympathetic neurons; blood pressure; NMDA receptors

S6.65

Effects of the peroxynitrite decomposition catalyst (MnTMPyP) on dexmedetomidine-induced contractions of the aorta of the LPS treated rat

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Aim: To investigate whether or not peroxynitrite scavenging improves dexme-

detomidine-induced contractions of rat aortae after 24 and 96 h treatment with lipopolysaccharide (LPS). **Methods:** Eight to ten weeks old male SD rats were injected intraperitoneally with (1 mg/kg) of LPS or normal saline. Twenty-four and ninety-six hours later, their aortae were harvested. Aortic rings were suspended for isometric tension recording in organ chambers and incubated in the presence or absence of *L*-NAME (100 μmol/L), indomethacin (10 μmol/L), 1400W (10 μmol/L), and metalloporphyrin manganese tetrakis (4-N-methylpyridyl) porphyrin (MnTMPyP) (10 μmol/L); concentration-contraction curves to dexmedetomidine (10 nmol/L to 10 μmol/L) were obtained. IL-6 and TNF-α were measured in plasma before and after LPS. Aortic tissues were assessed using immunofluorescence to 3-nitrotyrosine and protein expression of iNOS thru Western blotting. **Results:** Plasma cytokines remained below detection levels after 24 and 96 h exposure to LPS. Dexmedetomidine-induced contractions were attenuated in aortic rings without endothelium from LPS-treated animals and this attenuation was less in the presence of MnTMPyP. Protein expressions of iNOS were increased after LPS treatment. **Conclusion:** *In vivo* LPS administration attenuates the contractile responses to dexmedetomidine in aortic rings without endothelium because of induction of iNOS. The present findings indicate that contractile responses in such aortic rings were improved by *ex vivo* treatment with MnTMPyP and 1400W.

S6.66

Dihydromyricetin prevents Ang II-induced rat cardiomyocyte hypertrophy through NO-depentment pathway

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Aim: Dihydromyricetin displays a range of biological properties. The aim of study was to investigate whether dihydromyricetin had the potential therapeutic value to protect angiotensin II-induced cardiomyocyte hypertrophy of rat. **Methods:** Degree of hypertrophy was evaluated by cell surface analysis. Expression of ANP and β-MHC mRNA was measured with real-time PCR. Production of NO in myocardial cells was assessed using the NO-specific fluorescent dye 3-amino,4-aminomethyl-2,7-difluorescein, diacetate (DAF-FM DA). **Results:** Pre-incubation with dihydromyricetin significantly attenuated Ang II-induced myocardial cells hypertrophy in a dose-dependent manner. But dihydromyricetin did not show significant influence on MTT value and LDH activity, which suggested that the inhibition of dihydromyricetin on myocardial hypertrophy was not due to cellular viability or cytotoxicity. There were also a slight decreased NOS activity in myocardial cells after Ang II stimulation, while pre-incubation with dihydromyricetin evoked a relatively higher activity of NOS. Accordingly, both of intracellular and extracellular NO level also increased. Meanwhile, the mRNA expression of ANP and β-MHC, as cardiomyocyte hypertrophy index, was also down-regulated after dihydromyricetin pre-incubation. But *L*-NAME, as a NOS inhibitor, reverses the attenuating effect of dihydromyricetin on Ang II-induced myocardial cells hypertrophy. **Conclusion:** Taken together, these results suggest that dihydromyricetin prevents AngII induced rat cardiomyocyte hypertrophy through NO-dependent pathway.

Keywords: dihydromyricetin; NO; cardiomyocyte hypertrophy

S6.67

Compound ICA-105574 prevents arrythmias induced by cardiac delayed repolarization

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Aim: Impaired ventricular repolarisation can lead to LQTS with a high risk of lethal ventricular tacharrhythmias (VT). The hERG activators may offer a promising therapeutic strategy for LQTS and VT. This study was designed to investigate antiarrhythmic properties of ICA-105574, which enhances hERG through removing inactivation of the channel. Another compound NS1643 was parallel assessed. **Methods:** By patch-clamp technique, the study examined the effects of ICA-105574 and NS1643 on blocked hERG channel in HEK293 cells, APD and prolonged APD in guniea-pig ventricular myocytes. On Langendorff-perfused guinea-pigs hearts models of LQT1 and LQT2, VT were induced by I_{Kr} and I_{Ks} inhibitor combined with hypokalemia (2.1 mmol/L). The study investigated the antiarrythmic effects of ICA-105574 and NS1643 on the two models. **Results:** Both ICA-105574 and NS1643 shortened APD of ventricular myocytes, and QT or QTc intervals in isolated guinea-pig hearts in a concentration-dependent manner. Above effects of ICA-105574 was more potent than NS1643. ICA-105574 (3 μmol/L) and NS1643 (10 μmol/L) reversed the prolongation of APD by I_{Kr}, I_{Ks} inhibitor, and hypokalaemia. ICA-105574, but not NS1643, completely prevented VT in intact guinea-pig hearts caused by I_{Kr} and I_{Ks} inhibitors. Meanwhile, ICA-105574 at higher concentrations (10 μmol/L) shows a potential proarrhythmic risk in normal hearts. **Conclusion:** ICA-105574 has more potent antiarrythmic activity than NS1643. However, its potential proarrhythmic risk should be taken consideration for further developing this type of hERG activators.

Keywords: antiarrhythmic activity; hERG activator; ICA-105574; NS1643; proarrhythmic risk

S6.69

β_1- and β_2-adrenoceptor – phosphodiesterase control over human heart contractility

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There are two cardiostimulatory β-adrenoceptors (βAR) in human heart, β_1AR and β_2AR. Both β_1AR and β_2AR are coupled to the Gsα-cyclic AMP-PKA pathway in human heart with phosphorylation of key proteins responsible for contraction and relaxation. Phosphodiestereases (PDE), PDE3 and PDE4 metabolize cyclic AMP and are present in human heart. We sought to determine which PDEs are responsible for the metabolism of β_1AR and β_2AR mediated cyclic AMP in human heart. We studied hearts of patients with end-stage heart failure undergoing heart transplantation. Activation of both β_1AR with noradrenaline and β_2AR with adrenaline caused increases in contractile force and hastening of relaxation associated with phosphorylation of phospholamban, troponin I and C-protein. Inotropic and lusitropic effects of both noradrenaline and adrenaline were potentiated in patients chronically administered the β-blocker metoprolol. Inotropic and lusitropic effects were potentiated by inhibition of PDE3, but not PDE4 in metoprolol treated patients, but not non-β-blocker treated patients. Furthermore the potentiation was greater for adrenaline through activation of β_2AR than noradrenaline through activation of β_1AR. Our studies indicate that metoprolol induces a control by PDE3 of ventricular effects mediated through both β_1- and β_2-adrenoceptors, thereby further reducing sympathetic cardiostimulation in patients with terminal heart failure.

Keywords: human heart; β-adrenoceptors; phosphodiestereases; β-blockers

S6.70

Role of p38 MAP kinase in IFN-γ-induced HUVEC hyperpermeability

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Aim: Although cytokines are frequently known to mediate the progression of inflammatory diseases, however, by understanding their actions and signal transduction pathways, it can be emerged as a promising target for the therapy of various diseases. In the current work, the effect of a pro-inflammatory cytokine, interferon-γ (IFN-γ), on altering endothelial permeability to macromolecules and the potential signaling molecule involved in this event were determined. **Methods:** The viability of human umbilical vein endothelial cell (HUVEC) exposed to IFN-γ at doses ranged from 200–3.125 ng/mL for 24 h was assessed by using MTT assay. The effect of IFN-γ in inducing HUVEC hyperpermeability was studied by using FITC-dextran permeability assay. The involvement of p38MAP kinase in IFN-γ induced hyperpermeability was determined by application of pharmacological inhibitor, SB203580 (10 μmol/L, 30 min). **Results:** IFN-γ at doses ranged from 200–3.125 ng/mL did not cause significant cell death as compared to normal control. Exposure to IFN-γ with doses 100–0.1 ng/mL led to a significant increase in permeability to FITC-dextran where the maximum increase in permeability was observed at 8 h. Pretreatment with SB203580 attenuated IFN-γ-induced increase in permeability. **Conclusion:** IFN-γ induced endothelial hyperpermeability was independent of cell death. The results demonstrated the involvement of p38MAP kinase in IFN-γ increased HUVEC permeability and suggest that p38MAP kinase could be a potential pharmacological target for prevention of IFN-γ-induced endothelial cell barrier disruption and IFN-γ associated diseases such as asthma, rheumatoid arthritis and inflammatory bowel disease.

Keywords: p38 MAP kinase; IFN-γ; permeability; cytokine; HUVEC

S6.71

Ursolic acid ameliorates cardiac insufficiency against oxidative stress and inflammation in diabetic cardiomyopathy rat induced by streptozotocin

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Aim: Ursolic acid (UA), a pentracyclic triterpene, is reported to have an antioxidant and anti-inflammatory activity. To investigate the influence of UA on the develop-

ment of diabetic cardiac dysfunction, we assessed effects of UA on oxidative stress and inflammation in diabetic rat heart. **Methods:** Diabetes was induced in male Sprague-Dawley rats by an injection of streptozotocin (35 mg/kg, iv). Sustained blood glucose levels (>16.7 mmol/L) were considered as diabetic and selected for experimentation. Thirty rats were randomly divided into three groups: control, diabetic, diabetic+ UA (30 mg/kg/d, ig) administered for 6 weeks from 5th to 10th week. Experiment was carried out at the beginning of 13th week. Blood glucose, hemodynamic parameters, heart tissue levels of SOD, MDA, and GSH-Px concentration were measured. Heart tissue morphology was observed by light microscopy. Immunohistochemisty and Western blot were employed to determine the proteins levels of TNF-α, IL-1β, and MCP-1. **Results:** Cardiac function was significantly impaired in diabetic animals. This was accompanied by a significant up-regulation of expression of TNF-α, IL-1β, and MCP-1. In addition, SOD, GSH-Px activities were reduced and MDA levels was increased.The histopathological examination showed that myocardial cells had disordered arrangement, swelling and the infiltration of inflammatory cells in model group. These changes were significantly attenuated in the diabetic group treated with UA. **Conclusion:** UA can evidently relieve cardiac dysfunction in rats with diabetic cardiomyopathy induced by STZ, which might be related to modulating oxidative stress and protein expressions of TNF-α, IL-1β, and MCP-1 in diabetic heart.

Keywords: diabetic cardiomyopathy; ursolic acid, inflammation, oxidative stress
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S6.72

Effects of Huoxue Huayu Rule on inflammation cytokines network in SHR

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Aim: To study the mechanism of Huoxue Huayu method, which can promote circulation and treat hypertensive left ventricular hypertrophy. We observed the effects of Xuefu Zhuyu capsule (XFZY) and Yiqi Huoxue fomula (YQHX) on inflammation cytokines in spontaneous hypertensive rat (SHR). **Methods:** 48 SHRs were randomly divided into six groups according to their blood pressure: model control group, XFZY high dose group 8 g/kg, XFZY low dose group 4 g/kg, YQHX high dose group 2.4 g/kg, YQHX low dose group 1.2 g/kg, benazepril hydrochloride group 1 mg/kg. Each group had 8 rats. Eight Wistar rats was in normal control group at same time. They were consecutively administered for 14 weeks, serum content of IL-17, IL-1β, TNF-α, and IL-10 were detected with ELISA kit. **Results:** IL-17, IL-1β, TNF-α, and IL-10 had significant change in SHR, and XFZY capsule and QIHX fomula both changed cytokines contents. **Conclusion:** Huoxue huayu method can inhibit hypertensive myocardial fibrosis by regulating inflammation cytokines network.

Keywords: Huoxue Huayu rule; Xuefu Zhuyu capsule; yiqi huoxue fomula; inflammation cytokines; hypertension; myocardial fibrosis

S6.73

Ameliorated effects of aspirin on abnormal cardiac fibroblasts (CFs) induced by aldosterone *in vitro*

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Aim: Aspirin were widely used in cardiovascular diseases in clinic. At present research, the regulating effects and the mechanism of aspirin were investigated in the neonatal SD rat CFs abnormal induced by aldosterone (Ald). **Methods:** The CFs was digested by 0.08% trypsin with 1–3 d neonatal rat, and then was purified by differential adhension method. The optimal concentration of Ald was explored to different concentration explored to cardiac fibroblast by MTT methods assay. The four groups were allotted as following: DMEM medium blank control group, model group, Ald 1×10^{-8} mol/L, the aspirin group was 1.11×10^{-6} mol/L, spironolactone1×10^{-6} mol/L. The morphology of cardiac fibroblast was assayed by Giemsa, HE and Masson staining. Type I collagen and type III collagen were detected by ELISA kits. The hydroxyprolin was measured by colorimetric method. The samd 2, 3, 4 were determined by Western blotting assay. **Results:** The results confirmed Ald induced abnormal CFs. The aspirin can effectively inhibit the Ald-induced CFs proliferation by MTT, HE Giemsa and Masson staining. The Aspirin significantly inhibit I and III collagen secretion by Elisa and hydroxyproline assay. Western blotting results confirmed that aspirin can inhibit aldosterone-induced Smad2, 3, and 4 protein expression increase in CFs. Spironolactone can ameliorate all of the cardiac fibroblast abnormal induced by Ald. **Conclusion:** The Ald induced abnormal CFs may be involved in activate TGF-β/smad following signal closely. The aspirin can inhibit aldosterone-induced abnormal cardiac fibroblasts through down-regulated Smad2, Smad3, and Smad4 protein expression.

Keywords: aldosterone; aspirin; cardiac fibroblasts; TGF-β-Smad
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S6.74

Evodiamine inhibits angiotensin II -induced cardiomyocyte hypertrophy *in vitro*

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Aim: To investigate the effects of evodiamine (Evo), a component of *Evodiarutaecarpa* (*Juss*) Benth, on cardiomyocyte hypertrophy induced by angiotensin II (Ang II) and further explore the potential mechanisms. **Methods:** The cultured cardiomycytes from Sprague Dawley neonatal rats were randomly divided into control, model (Ang II 0.1 μmol/L), Evo (0.03, 0.3, and 3 μmol/L) groups. The cardiomyocyte surface area, protein level, intracellular free calcium ($[Ca^{2+}]_i$) concentration, activity of nitric oxide synthetase (NOS) and content of nitric oxide (NO) were measured, respectively. The mRNA expression of atrial natriuretic factor (ANF), calcineurin (CaN) and endothelial nitric oxide synthase (eNOS) of cardiomycytes were analyzed by real time RT-PCR. The protein expression of calcineurin catalytic subunit (CnA) was detected by Western blot analysis. **Results:** AngII significantly induced cardiomyocytes hypertrophy. Evo (0.03, 0.3, and 3 μmol/L) significantly attenuated AngII-induced cardiomyocyte hypertrophy, decreased the $[Ca^{2+}]_i$ concentration, CaN mRNA and CnA protein expressions, but increased the NOS activity and NO production, up-regulated the eNOS mRNA expression, respectively. **Conclusion:** Evo significantly attenuated AngII-induced cardiomyocyte hypertrophy, and this effect is partly due to the promotion of NO production, the reduction of $[Ca^{2+}]_i$ concentration and the inhibition of CaN signal transduction pathway.

Keywords: evodiamine; cardiomyocyte hypertrophy; Ang II; NO; CaN

S6.75

Activation of endothelial cells by complement activation of the alternative pathway and intervention by inhibitors

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Aim: To investigate the effect of complement activation of the alternative pathway on human microvascular endothelial cells and provide potential targets for intervention and drug screening. **Methods:** Normal human serum complement was activated by cobra venom factor. After exposure of endothelial cells to activated complement for various times, expression of adhension molecules, chemokine, and fibrinolytic activity, the activation of NF-κB, p38MAPK, and JAK2, and intervention by inhibitors were assayed. LDH, caspase-8, NO, and cell viability were also measured. **Results:** P-selectin was rapidly expressed by endothelial cells after exposure to activated complement. Then expression of E-selectin, ICAM-1, MCP-1, IL-8, tPA, and PAI-1 were up-regulated. JAK2, p38MAPK, and NF-κB were activated. Activation of the pathways was inhibited by AG490, SB203580, and PDTC, respectively. But only AG490 effectively inhibited the expression of ICAM-1. Increased LDH leakage and caspase-8 activity, and decreased NO release and cell viability were detected. **Conclusion:** Complement activation of the alternative pathway can induce activation and injury of endothelial cells, thus may lead to local inflammation and tissue injury in endothelium, and JAK2 may be a potential target for intervention.

Keywords: complement; endothelial cell activation; inflammation; signaling pathway; screening model
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S6.76

Ischemic injury promotes Keap1 nitration and disturbance of antioxidative responses in endothelial cells: a potential vasoprotective effect of melatonin

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Aim: Accumulating evidence implicates that the endothelial injury contributes to the ischemic neurovascular damage. This study is to assess role of Keap1/Nrf2 associated downstream nitric oxide redox signaling in ischemia-induced endothelial cells injury. **Methods:** EA.hy926 cells were exposed to oxygen-glucose deprivation (OGD) for 1, 3, 6, and 12 h. *S*-nitrosylation content of Keap1 protein was determined by biotin-switch method using the protein *S*-nitrosylation detection kit. Immunoprecipitation, immunoblotting and immunofluorescence were used for related proteins analysis. **Results:** The nuclear import of Keap1 rose after OGD or addition of peroxynitrite, which interacted with nuclear-localized Nrf2. And nitrotyrosine immunoreactivity in endothelial cells also increased. Importantly, these changes can be partially inhibited by melatonin. Moreover, there were no significant changes in S-nitrosylation of Keap1 when OGD-induced tyrosine nitration of Keap1 was blocked by melatonin. Weak expression of ZO-1 and heme oxygenase-1 levels during OGD can be reduced by melatonin pretreatment. **Conclusion:** Our results emphasize that upon nitrosative stress, the protective effect of melatonin on endothelial cells is likely mediated at least in part by inhibition of ischemia-evoked protein nitration of Keap1, hence contributing to relieve the disturbance of Nrf2/Keap1 antioxidative signaling.

Keywords: endothelial cells; Keap1; melatonin; oxygen–glucose deprivation; *S*-nitrosylation; tyrosine nitration

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S6.77

Noval arylsulfonamide derivative MPT0G013 inhibits angiogenic effect by targeting HDAC activity

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Aim: The role of histone deacetylases (HDACs) and the potential of these enzymes as therapeutic targets for cancer is an area of rapidly expanding investigation. In addition to histones, HDACs have many nonhistone protein substrates which have a role in regulation of gene expression, cell proliferation, cell migration, cell death, and angiogenesis. In this study, the anti-angiogenic effects of a novel synthetic HDAC inhibitor, MPT0G013, in HUVECs were investigated. **Methods:** Crystal violet assay, flow cytometric analysis, fluorogenic HDAC activity assay, Western blotting assay, tube formation assay, migration assay, Xenograft mouse model were used. **Results:** This study first demonstrates that MPT0G013 induced acetylation of H3 in human umbilical vein endothelial cells (HUVECs) and has a stronger inhibition effect of pan-HDAC activity than suberoylanilide hydroxamic acid (SAHA). It has also shown an antiproliferative effect in a dose-dependent manner. Following MPT0G013 treatment, cells were arrested in G_0/G_1 phase and had significant ip21 expression. Moreover, tube formation and migration were abrogated in a dose-dependent manner. Noncytotoxic concentrations of MPT0G013 inhibited endothelial phosphorylation of AKT, extracellular signal-regulated kinase1/2, p38, and β-catenin expression. Most importantly, MPT0G013 exhibited the tumor-inhibitory activity in HCT116 mouse xenograft models. **Conclusion:** Our study has highlighted the potential application of MPT0G013, a new potent HDAC inhibitor, in angiogenesis-related diseases.

Keywords: HDAC; HUVECs; angiogenesis

S6.78

Therapeutic potential of FTY-720 for treating cardiac hypertrophic remodeling

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Aim: Cardiac hypertrophy is regarded as a clinical determinant of heart failure. The average age of cardiac hypertrophy onset is his/her 50s and the average patient with heart failure is late 70s. This leaves a relatively large time window where a patient displaying early cardiac hypertrophy could be treated to slow or even prevent the progression of hypertrophic remodeling; therefore studies to discover regulatory mechanisms underlying hypertrophic remodeling and to identify potential therapeutic targets for treating heart failure are of paramount importance. **Methods:** Over the past few years using genetically modified mouse models and pharmacological agents our group has investigated the roles of a number of intracellular signaling proteins in cardiac hypertrophy, and recently identified a novel anti-hypertrophic molecule, p21-activated kinase-1 (Pak-1). **Results:** In the heart our studies demonstrate Pak1 protection against stress-induced hypertrophic remodeling and targeting of Pak-1 in heart muscle by FTY-720 (2-amino-2-[2-(4-octylphenyl) ethyl]-1,3-propanediol hydrochloride) via Gi coupled sphingosine receptors is able to prevent the induction of stress-induced hypertrophy and reverse existing hypertrophy and fibrosis in wild-type mice, without compromising the cardiac functions. FTY-720 is known as a sphingosine-like analogue, derived from myriocin, a component of the natural product *Isaria sinclairi*. **Conclusion:** Our data provides a scientific basis of development of FTY-720 or its analogues for clinical applications in the treatment of cardiac hypertrophic remodeling.

Keywords: FTY-720; Pak1; cardiac hypertrophy; heart failure

S6.79

Nicorandil enhances Na^+/Ca^{2+} exchanger activity via guanylate cyclase activation in cardiac ventricular myocytes

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Aim: Nicorandil exhibited cardioprotective and antiarrhythmic effects during the process of ischemia/reperfusion in heart. However, the mechanism through which nicorandil produces these actions remains to be further clarified. Na^+/Ca^{2+} exchanger (NCX) is involved in both the generation of action potential and ischemia/reperfusion-induced Ca^{2+} overload in cardiac myocytes. Here we investigated the effect of nicorandil on NCX activity. **Methods:** NCX activity was elucidated as determining Na^+/Ca^{2+} exchange current (I_{NCX}) and extracellular Na^+-removal-induced Ca^{2+} influx in both isolated guinea pig ventricular myocytes and CCL39 fibroblasts expressing wild type or mutant NCX1, as examined by whole-cell patch clamp and Fura-2/AM-based microfluorometry, respectively. **Results:** Nicorandil enhanced I_{NCX} and Na^+-removal-induced Ca^{2+} influx. A membrane permeable analog of cGMP, 8-bromo-cGMP, mimicked the effect of nicorandil on I_{NCX}, while a guanylate cyclase inhibitor ODQ, antagonized the nicorandil-induced promotion of NCX activity. Furthermore, in CCL39 fibroblasts, nicorandil enhanced NCX activity in ectopic wild type NCX1but not in $^{\triangle}$N1 mutant NCX1. **Conclusion:** The present findings indicate that nicorandil enhances NCX activity through simulating guanylate cyclase, and the promotion of NCX activity may partly contribute to the cardioprotection of nicorandil through accelerating Ca^{2+} exit via NCX.

Keywords: nicorandil; Na^+/Ca^{2+} exchanger; guanylate cyclase; myocyte

S6.80

Cadiac cannabinoid system accentuate atrial natriuretic peptide release

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Aim: The purpose of the present study was to define the effects of *N*-arachidonylethanolamine (anandamide), an endogenous ligand for cannabinoid receptors CB_1 and CB_2 on the regulation of atrial natriuretic peptide (ANP) release. **Methods:** Isolated perfused rabbit atria were prepared. Immunoreactive ANP in the perfusate was measured by a specific radioimmunoassay. **Results:** Anandamide increased ANP secretion and ANP concentration in terms of extracellular fluid (ECF) translocation which reflects atrial myocytic ANP release concomitantly with a decrease in atrial dynamics. SR141716, an inhibitor of CB1 receptor but not SR144528, an inhibitor of CB2 attenuated anandamide-induced accentuation of ANP release. AM 404, an inhibitor of anandamide transport mimicked the effect of anandamide. **Conclusion:** These results suggest that cardiac cannabinoid system is involved in the regulation of ANP release.

Keywords: cannabinoid; anandamide; atrial natriuretic peptide; atrial dynamics; cannabinoid receptors

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S6.81

Angiotensin II type I receptor-mediated oxidative stress at the rostral ventrolateral medulla underlies blood pressure elevation after binge methamphetamine

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Aim: Methamphetamine (METH), a potent central nervous system stimulant, is a common illicit drug abused in Taiwan, China. A majority of the hitherto investigations on METH focus on the behavior and psychology but least studies on cardiovascular responses. The present study aimed to delineate the participation of Ang II type I receptor (AT1R) at rostral ventrolateral medulla (RVLM), which plays a crucial role in central regulation of blood pressure, in cardiovascular responses elicited by binge methamphetamine exposure, a favorable method used by METH abusers. **Methods:** Binge METH (5 or 10 mg/kg) via four injections at 2-h intervals was intraperitoneally (ip) administered to Sprague-Dawley rats. The blood pressure (BP) and heart rate (HR) of conscious rats was monitored by radio-telemetry method. RVLM tissues were analyzed by a RT-PCR array for 84 gene related to hypertension or by ELISA, Western blot and chemiluminescence method for the METH concentration, AT1R protein expression, and superoxide. Systemic administration of AT1R antagonist candesartan or valsartan or microinjection of candesartan or AT1R siRNA into RVLM was applied before binge METH administration. **Results:** Binge METH elicited a dose- and time-dependent elevation of BP, HR, and mortality, alongside with up-regulation of METH concentration and 56 genes in RVLM, in which *agtr1a* and *agtr1b* were two of the most upregulated mRNA. METH also increased AT1R protein expression and superoxide production in RVLM, which were antagonized by pretreatment of candesartan or AT1R siRNA but not by valsartan. **Conclusion:** These results suggested that the AT1R-mediated oxidative stress in the RVLM underlies binge METH-induced elevation of cardiovascular responses.

Keywords: methamphetamine; rostral ventrolateral medulla; cardiovascular responses; angiontensin II type I receptor; oxidative stress

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S6.82

Effects of rutaecarpine on myocardial adenosine triphosphatase and calmodulin neural phosphatase in left ventricular hypertrophy rats

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Aim: To examine the effects of rutaecarpine (Rut) on myocardial hypertrophy rats, focusing on myocardial Na^+-K^+ ATPase (ATPase), Ca^{2+}-ATPase and calmodulin nerve phosphatase (CaN). **Methods:** Left ventricular hypertrophy was induced by abdominal aortic constriction (AAC) in rats, followed by Rut treatment (20-40 mg/kg, *po*) for 4 weeks. The blood pressure, the left ventricular heart index (LVHI), and histopathology were examined. The activities of ATPase, Ca^{2+}-ATPase and CaN and the mRNA expression of CaN and atrial natriuretic peptide (ANP) were determined. **Results:** Compared with the sham-operation rats, the blood pressure and LVHI in model rats were increased and activities of myocardial ATPase and Ca^{2+}-ATPase were decreased, while CaN increased. Histopathology showed that AAC-induced cardiomyeocytes hypertrophy with disarrayed and broken cardiac fibers. Rut treatment ameliorated AAC-induced left ventricular hypertrophy, as evidenced by decreased blood pressure, LVHI, and ameliorated AAC-induced pathological lesions. AAC-depressed myocardial ATPase and Ca^{2+}-ATPase activities were recovered by Rut. The AAC-induced mRNA expression of CaN and ANP and activity of CaN was attenuated by Rut. **Conclusion:** Rutaecarpine is effective against AAC-induced left ventricular hypertrophy in rats. The mechanisms of the protection are due, at least in part, to increased activities of myocardial ATPase and Ca^{2+}-ATPase and decreased activity of CaN.

Keywords: abdominal aortic constriction; myocardial hypertrophy; rutaecarpine; adenosine triphosphatase; calmodulin neural phosphatase

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S6.83

The vasorelaxant mechanisms of a Rho kinase inhibitor DL0805 in rat mesenteric artery rings

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Aim: Rho-kinase has been suggested as a therapeutic target in the treatment of cardiovascular diseases. The Rho kinase signaling pathway is substantially involved in vascular contraction. The aim of this study was to evaluate the vasorelaxant effects of Rho kinase inhibitor, 5-Nitro-1(2)*H*-indazole-3- carbonitrile (DL0805), in isolated rat mesenteric artery rings and to investigate its possible mechanisms. **Methods:** Mesenteric artery rings pre-contracted with high KCl (60 mmol/L), and once the plateau was attained, DL0805 (10~120 μmol/L) was added cumulatively to obtain the concentration-response curves. The microvessels were incubated with these inhibitors (*L*-NAME, methylene blue or indomethacin) or vehicle prior to maximal contraction induced by KCl, then the endothelial response to the DL0805 was determined. 4-aminopyridine (4-AP) was also used to demonstrate the role of K^+ channels on the relaxation induced by DL0805. **Results:** DL0805 exerted vasorelaxation in a dose-dependent manner in KCl-induced sustained contraction and partial loss of the vasorelaxation under endothelium-denuded rings. The DL0805-induced vasorelaxation was significantly reduced by the nitric oxide synthase inhibitor *L*-NAME and the cyclooxygenase inhibitor indomethacin. The voltage-dependent K^+ channel blocker 4-AP remarkably attenuated DL0805-induced relaxations in mesenteric artery. **Conclusion:** We find that DL0805 is a novel vasorelaxant compound associated with inhibition of Rho/ROCK signaling pathway. The NO-cGMP pathway may be involved in the relaxation of DL0805 in endothelium-intact mesenteric artery. The vasorelaxant effect of DL0805 is partially mediated by the opening of the voltage-dependent K^+ channels.

Keywords: DL0805; Rho kinase; vasorelaxation; mesenteric artery; endothelium; nitric oxide

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S6.84

Stilbene glycoside attenuates human platelet aggregation and secretion

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Aim: Stilbene glycoside (SG) is a water-soluble component of the rhizome extract from the traditional Chinese herb *Polygonum multiflorum*, exhibits anti-oxidative and anti-inflammatory effects. In this study, we investigated its anti-platelet aggregation and secretion. The purpose of the study is to explore its anti-platelet effect and mechanism. **Methods:** Using the lumiaggregometer (Chrono-log 700, Havertown, PA, USA) , we investigated the anti-platelet activity of SG on platelet aggregation and ATP secretion induced by collagen (2 μg/mL) and ADP (4 μmol/L). P-selectin expression were measured by flow cytometry. **Results:** SG inhibited platelet aggregation and ATP secretion induced by collagen, but not by ADP. It inhibited platelet P -selectin expression induced by thrombin. **Conclusion:** Our study indicates that SG is likely to exert protective effects in thromboembolic-related disorders by modulating human platelet activity.

Keywords: Stilbene glycoside; platelet; aggregation; secretion

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S6.85

Genitein's effect on AngII and ANP content in isolated myocardial tissue with ischemia/reperfusion injury

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Aim: To study the effects of genistein on isolated myocardial ischemia/reperfusion (I/R) injury rats and its effects on the levels of AngII and ANP. **Methods:** An isolated rat myocardial ischemia/reperfusion injury was made and genistein (low dose, medium dose and high dose) was given in reperfusion period. The heart rate (HR), mean left ventricular systolic pressure (mLVSP) or diastolic pressure (mLVDP), coronary flow (CF) and angiotensin II (AngII), trial natriuretic peptide (ANP) in myocardial tissue were observed. **Results:** In isolated rats heart treated with genistein, the CF was increased, but that the HR decreased, and mLVSP was raised but down about mLVDP compared with model rats. In addition, it's shown that genistein obviously decreased the levels of AngII but increased ANP in myocardial tissue. **Conclusion:** Genistein has a protective effect on isolated myocardial ischemia/reperfusion injury by expanding the coronary artery and enhancing the myocardial contractile force, which may be related to reduce the content of AngII and elevate ANP content.

Keywords: genistein; ischemia/reperfusion injury; AngII; ANP
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S6.86

Effect of isorhynchophylline on platelet release and platelet aggregation *in vitro*

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Aim: To investigate the antiplatelet release and aggregation effects of isorhynchophylline(Isorhy) *in vitro* and to examine its mechanisms of action. **Methods:** The effects of Isorhy on platelet serotonin (5-HT) release, and on platelet factor4 (PF4) release induced by collagen, on the release of malondialdehyde (MDA) caused by thrombin, on rabbit platelet aggregation induced by collagen and arachidonic acid (AA) were determined by fluorescence spectrophotometric method, ELISA method, thiobarbituric acid method, and Born'method, respectively. **Results:** Heparin thrombin clotting time (HTCT) was significantly lengthened by Isorhy. Moreover, Isorhy concentration-dependently depressed platelet 5-HT release produced by collagen. Isorhy showed a concentration-dependent depressant effect on rabbit platelet MDA and PF_4 release induced by thrombin and collagen and on rabbit platelet aggregation induced by collagen and AA, respectively. **Conclusion:** The present study indicate that Isorhy possesses anti-platelet release and aggregation effects, and may be a new anti-platelet drug.

Keywords: isorhynchophylline (Isorhy); platelet release; platelet aggregation; Malondialdehyde (MDA); platelet factor4 (PF4); collagen; thrombin

S6.87

Preparation of Eucommia polysaccharide effervescent granules and its protective effect on ischemia-reperfusion brain injury in rats

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Aim: Eucommia polysaccharide exhibit high degree of anti-inflammation, anti-oxidation, and so on. In this study, we verified the way of making Eucommia polysaccharide effervescent granules (EPEG) and its protective effect on ischemia-reperfusion brain injury in rats. **Methods:** Wet granulation in EPEG was prepared. Through longa's neural function learn point rating method was used to detect animal nerve damage degree, chlorinated four wow dyeing method pathological and index determination of EPEG was carried to to observe to cerebral ischemia. **Results:** EPEG was grey white particles, rapidly dissolving in water generating dark brow transparent solution. TTC dyeing showed that rats in the model group had severe ischemia in white areas. EPEG treatment reduced the ischemia size in the white area. In model group MDA content was significantly higher than blank group (P<0.01). Cerebral ischemia-reperfusion injury increased MDA content increased. In EPEG treatment group MDA content was significantly lower than model group (P<0.01). Model group had decreased super-oxide dismutase (P< 0.01), and in EPEG group the SOD activity increased significantly and NO release decreased significantly (P<0.01). **Conclusion:** EPEG ameliorated rat ischemia-reperfusion injury induced by MCAO. The mechanisms were likely due to anti-oxidation besides inhibiting excessive release of NO.

Keywords: eucommia polysaccharide effervesoent granules; ischemia-reperfusion injury; protection effect

S6.88

Protection by pitavastatin from doxorubicin-induced acute cardiotoxicity through suppression of oxidative stress

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Aim: Doxorubicin (DOX) is an effective chemotherapeutic drug. However, its clinical application may be hampered by dose-dependent cardiotoxicity. The mechanisms of DOX-induced cardiotoxicity are not fully understood, but DOX-relative cardiotoxicity induced by other than those mediating their anticancer effectiveness. In the present study, we use a rat model to test whether the pitavastatin (PTT) can exert protective effects against DOX-induced cardiotoxicity. **Methods:** Rats received daily treatment of either distilled water (4 mL/kg) or PTT (0.3, 1, and 3 mg/kg) for 12 d and then followed by an intravenous injection at d 5 of either saline (10 mL/kg) or DOX (10 mg/kg). **Results:** DOX led to significant decrease (P<0.05) in body weight, heart weight and heart-to-body weight ratio compared with that of control group. There was a progressive increase in heart weight and body weight consistent with a good dose-effect relationship seen in the groups of rats pretreated with 0.3, 1, and 3 mg/kg of PTT. DOX treatment also increased MDA and H_2O_2 and decreased SOD and GSH-Px activity in cardiac tissues. Pretreatment with 1 and 3 mg/kg PTT significantly reduced DOX-induced oxidative injury in cardiac tissue, suggesting by the fact that PTT significantly attenuated DOX-induced cardiac myofibrillar disarrangement. **Conclusion:** In summary, our evidence indicates that PTT elicited a typical protective effect on DOX-induced acute cardiotoxicity via suppressing oxidative stress.

Keywords: doxorubicin; cardiotoxicity; pitavastatin; oxidative stress
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S6.89

Ginsenoside Rg1 promoted the proliferation of mesenchymal stem cell in bone marrow and peripheral blood in cyclophosphamide-induced myelosuppression mice

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Aim: We previously demonstrate that ginsenoside Rg1 can improve the hematopoietic function (Eur J Pharmacol, 2012). The current study further investigates the effect of ginsenoside Rg1 on the proliferation of mesenchymal stem cells (MSCs) in cyclophosphamide-induced myelosuppression mice. **Methods:** Mice were given cyclophosphamide (150 mg/kg, ip for 3 d) to produce bone marrow depression. Rg1 was then administered at 7.5 and 15 mg/kg by ip for 7 d. Bone marrow cells were counted and the percentage of mesenchymal stem cells ($Sca\text{-}1^-CD44^+CD166^+$) was quantified by flow cytometry. The contents of stem cell factor (SCF), stromal cell derived factor 1 (SDF-1) and interleukin-3 (IL-3) were determined in bone marrow intercellular fluid and peripheral blood by ELISA. **Results:** Rg1 increased bone marrow cell numbers in cyclophosphamide-induced bone marrow depression mice. The percentage of $Sca\text{-}1^-CD44^+CD166^+$ cells was lower in cyclophosphamide group, but returned towards normal after Rg1 treatment in both bone marrow and peripheral blood. The contents of SCF, SDF-1, and IL-3 in bone marrow intercellular fluid and serum were depressed in the model group, but revived after Rg1 15 mg/kg treatment. **Conclusion:** Ginsenoside Rg1 promotes the proliferation of MSCs in bone marrow and peripheral blood in cyclophosphamide-induced myelosuppression mice.

Keywords: ginsenoside Rg1; MSCs; proliferation; microenvironment
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S6.90

Pioglitazone may alleviate high homocysteine induced atherosclerosis through VCAM-1△

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Aim: To investigate the effect of atherosclerosis induced by homocysteine (hHcy). **Methods:** 60 SD rats were divided into five groups including ① control group (ordinary diet), ② high fat group (cholesterol 3%, sodium cholate 0.5%, propylthiouracil 0.2%, sugar 5%, lard 10%), ③ high homocysteine group (methionine 3%), ④ high fat and high homocysteine group (② and ③), ⑤ high homocysteine and pioglitazone group (③, pioglitazone 0.3%), respectively. After 12 weeks, the level of homocysteine in plasma of model rats was tested by ELISA. The lipid degeneration of vascular intima was coloured by sudan III. The immunohistochemistry (IHC) was used to examine the expression of VCAM-1 of model rats artery. **Results:** The level of plasma homocysteine of tested groups from high to low was high fat and high homocysteine group, high homocysteine group, high fat group, high homocysteine and pioglitazone group, control group. Sudan staining results show that there was no obvious orange colour both in the endangium and the adventitia of the rats in the control group. The degree of lipid degeneration of both in the endangium and the adventitia of artery from serious to light was high fat and high homocysteine group, high homocysteine group, high fat group, high homocysteine and pioglitazone group. The expression of VCAM-1 from high to low was high fat and high homocysteine group, high homocysteine group, high fat group, high homocysteine and pioglitazone group, respectively, control group. **Conclusion:** High fat and high homocysteine may improve the expression of VCAM-1 in the endangium, and VCAM-1 was an important factor in the development of the atherosclerosis.

Keywords: pioglitazone; homocysteine; atherosclerosis; VCAM-1

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S6.91

Vasodilation effect and mechanisms of DL0805-1, a Rho kinase inhibitor, on the rat thoracic aorta

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Aim: Accumulating experimental and clinical evidence indicate that Rho/Rho-kinase is an important new target for the treatment of cardiovascular diseases. The aim of this study was to investigate the *in vitro* effect of DL0805-1, a Rho kinase inhibitor, on isolated rat thoracic aorta and its mechanism. **Methods:** Tension was measured to evaluate the vasodilation effect of DL0805-1 on rat endothelium-intact and endothelium-denuded thoracic aorta rings. The nitric oxide synthase inhibitor N^{ω}-nitro-*L*-arginine methyl ester (*L*-NAME), guanylate cyclase inhibitor methylene blue, cyclooxygenase inhibitor indomethacin, calcium-activated potassium channel blocker tetraethyl ammonium (TEA), ATP-sensitive potassium channel blocker glibenclamide and voltage-dependent potassium channel blocker 4-aminopyridine (4-AP) were used to illustrate the mechanisms of vasodilation effect of DL0805-1. **Results:** DL0805-1 relaxed aortic rings pre-contracted with norepinephrine (NE 1 μmol/L) or high KCl (60 mmol/L). Pretreatment with DL0805-1 noncompetitively inhibited contractile responses of aorta to NE or KCl. Pretreatment with L-NAME significantly reduced the DL0805-1 induced vasodilation. However, melhylene blue and indomethacin showed no significant affect vasodilation of DL0805-1. In endothelium-denuded rings, TEA significantly attenuated the vasorelaxant effect of DL0805-1, while glibenclamide and 4-AP had no impact on it. **Conclusion:** These results suggested that DL0805-1 induced relaxation in rat aortic rings through an endothelium-dependent pathway. The opening of calcium-activated K^+ channels and blocking of Ca^{2+} channels in vascular smooth muscle cells may be one of the mechanisms in vasorelaxation of DL0805-1.

Keywords: Rho-kinase; DL0805-1; thoracic aorta; endothelium; K^+ channels; Ca^{2+} channels

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S6.92

Role of microRNAs in heart diseases and translational research

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Aim: Deregulated myocardial fibrosis is a major cause of malignant arrhythmias. The focus of this investigation was to elucidate the importance of miRNAs in the control of pathophysiological processes following cardiac fibrosis. **Methods:** Gain of function and loss of function approaches in a specific miRNA transgenic mouse models were employed in the present study. Protein expression was examined with Western blot and miRNA level was quantified by Real-time PCR. **Results:** First, we found that the level of the miRNA is aberrantly up-regulated in the border zone of infarcted heart of wild type (WT) mouse following cardiac fibrosis by occlusion of the left coronary artery (LCA). Moreover, myocardial interstitial fibrosis has been dramatically developed in the genetic mouse model with cardiac-specific over-expression of the miRNA. This phenomena in cardiac fibrosis was followed by dilated cardiomyopathy and subsequent cardiac failure. In contrast, extent of fibrosis is significantly reduced by the genetic mouse model with cardiac-specific knockdown of the miRNA by occlusion of LCA and elimination of the miRNA by its antisense oligoribonucleotides (AMO) in cultured cardiac fibroblasts significantly results in reduction of secretion of collagen. In terms of its effect mechanisms, the miRNA directly targets at transforming growth factor-beta receptor (TGFBR), which acts as an inhibitor of TGF-β signals, subsequently promotes the formation of type I - type II receptor complex and activities TGF-β signaling pathway. Up-regulation of TGF-β signaling by the miRNA targeting TGFBR in turn induces secretion of collagen in fibroblast and down-regulation of TGFBR is also abolished by the AMO in cultured cardiac fibroblasts. **Conclusion:** Our finding indicates that miRNA can function as a potent regulator of myocardial interstitial fibrosis and represents a potential therapeutic target for malignant arrhythmias in general.

Keywords: microRNA; heart disease; myocardial fibrosis; TGF-β; translational medicine

S6.93

Electrophysiological effects of ranolazine on human atrial myocytes at therapeutically relevant concentrations

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Aim: Ranolazine is an antianginal agent. However, the ionic mechanisms involved in the effects of ranolazine at therapeutically relevant concentrations in human cardiac myocytes are unclear. The present study sought to investigate the effects of ranolazine on fast Na^+(I_{Na}), L-type Ca^{2+} (I_{Ca}), transient outward K^+ (I_{to}), ultra-rapid delayed rectifier K^+ (I_{Kur}) and action potential (APD) currents, as well as on action potentials, in human isolated atrial myocytes. **Methods:** Atrial myocytes were isolated enzymatically from specimens of human atrial appendage obtained from patients undergoing coronary artery bypass grafting. The action potential and membrane currents were recorded in both current- and voltage-clamp modes using the patch-clamp technique. **Results:** The duration of the action potential at 50% and 90% repolarization (APD_{50} and APD_{90}, respectively) was increased by 10 μmol/L ranolazine. The percentage reduction in APD_{50} and APD_{90} was 27.5% and 14.2%, respectively. Ranolazine had no effect on resting membrane potential or action potential amplitude. The steady state activation of I_{Na} were not affected by ranolazine. The steady state inactivation curves of I_{Na} were significantly shifted to the left by ranolazine. The $V_{0.5}$ and slope factor for inactivation averaged -103.5±3.9, and -6.5±0.8 mV, respectively, for control and -92.9±7.8 and -6.7±0.7 mV, respectively, for ranolazine. Ranolazine had no effect on I_{Ca}, I_{Kur}, or I_{to}, but it reversibly shortened the duration of the action potential in human atrial myocytes. **Conclusion:** Ranolazine, at a clinically relevant concentration, prolongs the action potential duration of the human atrial myocytes, probably by increasing I_{Na} inactivation.

Keywords: human atrial myocytes; ion currents; ranolazine

S6.94

Protective effects of EPO combined with GSH on hepatic ischemia reperfusion injury

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Aim: To investigate the effects of erythropoietin (EPO) combined with reduced glutathione (GSH) on hepatic ischemia reperfusion injury, and their optimal combination ratio. **Methods:** Models of mice with hepatic ischemia-reperfusion were established. Six formulas with different combination ratios were designed according to the weighted modification method. After ischemia for 30 min and reperfusion for 4 h, the blood and hepatic tissue was collected. The results were analyzed for obtaining the theoretical optimal combination ratio, according to the decreased activity of AST, ALT. Then the further confirmation test was conducted for investigating the relation between EPO and GSH. The activities of AST, ALT, MDA, SOD, GSH-P_X were measured. **Results:** Compared with those in I/R group, the activities of AST, ALT were significantly lower in EPO, EPO+GSH (M) and EPO+GSH (H) groups (P<0.01), and EPO+GSH (M) group showed the more significant effect than EPO group. When the ratio was 160 U:1 mg, EPO and GSH could decrease the activity of AST, ALT significantly. MDA concentration decreased, while the activities of SOD and GSH-P_X were increased obviously in EPO+GSH (M) and EPO+GSH (H) groups (P<0.05). **Conclusion:** Combination of EPO with GSH could produce synergistic protective effects on hepatic ischemia reperfusion injury, and the optimal combination ratio of EPO and GSH is 160 U:1 mg. The mechanism is partly related to the enhancement of antioxidation.

Keywords: EPO; GSH; hepatic ischemia reperfusion injury; weighted modification method

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S6.95

Role of Kir6.2 subunits of ATP-sensitive potassium channels in sepsis-induced cardiac dysfunction

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Aim: Cardiac dysfunction is well-described in sepsis and diagnosed in up to 60% of patients with septic shock. ATP-sensitive potassium (K_{ATP}) channels are critical to cardiac function. This study investigates the role of Kir6.2 subunits of K_{ATP} channels in cardiac dysfunction in LPS-induced sepsis. **Methods:** Kir6.2 subunits knockout (Kir6.2$^{-/-}$) and wild-type (WT) mice were injected with LPS to induce sepsis. Cardiac function was monitored by echocardiography. Left ventricles were taken for microscopy (both light and electron) and TUNEL examination. Serum lactate dehydrogenase (LDH) and creatine kinase (CK) activities, and tumor necrosis factor-α (TNF-α) levels in both serum and left ventricular tissues were determined. **Results:** Compared to WT, Kir6.2$^{-/-}$ mice showed significantly declined cardiac function 360 min after LPS administration, aggravated myocardial damage and elevated serum LDH and CK activities. Apoptotic cells were obviously increased in heart tissues from Kir6.2$^{-/-}$ mice at 90, 180, and 360 min. TNF-α expression in both serum and heart tissues of Kir6.2$^{-/-}$ mice was significantly increased. **Conclusion:** We conclude that Kir6.2 subunits are critical in resistance to sepsis-induced cardiac dysfunction through reducing myocardial damage by inhibition of apoptosis and inflammation. K_{ATP} channels blockers are extensively used in the treatment of diabetes, their potential role should therefore be considered in the clinic when patients treated with antidiabetic sulfonylureas are complicated by sepsis.

Keywords: sepsis; cardiac dysfunction; Kir6.2 subunits

S6.96

Pharmacological effects of CestrumNocturum n-butyl alcohol extract on guinea pig atria

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Aim: To investigate the contractile effect of Cestrum Nocturumn-Butyl Alcohol Extract (CNAE) on contractile characteristics of atria in isolated guinea pig. **Methods:** The effect of CNAE in contractive amplitude, contractile velocity and spread velocity were observed in isolated guinea pig right atria. **Results:** CNAE (0.1, 0.3, and 1 mg/mL) possessed of the effect of increasing the contractile amplitude, contractile velocity and spread velocity of isolated guinea pig right atria. but for the CNAE (10.0, 30, and 100 mg/mL) prominently inhibited the contractive amplitude and decreased contractile velocity and spread velocity. **Conclusion:** CNAE possessed the biphasic manner effect on the contractile amplitude, contractile velocity and spread velocity of isolated guinea pig right atria.

Keywords: Cestrum Nocturumn-Butyl Alcohol Extract (CNAE); heart atria; contractility; biphasic manner effect

S6.97

Taurine magnesium coordination compound inhibits I_{Na} and I_{to} but increases $I_{Ca,L}$ in rat ventricular cardiomyocytes

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Aim: To investigate the antiarrhythmic mechanism of taurine magnesium coordination compound (TMCC) on sodium current (I_{Na}), L-type calcium current ($I_{Ca,L}$) and transient outward potassium current (I_{to}) in single ventricular cardiomyocytes of rat. **Methods:** Whole-cell patch clamp was used to record I_{Na}, $I_{Ca,L}$, and I_{to} in single ventricular cardiomyocytes of rat. **Results:** I_{Na} and I_{to} was inhibited by 100, 200, and 400 μmol/L TMCC concentration-dependently, while $I_{Ca,L}$ was increased by 400 μmol/L TMCC and the steady-state inactivation curve of $I_{Ca,L}$ was shifted to right, however, the steady-state activation curve of $I_{Ca,L}$ remained unchanged. **Conclusion:** TMCC inhibits I_{Na} and I_{to} concentration-dependently, which may contribute to the the antiarrhythmic effect. The effect on $I_{Ca,L}$ makes TMCC different from traditional antiarrhythmic drugs.

Keywords: taurine magnesium coordination compound (TMCC); arrhythmias; ion channel; whole-cell patch clamp technique

S6.98

Rg1 improved hematopoiesis function recovery combined with human cord blood stem cells transplantation in radiation damaged SCID mice

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Aim: To study the effects of ginsenosides Rg1 combined with human cord blood stem cells (HSCs) transplantation on hematopoiesis function recovery in radiation damaged SCID mice. **Methods:** The contents of human cord blood mononuclear cells (MNCs), HSCs and blood cell subpopulations were detected by flowcytometry. Severe radiation damage SCID mice were prepared by sublethal dose X ray radiation. The MNCs were implanted by vena caudalis. Hemogram and free intracellular calcium concentration ($[Ca^{2+}]_i$) were detected. The anti-human cell nucleolus antibody-FITC labeled MNCs were observed in myeloid tissue. **Results:** Compared with MNCs transplantation along, Rg1 could recover the number of neutrophils, platelet and white blood cells at normal level after transplantation of MNCs. The duration of recovery was obviously in advance. All mice well survived, but all of model mice were dead in a week. The proportion of LSK and CD133$^+$flk-1$^+$ cells were higher. Myeloid tissue disorganization was obviously improved. The levels of $[Ca^{2+}]$i were increased by Rg1 0.1–3 μmol/L in bone marrow karyocytes. Rg1 3 μmol/L improved MNCs immigration and adherence to fibronectin coating plate. At 4 weeks after MNCs transplantation, anti-human cell nucleus antibody-FITC labeled cells were observed in myeloid tissue. **Conclusion:** Rg1 obviously improved recovery of hematopoiesis function and shortened recovery duration of neutrophils and platelets in irradiated SCID mice with MNCs transplantation. The results are related to increasing $[Ca^{2+}]_i$ level in bone marrow karyocytes and proportions of HSCs and EPCs.

Keywords: ginsenosides Rg1; mouse; haemopoietic stem cell; bone marrow

S6.99

Effects of curcumin on neonatal rat cardiac myocytes exposed to high-glucose

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Aim: To investigate the effects of curcumin on neonatal rat cardiac myocytes exposed to high-glucose. **Methods:** Neonatal rat cardiomyocytes were cultured in normal-glucose (NG, 5.5 mmol/L) or high-glucose (HG, 30 mmol/L) medium. The HG-cultured cardiomyocytes were treated with 10 μg/L curcumin. Prior to and after the high-glucose injury the cell viability was assayed by CCK8, LDH activity and ROS generation was examined by biochemical tests, and apoptosis were detected by and FCM. **Results:** Compared with NG-cultured cardiomyocytes, the LDH level and ROS generation were increased significantly in HG-cultured cardiomyocytes. Furthermore, HG induced apoptotic cell death. Curcumin significantly increased the cell viability, decreased the activity of LDH and inhibited the ROS generation. **Conclusion:** These results suggest that curcumin could reduce cardiac myocytes apoptosis exposed to high-glucose by scavenging ROS and attenuating cell death.

Keywords: curcumin; apoptosis; high-glucose; ROS

S6.100

Stemness and differentiation of pre-existing cardiosphere-derived cells were modulated by telocyte microenvironment

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Aim: Many studies have shown the existence of telocytes as a distinct type of cell in human and mammalian myocardium, which play an important role for supporting the cardiomyocytes three-dimensional organization and could secrete some grow factor to influence the microenvironment. Recently, cardiosphere-derived cells (CDCs) were found to be unusual pre-existing cells. Compared with other kinds of stem cells, CDCs showed the greatest cardiomyocytes differentiation potency. This study is to explore whether the microenvironment provided by telocytes can sustain the stemness of CDCs and improve cardiomyocytes differentiation efficiency of CDCs. **Methods:** The experiment was divided into control group, T-group (CDCs co-cultured with adult rat telocytes), N-group (CDCs co-culture with neonatal rat caridomyocytes). The observation was carried out after co-culturing for 3, 5, 7, 9, and 11 d, respectively. Q-PCR was used to check the change of stem cell markers (Nanong, Isl-1) and cardiomyocyte markers (Gata4, CTNI). Immunofluorescence was used to detect the expression of CTNI protein in each group. **Results:** After co-culturing for 3d, the outline of CDCs in the T-group was clearer than the control group and N-group. The expression of Isl-1in T-group was higher than other groups at d 3, Nanong was higher at 5 d, Gata4 and CTNI were higher at d 9. There are almost no expression of CINI protein in control group and N-group, but higher expression of CINI protein in T-group. **Conclusion:** The microenvironment provided by telocytes can maintain the stemness of CDCs at the early stage, while improve the cardiomyocytes differentiation efficiency at the last stage.

Keywords: microenvironment; telocyte; cardiosphere-derived cells; stem cells; differentiation

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S6.101

Advanced glycation end products impaired the activity of Na^+/K^+- ATPase in diabetic cardiomyopathy: role of AMPK/ SIRT1 pathway

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Aim: The activity of Na^+/K^+-ATPase is decreased in heart of diabetic animals. We investigated whether the advanced glycation end product (AGEs) impaired the stability of Na^+/K^+-ATPase through regulating the AMPK/SIRT1 pathway in the progress of diabetic cardiomyopathy (DCM). **Methods:** The rat model of type 1 diabetic mellitus (T1DM) was established by intraperitoneal injection of streptozotocin (STZ, 65 mg/kg) for one time. Neonatal rat cardiomyocytes were cultured. The function of heart was detected by Doppler and the protein expression of SIRT1 and AMPK were dectected by immunohistochemistry and Western blot. The activity of Na^+/K^+-ATPase was detected. **Results:** *In vivo* research, the results showed the activity of Na^+/K^+-ATPase decreased with down-regulated expression of AMPK and SIRT1. *In vitro* research, AGEs impaired the Na^+/K^+-ATPase activity accompanied by the decreased expression of AMPK and SIRT1. Over-expression of SIRT1 increased the Na^+/K^+-ATPase activity. 5-Aminoimidazole-4-carboxamide-3-ribonucleoside (AICAR) up-regulated the SIRT1 expression. It improved the Na^+/K^+-ATPase activity which was partly abolished by splitomicin. **Conclusion:** AGEs-induced Na^+/K^+-ATPase activity impairment participated diabetic cardiomyopathy, which was related with AMPK/SIRT1 pathway.

Keywords: diabetic cardiomyopathy; SIRT1; AMPK; Na^+-K^+-ATPase

S6.102

The therapeutic effects of Genistein Sulfonate Sodium on isolated myocardial ischemia/reperfusion injury and its relation with anti-oxidation

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Aim: The aim of this study was to examine the efficacy of Genistein Sulfonate Sodium (GSS) on isolated myocardial ischemia/reperfusion injury and the relation of its mechanism to anti-oxidation. **Methods:** An isolated rat myocardial ischemia/reperfusion (I/R) injury was made by employing Langendorff. The hearts were treated with GSS or NS at the reperfusion period. The heart rate (HR), maximum 1eft ventricular systolic pressure (mLVSP), the myocardial diastolic/systolic velocity, and the content of malonaldehyde (MDA) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in tissue were measured. **Results:** Compared with model group, function of the I/R heats treated with GSS were significantly improved, and showed a decrease in content of MDA and a obvious increase in the enzymatic activities of SOD and GSH-Px in the myocardial tissues. **Conclusion:** GSS has a therapeutic effect on ischemia/reperfusion injury in isolated rat hearts, and this protection was through enhancing anti-oxidative activity in myocardial tissues.

Keywords: Genistein Sulfonate Sodium; myocardial ischemia/reperfusion injury; anti-oxidation

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S6.102

The therapeutic effects of Genistein Sulfonate Sodium on isolated myocardial ischemia/reperfusion injury and its relation with anti-oxidation

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Keywords: Genistein Sulfonate Sodium; myocardial ischemia/reperfusion injury; anti-oxidation

Acknowledgements: This work was supported by grants from the Bureau of Education (GJJ11571) and Health of Jiangxi Province (20093191).

S6.104

Effect of TAT-tCNTF to HUVECs' injury caused by cytochalasin D

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Aim: To investigate the effect of TAT-tCNTF on injury of human umbilical vein endothelial cells (HUVEC) induced by cytochalasin D (CD) and the action mechanism. **Methods:** TAT-tCNTF and CD affected cell viability by CCK-8. Using fluorescence microscope we observed changes of F-actin resulted by CD and TAT-tCNTF. Level of intracellular free calcium concentration was detected by Fluo 4-AM loading by the fluorescence microscopy and flow cytometry. Western blot was used to detect F-actin protein expression. **Results:** HUVECs treated with 1 μg/mL CD showed obvious growth inhibition. After TAT-tCNTF treatment HUVECs grew actively. TAT-tCNTF decreased HUVEC intracellular free calcium concentration enriched by CD. Distribution of F-actin in HUVECs after CD treatment was massively reduced while crowded together after subsequent treatment with TAT-tCNTF. Western blot showed F-actin protein had almost no change. **Conclusion:** TAT-tCNTF can confront and reverse the injury caused by CD, whose mechanism might be associated with increasing the cell viability and maintaining actin cytoskeleton and with direct action with CD.

Keywords: TAT-tCNTF; cytochaladin D; F-actin; Ca^{2+}; HUVEC

S6.105

Clematichinenoside, an anti-inflammatory monomer, attenuates myocardial infarction in ischemia/reperfusion injury both *in vivo* and *in vitro*

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Aim: Clematichinenoside is a triterpenoid saponin isolated from the roots of Clematis chinensis Osbeck (Ranunculaceae). Oxidative stress is thought to play a considerable role in ischemia/reperfusion (I/R) injury that impairs cardiac function. The present study examined protective effect of clematichinenoside on regional and global I/R injury and myocytes. **Methods:** Regional myocardial I/R (MI/R) injury of rats was induced by the occlusion of left anterior descending coronary artery, and isolated guinea pigs heart using Langendorff apparatus model was served as a global I/R injury. Primary cultured neonatal ventricular myocytes were further applied to explore the anti I/R injury property *in vitro*. Infarct size was measured with TTC stain; enzyme activities such as lactate dehydrogenase (LDH), creatine kinase (CK), superoxide dismutase (SOD), maleic dialdehyde (MDA) and NO were analyzed with assay kits; iNOS and eNOS expressions were determined by Western blot. **Results:** Clematichinenoside attenuated infarct size, decreased LDH, CK, MDA levels and enhanced SOD activity. Clematichinenoside improved hemodynamics indexes, such as LVDP, $\pm dp/dt_{max}$, in the isolated guinea pigs heart after reperfusion. Clematichinenoside enhanced SOD activity, reduced MDA content, and inhibited excessive production of NO through down-regulating iNOS as well as up-regulating eNOS during I/R injury. **Conclusion:** Clematichinenoside attenuates I/R injury both *in vivo* and *in vitro* via anti-oxidant and restoring the balance between iNOS and eNOS.

Keywords: clematichinenoside; myocardial ischemia/reperfusion; anti-oxidant; nitric oxide balance

S6.106

Tumor vascular disrupting agent DMXAA inhibits platelet activation and thrombosis via inhibition of TXA2 signaling and phosphodiesterase

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Aim: 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) is a tumor vascular disrupting agent under clinical trials as an adjacent antitumor agent. DMXAA is structurally similar to flavone-8-acetic acid (FAA), an old tumor vascular disrupting agent with anti-platelet and anti-thrombotic effects. In contrast to FAA, which causes bleeding in tumor patients, no bleeding has been reported in patients receiving DMXAA. Whether DMXAA also affects platelet function is not clear. To determine the effects of DMXAA on platelet function and explore the underlying mechanisms. **Methods and results:** DMXAA concentration-dependently inhibited human platelet aggregation and ATP release induced by U46619, arachidonic acid, ADP, collagen or ristocetin. Furthermore, DMXAA inhibited phosphorylation of Erk1/2 and Akt downstream of TXA2 signaling inhibition. DMXAA also inhibited human platelet phosphodiesterase (PDE). The anti-platelet effects were further confirmed using mice intravenously given DMXAA. DMXAA dramatically inhibited thrombus formation in $FeCl_3$-injured mouse mesenteric arterial thrombus model and laser-injured mouse cremaster arteriole thrombus model. Notably, at a dose exhibiting antithrombotic effects similar to clopidogrel in mice, DMXAA did not significantly increase bleeding. **Conclusion:** For the first time, we found that tumor-vascular disrupting agent, DMXAA has potent anti-platelet and anti-thrombotic effects without any bleeding diathesis. As DMXAA inhibits platelet activity with safe profile, DMXAA could be used as an efficacious and safe anti-platelet drug.

S6.107

Role of polyamines on MPTP in myocardial ischemia reperfusion injury

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Aim: To investigate role of polyamine on MPTP in myocardial ischemia reperfusion injury. **Methods:** The ischemia/reperfusion model of heart was made by global ischemia for 30 min and reperfusion for 120 min. The left ventricular function, polyamine content, the lactate dehydrogenase (LDH), nitricoxide synthase (NOS), and superoxide dismutase (SOD) activity, MPTP opening of isolated mitochondria were measured, respectively. **Results:** Compared to the I/R group polyamine content was decreased both in ischemia and reperfusion period. Both treatment with MPTP specific inhibitor cyclosporine A (CSA 0.2 μmol/L) and polyamine (0.1, 0.2, 1, and 5 μmo/L) reduced the infarct size and improved the contractile function of heart with increased LVSP, increased $\pm dp/dt_{max}$ and decreased LVEDP; the LDH releasing levels in coronary effluent and mitochondria was effectively decreased, Mn-SOD content in mitochondria was increased, the T-NOS content was decreased. However, polyamine treatment (10 and 15 μmol/L) had a decreased recovery of LVSP, $\pm dp/dt_{max}$, and increased LVEDP, which were associated with a increased infarct size, decreased Mn-SOD content, increased T-NOS content. Polyamine with high doses (10 and 15 μmol/L) increased the opening, and polyamine with relative low doses (0.1, 1, and 5 μmol/L) inhibited the MPTP opening. **Conclusion:** Polyamine may provide either cardioprotection by inhibiting MPTP or induce myocardial injury by increasing MPTP opening, and which is dependent on the concentration of polyamine used.

Keywords: polyamine; mitochondrial permeability transition pore

S6.108

Role of miR-17-3p in vascular inflammation

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Aim: MicroRNAs (miRNAs) are a class of small, noncoding RNAs of ≈22 nucleotides that negatively regulate gene expression. Upon binding to the 3′-UTR of mRNA, they induce mRNA degradation or repress mRNA translation. MiRNAs appear to play a role in the development/progression of many disorders, including inflammation, cardiovascular diseases and endothelial dysfunction. The current project aims to study the role of miR-17-3p in vascular inflammation. **Methods:** Human umbilical vein endothelial cells (HUVECs) were transfected with the miR-17-3p agomir (miR-17-3p mimetic) or its negative control using Lipofectamine 2000. They were incubated with lipopolysaccharide (LPS, 10 ng/mL) for 16 h to induce inflammatory reactions. The level of miR-17-3p and the expression of inhibitor of kappa B protein alpha (IκBα, the cytoplasmic inhibitor of nuclear factor kappa B) were measured by quantitative PCR and Western blotting, respectively. The amount of interleukin-8 (IL-8, an inflammatory marker) released in the culture medium was detected with ELISA kit. **Results:** The level of miR-17-3p and IL-8 in HUVECs were increased but the expression of IκBα was reduced following LPS stimulation. LPS-induced increase in IL-8 level and decrease in IκBα expression were smaller in HUVECs transfected with miR-17-3p agomir than in those transfected with the negative control. **Conclusion:** MiR-17-3p is up-regulated in LPS-induced inflammation. It may produce an anti-inflammatory effect by reducing IL-8 production and preventing the down-regulation of IκBα.

Keywords: inflammation; miR-17-3p; IL-8; IκBα

S6.109

Phosphorylation of nuclear myosin II and myocardial ischemia-reperfusion oxidative injury

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Aim: Nuclear myosin II (NMII) was reported to be a core transcription factor. Through phosphorylation/dephosphorylation of its regulatory light chain (MLC_{20}), NMII can regulate its targeting gene expression. In this study, the correlation between phosphorylation /dephosphorylation of MLC_{20} and NADPH oxidase 2 (NOX2) expression following myocardial ischemia-reperfusion (I/R) was investigated. **Methods:** In a rat model of myocardial IR, the left main coronary artery of rat hearts was subjected to 1 h occlusion followed by 3 h reperfusion. The infarct size, apoptosis, the activities of creatine kinase (CK), myosin light chain kinase (MLCK) and NOX, the phosphorylation level of MLC_{20}, the expression of NOX2 and the level of H_2O_2 were measured. **Results:** Following myocardial I/R, the infarct size in myocardium was significantly increased accompanied by elevated activities of CK, caspase-3, MLCK and NOX, up-regulated phosphorylation level of nuclear MLC_{20}, NOX2 expression and high numbers of myocardial apoptotic cells; these effects were attenuated by pretreatment with the specific inhibitor of MLCK. **Conclusion:** NMII might be involved in regulation of NOX2 expression through phosphorylation/dephosphorylation of MLC_{20} following myocardial

(I/R). Inhibition of the phosphorylation MLC_{20} can reduce I/R-induced myocardial oxidative injury.

Keywords: Myosin II; ischemia/reperfusion; oxidative stress injury; NADPH oxidase; myosin light chain kinase

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S6.110

Protective effects of essential oil of Alpinia Zerumbet against human umbilical vein endothelial cells (HUVECs) injury induced by LPS

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Aim: To investigate the protective effects of essential oil of Alpinia Zerumbet (EOFAZ) against human umbilical vein endothelial cells (HUVEC) injury induced by LPS. **Methods:** HUVECs were cultured *in vitro* and divided into 6 groups as following: control, model (LPS 15 μg/mL), EOFAZ (4, 1, and 0.25 μg/L, respectively), and aspirin (ASP 4 mmol/L). The third to fourth generations of HUVECs were used to evaluate the LPS-induced endothelial dysfunction. The morphology of HUVEC was observed by inverted phase contrast microscope. The pathological check was assayed by HE and Gimsa staining. The cell proliferation activity was assayed by MTT. The LDH and NO release was detected by colorimetric methods using commerical kits. The contents of TXA2, PGI2, ET-1, AngII, IL-1, IL-2, IL-6, and IL-8 in medium were measured by ELISA methods. **Results:** Exposure HUVECs to 15 μg/mL LPS for 12 h, there were significantly HUVECs injury compared with the control group. After preincubation with EOFAZ 1 h, cell morphology was remarkably ameliorated. MTT results and LDH release confirm EOFAZ improved the survival ratio. Furhter research indicated that EOFAZ increased NO and PGI2 contents in medium, however, significantly decreased the TXA2, ET-1, AngII, IL-1, IL-2, IL-6, and IL-8 contents. **Conclusion:** The EOFAZ ameliorate the HUVECs injury induced by LPS, the mechanism may involve regulation of the inflammatory factors.

Keywords: essential oil of Alpinia Zerumbet; HUVEC; LPS; endothelial injury

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S6.111

Inflammation during pregnancy in rats leads to early aorta morphological changes in adult offspring

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Aim: Previous findings from our laboratory have shown that exposure to inflammation stimuli induced by lipopolysaccharide (LPS) during pregnancy increases the risk of hypertension in adult offspring. This study aimed to evaluate the role of maternal LPS exposure on early aorta morphological changes in the adult offspring and further assess the contribution of gap junction during hypertension. **Methods:** Female Spraque-Dawley rats were administered 0.5 mL, ip, saline or 0.79 mg/kg, ip, LPS on gestation Days 8, 10, and 12. Histopathological observation and morphometric analysis of the thoracic aortas were performed by transmission electron microscopy, simultaneously, four connexin (Cx) molecules, namely Cx37, Cx40, Cx43, and Cx45 in the thoracic aortas were detected by immunofluorescence staining and Real-Time PCR in offspring. **Results:** In 3-month-old maternal inflammation offspring, the thoracic aortas exhibited lesions, including impaired endothelial cells, thickening and fibration of intimas, infiltration of inflammatory cells to the subendothelial space, and migration and proliferation of vascular smooth muscle cells to the intima. However, no detectable pathological changes were observed in the offspring without maternal LPS exposure. Immunofluorescence labeling revealed that the endothelial cells of thoracic aortas express abundant levels of Cx43, lower amounts of Cx37, 40, and 45. The analysis of mRNA suggested the expression of Cx43 mRNA increased obviously in offspring of maternal LPS exposure. **Conclusion:** In summary, our results indicate that maternal LPS exposure during pregnancy leads to vascular changes that predispose to hypertension in adult offspring, which may be related to abnormal gap junctions.

Keywords: inflammation; pregnancy; connexin

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S6.112

Peroxiredoxin II, an anti-oxidant protein, is a new regulator of cardiac contractility through phospholamban

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Aim: Peroxiredoxin II (prxII), a cytosolic form of the anti-oxidant peroxiredoxin family, has been reported to protect cardiomyocytes from oxidative stress-induced injury. Interestingly, we found that prxII expression levels were decreased to 60% in failing human hearts, compared to donors. However, the expression levels of this cellular peroxidase were significantly increased in the hyperdynamic hearts of two genetically modified mouse models with: a) phospholamban ablation; and b) over-expression of the active inhibitor-1 of protein phosphatase 1. **Methods:** To determine whether alterations in prxII levels may contribute to altered cardiac contractility, we generated adenoviruses with sense (Ad-prxII) and anti-sense prxII (Ad-prxII-AS) insertions and infected adult rat cardiomyocytes with these viruses. Myocyte contractility and calcium kinetics were then recorded after 24 h of infection. **Results:** Over-expression of Ad-prxII was associated with decreases in the basal rates of contraction and relaxation to 31% and 25%, respectively, of GFP control levels. The fractional shortening was also reduced to 36% of GFP controls. In parallel, calcium kinetics were inhibited as evidenced by 65% decreases in the peak of the calcium transient and prolongation in the time to 80% decay of calcium peak to 70% of controls. The caffeine-induced sarcoplasmic reticulum calcium content was also reduced to 80% of GFP controls. Isoproterenol stimulation abolished the inhibitory effects of prxII over-expression. On the other hand, Ad-prxII-AS infected cardiomyocytes exhibited enhanced contractile parameters and Ca-kinetics, compared to GFP controls under basal conditions, but the maximally stimulated parameters by Iso were similar among the 3 groups. Interestingly, the depressed or increased contractility by Ad-prxII or Ad-prxII-AS respectively was associated with parallel decreases or increases in phosphorylation of phospholamban (Ser16 and Thr17), compared to GFP-infected cells. There were no alterations in the expression levels of key SR calcium handling proteins: SERCA2, phospholamban, calsequestrin and ryanodine receptor in the infected cells. **Conclusion:** These findings indicate that prxII, an anti-oxidant protein, may regulate basal cardiomyocyte contractile performance through phospholamban phosphorylation.

Keywords: peroxiredoxin II; phospholamban; contractility; calcium transient

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Dihydrotanshinone I inhibits LPS induced LOX-1 expression *via* TLR4/ROS/NF-κB signal pathway in human umbilical vein endothelial cells

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Aim: Lectin-like receptor for oxidized low density lipoprotein (LOX-1) has been implicated as the main receptor for oxidized low density lipoprotein (ox-LDL) primarily expressed in endothelial cells. and it allows uptake of ox-LDL into endothelial cells. It is considered as a potential target for atherosclerosis. Dihydrotanshinone I (DT) is one of the major active components isolated from Chinese herb *Salvia miltiorrhiza*. In the present study, the effect of DT on LPS induced LOX-1 expression in human umbilical vein endothelial cells (HUVECs) and the possible molecular pathways were investigated. **Methods:** The cytotoxicity of DT to HUVECs was determined by MTT assay. HUVECs were treated with LPS for 24 h with or without DT pretreatment. The protein expression of TLR4, p65, and LOX-1 was examined by Western blotting; The intracellular reactive oxygen species (ROS) production was examined by flow cytometry with fluorescence probe DCFH-DA; Specific TLR4 siRNA was used to test the role of TLR4; the uptake of ox-LDL was evaluated with Dil-ox-LDL. **Results:** DT showed no toxic effect on

HUVECs at 0.01-1 μmol/L for 24 h. LPS induced LOX-1 expression in a time and concentration dependent manner. The protein expression of TLR4, p65 and LOX-1 was significantly enhanced by LPS, which was reversed by DT pretreatment. LPS induced LOX-1 expression was blocked by TLR4 siRNA, NF-κB inhibitor, and NADPH oxidase inhibitor DPI. Furthermore, both DT and DPI inhibited LPS induced ROS formation. In addition, DT dramatically inhibited ox-LDL uptake induced by LPS. **Conclusion:** These data suggested that DT inhibits LPS induced LOX-1 expression via TLR4/ROS/NF-κB signal pathway providing evidence that DT might benefit atherosclerosis by inhibiting LOX-1 expression in endothelial cells.

Keywords: dihydrotanshinone I; LOX-1; ROS ; endothelial cells

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Andrographolide inhibit isoproterenol-induced myocardial hypertrophy in rats by reducing the excessive activation of autophagy

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Aim: To explore the inhibition effect of andrographolide (AP) on myocardial hypertrophy induced by isoproterenol (Iso), and the mechanism about autophagy regulation. **Methods:** Myocardial hypertrophy animal model was induced in rats by subcutaneous injection of isoproterenol (1 mg/kg) for 10 consecutive days, once a day. The rats were subcutaneously injected with different concentrations of AP (0.1 and 0.3 μmol/kg), solvent or NS, for 14 consecutive days, once a day. At 12 h of fasting after last administration, the rats were sacrificed. We accurately weighed body weight, heart weight, left ventricular weight, and calculated heart mass index and left ventricular mass index; Western blot assay was used to detect the expression of BECN-1 and LC3-B in myocardial tissue, which were the marker protein of autophagy. **Results:** Compared with the normal group, myocardial hypertrophy rats showed a significantly increase in heart mass index (P<0.05), left ventricular mass index (P<0.05), and protein expression of BECN-1 and LC3-B in myocardial tissue (P<0.05). Compared with model group rats, AP treated rats showed a significantly decrease in heart mass index (P<0.05), left ventricular mass index (P<0.05), and protein expression of BECN-1or LC3-B (P<0.05). **Conclusion:** Andrographolide delays the process of isoproterenol-induced cardiac hypertrophy and heart failure in rats. The effects may be through reduction of the excessive activation of autophagy.

Keywords: andrographolide; isoproterenol; cardiac hypertrophy; autophagy

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Endogenous acetylcholine contributes to mild hypothermia induced endothelium-dependent relaxations in the SHR aorta

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The present study investigated whether or not endogenous acetylcholine contributes to endothelium-dependent relaxations induced by mild hypothermia. Aortic rings with or without endothelium of spontaneously hypertensive (SHR) and Wistar-Kyoto normotensive (WKY) rats were suspended in organ chambers for isometric tension recording. The rings were contracted with prostaglandin F2α and exposed to progressive mild hypothermia (from 37°C to 31°C). The latter induced endothelium-dependent relaxations which were inhibited by atropine, tubocurarine, acetylcholinesterase, bromoacetylcholine, hemicholinium-3 and vesamicol in SHR but not in WKY aortae. The endothelium of both SHR and WKY aortae took up choline from the extracellular environment and synthesized acetylcholine. Compared with WKY, SHR aortae expressed similar level of acetylcholinesterase and choline acetyltransferase, but a lesser amount of vesicular acetylcholine transporter, located mainly in the endothelium. These findings demonstrate that the endothelium of both normotensive and hypertensive rats can produce acetylcholine. Mild hypothermia causes endothelium-dependent relaxations which can be reduced by interfering with the metabolism or the action of acetylcholine in SHR aortae only. Thus, in the hypertensive rat, endothelial endogenous acetylcholine activate acetylcholine receptors and elicit endothelium-dependent relaxations.

Keywords: non-neuronal cholinergic system; endogenous acetylcholine; mild-hypothermia; endothelium-dependent relaxation