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S11.1

Aquaporin-1 dependent proximal tubular cells migration mediates reno-protective effect of acetazolamide against ischemia-reperfusion induced acute kidney injury

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Aim: The study is to investigate whether diuretic acetazolamide (AZA) protects against renal ischemia-reperfusion (I/R) injury, and its possible link with water channel aquaporin-1 (AQP1). **Methods:** *In vivo* study was conducted using AQP1 wild-type ($AQP1^{+/+}$) and knock-out ($AQP1^{-/-}$) mice, randomly divided into three groups: sham, I/R and AZA treatment group. Mice blood was collected for renal function measurement and kidneys were collected for F-actin staining, Western blot examination. *In vitro* study was examined by HK-2 cell line with hypoxia/reoxygenation injury. We observed the migration promoting effect of AZA using wound-healing and transwell assays. AQP1 specific siRNA knockdown and overexpression plasmid were also utilized. **Results:** In $AQP1^{+/+}$ mice, serum creatinine (Scr) and blood urea nitrogen (BUN) were increased in I/R group, while in AZA treated group these indicators dropped. By contrast, in $AQP1^{-/-}$ mice, AZA treatment failed to decrease either Scr or BUN level. Besides, in $AQP1^{+/+}$ mice we confirmed aquaporin-1 (AQP1) expression on cell membrane in I/R group decreased and returned after AZA treatment. F-actin staining demonstrated AZA could promote epithelial cells migration by increasing protrusion–like structures, however, this effect was blunted in $AQP1^{-/-}$ mice. *In vitro*, we showed AZA promoted HK-2 cell migration. In addition, AQP1 siRNA abolished promoting migration effect of AZA; AQP1 overexpression improved the migration of HK-2 cells after AZA treatment. **Conclusion:** Our results firstly indicated that AZA ameliorates renal IR injury via promoting epithelial cells migration through regulating AQP1.

Keywords: Aquaporin-1 (AQP1); ischemia-reperfusion; acute kidney injury; acetazolamide; migration

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S11.2

Determination of lapachol by liquid chromatographic-mass spectrometry and the application to pharmacokinetics in rats

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Aim: To study the quantification methods and pharmacokinetic application of lapachol. **Methods:** A sensitive and specific high-performance liquid chromatography-tandem mass spectrometry method (LC/ESI/MS) was developed and validated for quantification of the bioactive naphthoquinone lapachol in rat plasma after intragastric administration. The analytes were determined using the negative electrospray ionization mode in the selected reaction ion monitoring (SRM). The chromatographic separation was on a Capcell C_{18} column coupled with a C_{18} guard column using a mobile phase composed of acetonitrile-water containing 0.02% acetic acid. Simultaneous MS detection of lapachol and the internal standard was performed at the *m/z* 241 (lapachol) and *m/z* 255 (Isoliquiritigenin), and the SRM of the two compounds were at 186 (lapachol) and 135 (Isoliquiritigenin), respectively. **Results:** The lower limit of quantification was 10 ng/mL in rat plasma. The precision measured was obtained from 3.49 to 8.38%. Extraction recoveries were in the range of 84.79%–97.67%. **Conclusion:** This method was successfully applied to the quantification and pharmacokinetic study of lapachol in rats.

Keywords: lapachol; determination; pharmacokinetics; LC-ESI-MS^n

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S11.3

Telomere-independent Rap1, modulates NF-κB-dependent inflammation in macrophages

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Aim: The activity of nuclear factor (NF-κB) is modulated by cytoplasmic repressor activator protein 1 (Rap1) in cancel cells. Experiments were designed to test whether or not Rap1 is present in atheromatous lesions and affects NF-κB dependent inflammation in macrophages, the predominant cell type involved in the progression of atherosclerotic lesions. **Methods:** The expression of Rap1 and macrophages in human atheromatous lesions was detected by immunohistochemistry. The expression of lipopolysaccharide- (LPS, 50 ng/mL, 4 h) and tumor necrosis factor alpha (TNF-α, 100 ng/mL, 4 h) induced NF-κB dependent genes and proteins in wild type and Rap1 knockdown THP-1 cells were measured using real-time PCR and enzyme-linked immune sorbent assays. Western blotting was applied to determine the expression of p65 and its phosphorylation in wild type and Rap1 knockdown THP-1 cells. **Results:** Rap1 co-localized with macrophages and its staining positively correlated with graded human atherosclerosis. In THP-1 cells, Rap1 knockdown suppressed the phosphorylation of p65 and reduced the expression of LPS- or TNFα-induced NF-κB dependent pro-inflammatory cytokines at mRNA and protein levels. **Conclusion:** Telomere-independent Rap1 is present in human athermanous lesions. Cytoplasmic Rap1 within macrophages may impact on inflammation during atherosclerosis.

Keywords: Rap1; NF-κB; macrophage; inflammation; atherosclerosis

S11.4

PPARδ mediates the vascular benefits of metformin in diet-induced obese mice

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Aim: 5′ Adenosine monophosphate-activated protein kinase (AMPK) was reported to form transcriptional complex with peroxisome proliferator-activated receptor delta (PPARδ) to induce gene expression synergistically. This study investigated whether PPARδ mediates the effects of anti-diabetic drug metformin (AMPK activator) in ameliorating ER stress and endothelial dysfunction in high-fat diet-induced obese (DIO) mice. **Methods:** Endothelium-dependent relaxation (EDR) and protein expressions in aortae were measured by wire myograph and Western blotting, respectively. Fluorescence imaging determined the levels of reactive oxygen species (ROS) and nitric oxide (NO) under confocal microscopy. **Results:** DIO mice showed impaired EDR and elevated levels of ER stress markers and ROS in aortae. Chronic metformin treatment reversed the above-described effects in DIO *PPARδ* wild-type littermates but not in knockout mice. Metformin and PPARδ agonist GW1516 alleviated the tunicamycin (ER stress inducer)-induced impairment of EDR, ER stress and oxidative stress in mouse aortae as well as NO production in endothelial cells. Effects of metformin were abolished by the co-treatment of GSK0660 (PPARδ antagonist) whilst those of GW1516 were not affect by compound C (AMPK inhibitor). **Conclusion:** The present study supports that metformin curtails ER stress and oxidative stress and increases NO bioavailability upon activation of AMPK/PPARδ pathway, and subsequently combats against vasculopathy in obese diabetic mice.

Keywords: metformin; PPARδ; endothelial dysfunction; ER stress; obesity

S11.5

WS070117 ameliorates the formation of foam cells by regulating the expression of LOX-1 and ABCG1 in an AMP-activated protein kinase-dependent manner

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Aim: The present study investigated the effects of WS070117, a novel small molecule compound, on foam cell formation, and the possible underlying mechanism. **Methods:** J774.A1 murine macrophages were pretreated with different concentrations of WS070117 (25, 50, and 100 μmol/L) and then incubated with oxLDL for 24 h. Detection of foam cell formation is conventionally practiced by Oil Red O (ORO) and Filipin staining of lipid-laden macrophages. Other methods include DiI-labeled oxLDL (DiI-oxLDL) uptake and cholesterol efflux assay. Real-time PCR and Western blot were performed to measure the expression of ATP-binding cassette transporter G1 (ABCG1) and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in mRNA and protein levels. To investigate the mechanism on macrophage foam cell formation of WS070117, AMP-activated protein kinase (AMPK) selected inhibitor Compound C were added. **Results:** Treatment with WS070117 resulted in a decrease in oxidized low-density lipoprotein (oxLDL)-mediated cholesterol accumulation in macrophages, a

consequence that was due to a decrease in cholesterol uptake and an increase in cholesterol efflux. Additionally, WS070117 significantly down-regulated protein expression of LOX-1. However, WS070117 increased the mRNA and protein expression of ABCG1. Moreover, Compound C abolished the WS070117-induced protective effects on the expression of LOX-1 and ABCG1. **Conclusion:** Our data suggest that WS070117 suppresses foam cell formation by regulation of ABCG1 and LOX-1 in an AMPK depend manner.

Keywords: WS070117; foam cells; LOX-1; ABCG1; AMPK

S11.6

Therapeutic effects of ginsenoside metabolite compound K in adjuvant-induced arthritis via attenuating dendritic cells function

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Aim: An *in vitro* study has shown joint-protective effects of ginsenoside metabolite compound K (CK) in RA, but the effects of CK in RA animal model and the mechanisms are still unknown. In this study, the effects of CK on adjuvant-induced arthritis (AA) and the underlying mechanisms were investigated. **Methods:** Complete Freund's adjuvant (CFA) was used to induce AA rats. After the onset of arthritis, rats were given CK (5, 10, 20, 40, 80, and 160 mg/kg,) and MTX (0.5 mg/kg). To evaluate the severity of arthritis, arthritis global assessment and swollen joint count were evaluated every 3 d. Levels of serum antibodies were detected by ELISA. Proliferation of T cells was measured by ^{3}H-TdR. Subset of T cells, phagocytosis and the expression of co-stimulators and MHCII on dendritic cells (DC) were assayed by flow cytometry. Ability of DC in activation of T cell was measured by mixed lymphocyte reaction. Levels of CCL19 and CCL21 in spleen were assayed by immunology and histology chemistry. The expression of CCR7 on DC was assayed by Western-blot. **Results:** CK attenuated AA clinical signs, regulated the levels of antibodies in serum, alleviated the histopathology of spleen in AA rats. CK inhibited T cell proliferation, up-regulated the percentage of naïve T cell, down-regulated the percentage of activated T cells. In addition, CK inhibited levels of chemokines CCL19 and CCL21 in spleen, suppressed DC activation and the expression of CCR7 on DC. **Conclusion:** CK attenuation AA and the mechanisms were likely due to inhibit immune response by inhibiting DC activation via CCR7.

Keywords: ginsenoside metabolite compound K; dendritic cells; adjuvant-induced arthritis; immune response

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S11.7

4-HNE increases intracellular ADMA levels in cultured HUVECs through miR-21-dependent mechanisms

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Aim: To investigate whether 4-hydroxynonenal (4-HNE) regulates asymmetric dimethylarginine (ADMA) metabolism through affecting the expression of ADMA metabolizing enzymes and the involvement of microRNA (miRNA) miR-21 pathway. **Methods:** Cultured HUVECs were treated with 4-HNE (at concentrations of 1, 5, and 10 μmol/L, respectively) or 0.1% DMSO (vehicle control) for 24 h. MiR-21 inhibitor (final concentration of 100 nmol/L) was transfected at 1 h before 4-HNE treatment. HUVECs were also transfected with miR-21 (at concentrations of 50 nmol/L and 100 nmol/L) and cultured for 12, 24, and 48 h, respectively. DDAH mRNA and miR-21 expression were determined by semi-quantitative real time PCR. DDAH1 and DDAH2 protein expression were analyzed by Western blot. ADMA in the cell medium and cell lysates were analyzed by ELISA. **Results:** MiR-21 decreased DDAH1 and DDAH2 expression and ADMA metabolic activity of the cell lysates significantly, and increased intracellular ADMA content significantly in HUVECs. 4-HNE treatment (10 μmol/L) for 24 h upregulated pri-miR-21 and miR-21 expression and increased intracellular ADMA concentration, decreased DDAH1/2 mRNA and protein expression and ADMA metabolizing activity of the cell lysates significantly. MiR-21 inhibitor reversed the 4-HNE-induced inhibition on DDAH1 expression completely, and partially reversed the 4-HNE-induced changes in ADMA metabolizing activity and intracellular ADMA content. **Conclusion:** 4-HNE down-regulates DDAH1 expression and increases intracellular ADMA accumulation in HUVECs through a miR-21-dependent mechanism.

Keywords: microRNA 21 (miR-21); dimethylarginine dimethylaminohydrases (DDAH); asymmetric dimethylarginine (ADMA); 4-hydroxynonenal (4-HNE); human umbilical venous endothelial cells (HUVECs).

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S11.8

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

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Aim: Experiments were designed to determine the effects of thymoquinone, 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 inhibitor (L-N^{G}-nitroarginine methyl ester, L-NAME) or soluble guanylyl cyclase (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, ODQ). They were contracted with prostaglandin F2α 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 detaNONOate in L-NAME-treated rings restored and even increased the contractile response to thymoquinone. By contrast, treatment with 8-Bromo cyclic GMP of ODQ-treated preparations did not restore the augmentation by thymoquinone. **Conclusion:** These findings demonstrate that thymoquinone causes an endothelium-dependent augmentation similar to that seen in hypoxia (Chan *et al*, Am J Physiol 2011: H2313). This facilitation also requires endothelium-derived NO and activation of soluble guanylyl cyclase, but not the presence of cyclic GMP.

Keywords: 8-Bromo cyclic GMP; deta NONOate; hypoxia; nitric oxide; prostaglandin F2α

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S11.9

WS070133 effect on sleep-promoting neurons in the ventrolateral preoptic nucleus

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In clinical practice, sedatives are expected to induce the sedation without serious side effects. WS070133 ((3S4R,5R)-3,4-dihydroxy-5-(6-((4-hydroxy-3-methoxybenzyl)amino)-9H-purin-9-yl)tetrahydrofuran-2-yl) methyldecanoate is a new synthesized adenosine derivative. Previous behavioral study show that WS070133 induce sedative effects and sleep promoting effect. This research is concentrated to elucidate the possible effects of WS070133 on the sleep-promoting area in hypothalamus. Whole cell patch clamp-recording was performed to test the WS070133 induced excitation of VLPO neurons by increasing their spontaneous firing thereby in sleep function. The adenosine and its derivatives excite adenosine receptors in the neurons of ventrolateral preoptic nucleus (VLPO), and trigger sleep when excited VLPO neurons release inhibitory neurotransmitters to nearby wake-promoting area. Our results show that WS070133 excited VLPO neurons through a postsynaptic activation of type A_{2A} adenosine receptors. The firing rate of type 2 VLPO neurons was significantly increased under 10^{-5} mol/L WS070133, reveal that the WS070133 induce sleep via the activation of sleep-promoting neurons in VLPO. The results suggest that the activation of neurons in the ventrolateral preoptic nucleus could be a possible pattern for adenosine derivative WS070133 to induce sedation.

Keywords: WS070133; ventrolateral preoptic nucleus; sleep promoting; patch clamp

S11.10

Penetration of verapamil across blood brain barrier following cerebral ischemia depending on both paracellular pathway and P-glycoprotein transportation

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Aim: Blood brain barrier (BBB) dysfunction is a common facet of cerebral ischemia, and the alteration of drug transporter, P-glycoprotein (P-gp), has been documented. This study explores influence of damaged BBB and elevated P-gp on cerebral verapamil penetration after ischemia both *in vivo* and *in vitro*. **Methods:** Middle cerebral artery occlusion (MCAO) induced ischemia/reperfusion (I/R) of rats, and $Na_2S_2O_4$ induced hypoxia/reoxygenation (H/R) damage of rat brain mirovessel endothelial cells (RBMECs) respectively served as BBB breakdown model *in vivo* and *in vitro*. Evans-Blue (EB) extravagation and ^{125}I-albumin were used to quantify BBB dysfunction; UPLC-MS/MS analytical method was performed to determine accurately the concentration of verapamil in brain tissue and cell. Flow cytometry, immunohistochemisty and Western blotting were applied to evaluate transport function and protein expression of P-gp. **Results:** Overexpressed ICAM-1 and MMP-9 mediated BBB dysfunction after ischemia, which induced EB leakage and ^{125}I-albumin uptake increase. Enhanced accumulation of verapamil in brain tissue, but intracellular concentration reduced evidently after H/R injury. Transcellular transportation of verapamil elevated when P-gp function or expression was inhibited after H/R injury. **Conclusion:** These data indicated that BBB penetration of verapamil under ischemia condition was not only depending on BBB breakdown, but also regulated by P-gp.

Keywords: cerebral ischemia; blood brain barrier; P-glycoprotein; verapamil

S11.11

Hrd1 facilitates tau degradation by proteasome and lysosome pathways.

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Intraneuronal accumulation of abnormal phosphorylated tau (p-tau) is a molecular pathology in many neurodegenerative tauopathies, including Alzheimer's disease (AD), Progressive supranuclear palsy and frontotemporal dementia with parkinsonism-linked to chromosome 17 (FTDP-17). However, the underlying mechanism remains unclear. Here, we found that Hrd1 interacted with tau and promoted the degradation of total tau and p-tau as well. The degradation of tau depended on its Hrd1 E3 activity. Knockdown of endogenous Hrd1 with siRNA stabilized tau levels. In addition, inhibition of proteasome maintained tau level and increased Hrd1-mediated tau ubiquitination, suggesting the proteasome was involved in tau/p-tau degradation. Additionally, tau levels were decreased significantly on autophagy induced by rapamycin, which was reversed on inhibition of autophagy by 3-MA or NH4Cl, suggesting tau can be degraded by autophagy. Hrd1 over-expression decreased tau and p-tau levels while inhibition of autophagy activity by 3-MA or NH4Cl stabilized tau, but not p-tau. Moreover, Hrd1 facilitates the formation of autophagosome in tau-overexpressing cells, suggesting Hrd1-mediated tau degradation partly depends on autophagy-lysosome pathway. Furthermore, over-expression of Hrd1 significantly alleviated tau cytotoxicity and promoted cell survival. These results indicated that Hrd1 target tau for proteasome and autophagy degradation and promotes neuron survival. This study provides an important insight into the molecular mechanisms of human tauopathies.

Keywords: Alzheimer's disease; tau; tauopathies; Hrd1; ubiquitin-proteasome pathway; autophagy-lysosome pathway

S11.12

Pentamethylquercetin (PMQ) inhibited the growth of hepatic ascitic tumor cell H22 by improving metabolic environment in monosodium glumate (MSG)-induced obese mice

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Aim: Obesity is associated with higher risk and with poor prognosis of cancer. We investigate the effects of PMQ on the metabolic disorders and the tumor growth in MSG-induced obese mice. **Methods:** MSG-induced obese mice model was established. At 5 week of age, all mice were randomized to ten groups (n=10 per group): Control and MSG model+vehicle, PMQ 5, 10, and 20 mg/kg/d, metformin 300 mg/kg/d respectively and administrated by gastric gavage for 19 weeks. Twenty-two weeks old mice were injection sc with H22 hepatic ascitic tumor cells. After two weeks, animal were anesthetized and blood, tumor, liver, adipose tissues were harvested. **Results:** Compared with control mice, MSG treatment induced obesity, metabolic disorders, and much larger tumor in mice. In obese mice, the expression of Sirt6 and PTEN were down-regulated and the expression of TBK1, IKKε and c-Myc were up-regulated. PMQ dose-dependently reduced body weight gain and adipose weight, improved insulin resistance and metabolic disorders, and inhibited tumor growth in MSG mice. There is significant positive correlation between improved metabolism and inhibited tumor growth by chronic PMQ treatment. With treatment of PMQ, the expressions of Sirt6, PTEN, TBK1, IKKε, and c-Myc were totally inversed. **Conclusion:** PMQ improved metabolic disorders, inhibited tumor growth and inversed the changes of the expressions of Sirt6, PTEN, TBK1, IKKε, and c-Myc in MSG-induced obese mice. The results suggest that obesity and tumor would be simultaneously prevented by PMQ.

Keywords: pentamethylquercetin; obese mice; hepatic carcinoma H22; Sirt6; PTEN

S11.13

Methionine sulfoxide reductase A catalyzes antioxidation of *L-methionine in vitro* and *in vivo*

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Aim: Methionine was reported as an anti-oxidant *in vitro*. In this study, the anti-oxidation of *L*-methionine in hydrogen peroxide (H_2O_2)-induced CHO cell injury model and its mechanism were investigated. **Methods:** Free radical was induced by Fenton reaction and measured by electron paramagnetic resonance (EPR) to valuate *L*-methionine's radical scavenging property. H_2O_2 (250 μmol/L) was used to induce an oxidant stress model in CHO cells. Methionine (0.125, 0.25, 0.5, and 1 mmol/L) was added at 20 h before H_2O_2-induced-oxidative stress. DMSO (0.5%) was used to inhibit methionine sulfoxide reductase A (MsrA) activity. Mortality rate of CHO cells was assayed by MTT. And superoxide formation was tested by MitoSox fluorescence assay. **Results:** *L*-Methionine could reduce radical formation at a rather high concentration of 1 and 10 mmol/L. MsrA enzyme enhanced the radical scavenging property of methionine *in vitro* remarkably. Under H_2O_2 condition, cell death was remarkably increased. Methionine pretreatment decreased the percentage of cell death and superoxide formation induced by H_2O_2 in 0.125 and 0.25 mmol/L. DMSO, a competitive inhibitor of MsrA, can block the antioxidantion of *L*-methionine at low concentrations *in vivo*. **Conclusion:** *L*-methionine can reduce CHO cell injury induced by H_2O_2 and the mechanisms were likely due to a MsrA-catalyzed methionine oxidation.

Keywords: MsrA; methionine; oxidant stress; free radical; superoxide

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S11.14

Study on schizophrenic model induced by NMDA receptor antagonist in mice: role of oxidative stress

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Aim: Schizophrenia is a severe illness and its pathophysiolog is not fully understood. The present study aims to evaluate the role of oxidative stress in schizophrenic mouse model induced by ketamine, known as NMDA receptor antagonist, to further understand its pathophysiology. **Methods:** Locomotor activity, Y-maze task, novel object recognition and forced swimming test were used to assess behavior abnormalities associated with schizophrenia. Immunohistochemical method was used to study the change of neurons. SOD, MDA, NO and NOS in serum and supernatants of brain tissues were measured by commercial test kits. **Results:** Ketamine (25, 50, and 100 mg/kg, ip) administered acutely or repeatedly (for 7 d) can increase the locomotor number significantly. In Y-maze task, ketamine (25, 50, and 100 mg/kg) impaired spontaneous alternation after both acute and repeated treatments. In novel object recognition test, acute or chronic ketamine treatment showed no significant effect on mouse exploratory preference behavior. In forced swimming test, repeated treatment of ketamine (100 mg/kg) enhanced the immobility duration. Acute treatment of ketamine

(100 mg/kg) had no effect on neurons in the prefrontal cortex or hippocampus (1, 3, 5, and 7 d after the treatment). In contrast, repeated treatment of ketamine caused neuronal impairment in mouse hippocampus (3rd, 5th, and 7th d after the final administration). Further results showed that repeated administration of ketamine increased NO and NOS in prefrontal cortex, hippocampus and serum, while decreased SOD in hippocampus and serum. **Conclusion:** Chronic ketamine treatment to mice successfully mimics the core behavioral deficits in schizophrenia. Neuronal injury was associated with the chronic ketamine-induced schizophrenic model. Oxidative stress may contribute to the significant neuronal injury in mouse brain induced by chronic ketamine treatment.

Keywords: schizophrenia; ketamine; neurons; oxidative stress
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S11.15

Metabolome alteration of maternal-fetal unit biofluid in IUGR rats induced by prenatal caffeine ingestion and the exploration of maternal biomarkers for early prediction and alert

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Aim: Metabolic abnormalities were reported in maternal-fetal unit with intrauterine growth retardation (IUGR). Here we aimed to investigate the metabolomes in maternal-fetal biofluid of caffeine-induced IUGR and tried to explore maternal biomarkers. **Methods:** Pregnant rats were orally treated with caffeine (20, 60, and 180 mg/kg) from gestational days (GD) 11 to 20. Metabolome of maternal and fetal plasma on GD20 and maternal plasma on different times (GD11, GD14, and GD17) were analyzed by ^{1}H nuclear magnetic resonance-based metabonomics. Corticosterone (CORT) level in maternal and fetal plasma were determined by ELISA. **Results:** Caffeine increased maternal and fetal CORT levels which are negatively correlated with fetal bodyweights, respectively. Fetal metabolome were altered by caffeine, which presents as elevated glucose and decreased lactic acid, reduced fatty acids, triglyceride and β-hydroxybutyric acid (BHB), varied apolipoprotein and increased levels of several amino acids. The maternal metabolome indicated decelerated glucose while enhanced lipid metabolism and accelerated protein catabolism while some metabolites had altered since mid-gestation. **Conclusion:** IUGR maternal-fetal metabolome was changed by CORT elevation. Maternal CORT level and some metabolites namely α-, β-glucose, high-density lipoprotein-cholesterol, BHB, isoleucine and valine might be an integrated biomarker system for caffeine-induced IUGR.

Keywords: caffeine; IUGR; metabolome; glucocorticoid; biomarkers
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S11.16

Procaspase-3 activator PAC-1 and YCL0426 induce hypoxia reaction and DNA damage in tumor cells

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Aim: PAC-1 is the first small molecular compound identified to induce apoptosis by direct activation of procaspase-3 into caspase-3, the main executor of apoptosis. YCL-0426 is a potent procaspase-3 activating compound synthesized in our lab. In this study, the antitumor mechanism and the impact to other signal pathways of PAC-1 and YCL0426 were investigated. **Methods:** The apoptosis inducing property was evaluated by Cellomics multiparameter apoptosis 1 kit. Influence on other signal pathways was detected on a panel of genetically modified fluorescent cell lines. Fluorescent photographs were obtained and analyzed on IN Cell Analyzer 2000 High Content Analysis platform. **Results:** Only early signs of apoptosis were found when HepG2 cells were treated with 10 μmol/L of YCL0426 for 30 h, and apoptosis was not obvious too in 30 μmol/L PAC-1 group, However, 3 h of 10 μmol/L PAC-1 or 3 μmol/L YCL0426 treatment was sufficient to induce a significant Hif-1α stabilization under normoxic condition. Hif-1α stabilized in a time- and dose-dependent manner. PAC-1 and YCL0426 were also found to induce Rad51 foci formation in the nuclear significantly, which was a sign of DNA double strand breaks. A slight upregulation of pH2AX fluorescence intensity and p53 protein level were also observed. **Conclusion:** We found that PAC-1 and YCL0426 induced hypoxia reaction and DNA damage response in cancer cells. DNA damaging property might be the main antitumor mechanism.

Keywords: apoptosis; procaspase-3; Rad51 foci; Hif-1α; high content analysis

S11.17

Effects of hawthorn leaf procyanidins on calcium mobilization in HUVEC

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Aim: Intracellular calcim elevations, especially long term elevation are necessary for most activities of vascular endothelial cells. Hawthorn leaf procyanidins significantly increase the production of NO in vascular endothlial cells, which is dependent on intracellular calcium mobilization. This study is to observe the effects of hawthorn leaf procyanidins on $[Ca^{2+}]_i$ in HUVEC and study the underlying mechanism. **Methods:** $[Ca^{2+}]_i$ in HUVEC was detected by using Fura-2/AM in a live cell station for 30 min. **Results:** 6–50 mg/L oligometric procyanidins (OPC) and 12.5–50 mg/L polymeric procyanidins (PPC) concentration-dependently increased $[Ca^{2+}]_i$. The effects of OPC could be inhibited by deleting extracellular calcium with EGTA, inhibiting intracellular calcium release with TG, treating HUVEC with Ni^{2+} and heparin (blockors of cell membrane Na^+/Ca^{2+} exchanger and endoplasmic reticulum IP3 receptor, respectively), and deleting extracellular sodium. The effects of PPC could be inhibited by deleting extracellular calcium with EGTA, treating with Ni^{2+}, and deleting extracellular sodium, while not be affected by inhibiting intracellular calcium release. **Conclusion:** Hawthorn leaf procyanidins can induce calcium mobilization in HUVEC. The activity of OPC may be exerted through a mixed mode including Na^+/Ca^{2+} exchange-dependent calcium influx and intracellular calcium release; while the activity of PPC may be exclusively related to Na^+/Ca^{2+} exchange-dependent calcium influx.

Keywords: hawthorn leaf; procyanidins; HUVEC; intracellular calcium; mobilization

S11.18

The screening and preliminary evaluation of $A\beta_{1-42}$ aggregationinhibitors *in vitro*

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Aim: To screen the inhibitors of β-Amyloid (Aβ) aggregation and evaluate the active compounds at molecular level. **Methods:** The aggregation of $A\beta_{1-42}$ wasdetected by fluorescent dyes Thioflavin-T (Th-T). Upon the binding of fibrils, Th-T displays a dramatic shift of the excitation maximum (from 385 nm to 450 nm) and the emission maximum (from 445 nm to 482 nm). β-Sheet of Aβ was analyzed by circular dichroism (CD). **Results:** Nine compounds were screened in this study. Eight of them decreased the total fluorescence intensity of $A\beta_{1-42}$. Among all the compounds H118C had the lowest cell toxicity. Absorbance scanning (from 370 nm to 770 nm) showed that H118C had no absorbance at 440 nm and 485 nm. The emission spectrum scanning (Ex=440 nm, Em from 460 nm to 650 nm) showed that H118C did not increase the fluorescence intensity at 485 nm, indicated that H118C couldn't influence the binding of Th-T to $A\beta_{1-42}$. These results indicated that H118C had no nonspecific influence on fluorescence. CD spectroscopy showed H118C could reduce the β-sheet content of $A\beta_{1-42}$. **Conclusion:** H118C can effectively inhibit the aggregation of $A\beta_{1-42}$. Reducing β-sheet of $A\beta_{1-42}$ might be one of the mechanisms for H118C to disrupt $A\beta_{1-42}$ aggregation.

Keywords: Thioflavin-T; β-Amyloid; circular dichroism
Acknowledgements: This work was supported by the National Natural Science Foundation of China (81202505 and 81100239); Chinese National Technology Major Project of New Drug Development (2012ZX09J12201-002, BWS11J052).

S11.19

Protective effect of Shuangshenningxin preconditioning on mitochondrial function against ischemia/reperfusion injury in rat hearts

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Aim: To investigate the effect of Shuangshenningxin (SSNX) on mitochondrial functional defects and metabolic derangement in myocardial ischemia/reperfusion (MI/R) injury. **Methods:** Rats were treated with trimetazidine (10 $mg\cdot kg^{-1}\cdot d^{-1}$, ig), SSNX (22.5, 45, and 90 $mg\cdot kg^{-1}\cdot d^{-1}$, ig) or saline for 5 d. MI/R was induced by ligation of the left anterior descending coronary artery for 40 min and releasing

to promote reperfusion for 120 min on the last day of administration. Then, the activities of serum LDH and CK-MB were measured. Cardiac tissues were tested for the levels of ATP, ADP, and AMP by HPLC and transmission electron microscopy was used to observe the changes of mitochondrial ultrastructure. Mitochondria were isolated for determination of mitochondrial swelling and metabolic enzymes activities, respectively. **Results:** Both trimetazidine and SSNX could decreased the release of LDH and CK-MB after MI/R injury. This observed cytoprotection may be associated with an increase in myocardial ATP content and a preservation of mitochondrial structure, decreasing mitochondria swelling degree as well as increasing the activities of complex I and citrate synthase. **Conclusion:** These findings indicate that SSNX may reduce myocardial ischemia/reperfusion injury by protecting mitochondrial structure and function.

Keywords: Shuangshenningxin; ischemia/reperfusion injury; mitochondria; energy metabolism

S11.20

Effects of traditional Chinese medicine brucine on alcohol consumption and preference in alcohol-preferring FH/Wjd rats

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Aim: To evaluate pharmacological and toxicological effects on alcoholism of brucine (BRU), isolated from traditional Chinese medicine *Strychnos nux-vomica*. **Methods:** FH/Wjd rats were used to study the roles of BRU on alcohol, sucrose intake and their preference in two-bottle choice test. In the paradigm of 5-d alcohol deprivation, antagonism of BRU on alcohol craving was investigated. Besides, the effects of BRU on locomotion, feeding and body weights were measured. To test the abuse potential of BRU, Conditioned Place Preference (CPP) was performed. Up and Down Procedure was used to determine LD_{50} of BRU. Finally, BRU (60 mg/kg, sc bid) was injected for 30 d to observe body weight loss and survival rate. **Results:** BRU (10–30 mg/kg, sc bid), but not sucrose, dose-dependently reduced alcohol intake and alcohol-preference. Moreover, 30 mg/kg of BRU significantly suppressed the extra alcohol intake during the 4 h re-access after 5-d deprivation. The data indicated that BRU didn't affect locomotion, feeding and body weights at the same doses. CPP in FH/Wjd rats was not induced by 30 and 90 mg/kg BRU (sc). Its LD_{50} (sc) in male FH/Wjd rats is 264.6±17.7 mg/kg. Thirty-day BRU injection did not affect body weights and survival rate. **Conclusion:** BRU may be a promising drug with low toxicity and no abuse potential for the treatment of alcoholism.

Keywords: brucine; alcoholism; FH/Wjd rat

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S11.21

Hepatoprotective effects of *Gentiana manshurica* Kitagawa

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Aim: Gentiopicroside-containing extract and gentiopicroside from *Gentiana manshurica* Kitagawa (GM) was investigated for their hepatoprotective effects *in vivo*. **Methods:** We applied *in vivo* model of acetaminophen (APAP)-induced liver injury, *D*-galactosamine (GalN)/lipopolysaccharide (LPS)-induced feminant hepatic failure and ethanol-induced acute alcohol liver injury models using C57BL/6 mice. **Results:** GM significantly prevented the APAP-induced acute hepatotoxicity by enhancing the hepatic antioxidant activity, involving the inhibition of caspase-3 cleavage and JNK/ERK activation. GM prevented ethanol-induced acute liver steatosis, possibly through blocking CYP2E1-mediated free radical scavenging effects and SREBP-1-regulated fatty acid synthesis. And gentiopicroside also protects against GalN/LPS-induced liver injury, which prevents oxidative stress, apoptosis and MAPK phosphorylation. **Conclusion:** Thus, GM may be useful as a potential pharmacological therapy for the prevention of liver injury and represent a potential new source of drugs for hepatoprotection.

Keywords: *Gentiana manshurica* Kitagawa; feminant hepatic failure; alcohol steatosis

Acknowledgements: This study was supported by the National Natural Science Foundation of China (81260664 and 81160538).

S11.22

Anti-proliferative effect of MAM, a natural product on human breast cancer cell MCF-7 by inducing apoptosis through the mitochondria-dependent pathway

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Aim: The study was designed to investigate the anti-proliferative effect of MAM, a natural product isolated from *Polygonum cuspidatum*, on six different human cancer cells and the underlying mechanism on human breast cancer MCF-7 cells. **Methods:** The cytotoxic activity of MAM on six cancer cells (95D, A549, H1299, MCF-7, MDA-MB-231, and MDA-MB-461) was tested by MTT assay. Occurrence of apoptosis was detected by Hoechst 33342 staining. The profile of cell cycle and the level of reactive oxygen species (ROS) were detected by flow cytometry. The mitochondrial membrane potential was detected with the fluorescence probe JC-1. The protein expression of Bcl-2, Bax, cleaved-caspase-3, -7, -9 were examined by Western blotting. **Results:** MAM showed anti-proliferation activity on all the six type of cancer cells. The IC_{50} value for MCF-7 cells was 3.3 μmol/L after 24 h treatment. The JC-1 staining showed MAM significantly induced mitochondrial membrane potential loss. MAM treatment resulted in cell cycle arrested in S-phase. Hoechst 33342 staining revealed that MAM caused nuclear DNA fragmentation. Furthermore, the protein expression of Bcl-2 was significantly down-regulated and the ratio of Bax/Bcl-2 significantly up-regulated. The protein expression of cleaved-caspase-3, -7, -9 was also significantly up-regulated. **Conclusion:** This study demonstrated that MAM inhibit the proliferation cancer cells mediated by induction of cell cycle arrest and apoptosis.

Keywords: MAM; MCF-7 cell; cancer; apoptosis

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S11.23

Serum amyloid A differentially activates microglia and astrocytes via the PI3K pathway

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Aim: Microglia and astrocytes in the brain play an important role in the development and/or progression of Alzheimer's disease (AD). Serum amyloid A (SAA) is a major acute-phase protein produced locally in the brain and colocalized with senile plaques in AD patients. We investigated whether SAA plays a role in the development of AD. **Methods:** The viability of cultured primary microglia and astrocytes was measured by MTT; cell cycle and apoptosis analysis was also conducted. Cultured microglia and astrocytes were simulated with 1 μmol/L SAA for different periods of time (2, 4, 6, and 12 h) or treated with 1 μmol/L SAA with or without 15 min pretreatment of MAPK or PI3K inhibitors. Total RNA was extracted for qPCR analysis. **Results:** SAA induced morphological changes of primary microglia but not astrocytes. Interestingly, SAA increased the viability of microglia by inhibiting their apoptosis and reduced the viability of astrocytes by inducing G_1 cell cycle arresting. SAA treatment increased the mRNA levels of IL-6, TNF-α, IL12p40, IL23p19, and IL-10, with higher potency in microglia than in astrocytes. However, SAA induced more iNOS mRNA in astrocytes than in microglia. SAA induced these cytokines and iNOS expression by activating the PI3K pathway in both glial cells, but selectively activated the JNK pathway in microglia and the NF-κB pathway in astroctyes. **Conclusion:** These results suggest that SAA can stimulate a different reactive phenotype in microglia and astrocytes, and SAA regulates cell viability differently in these two glial cell in part through the PI3K pathway.

Keywords: Alzheimer's disease; serum amyloid A; microglia; astrocytes

S11.24

Adult hippocampal telomerase is implicated in mood disorders

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Aim: The aim of the present study is to determine the role of adult hippocampal telomerase in the pathology of mood disorders. **Methods:** We constructed telomerase reverse transcriptase subunit (TERT) knockout mice, TERT RNA interference virus (LV-TERT-shRNA) and TERT overexpression virus (AD-TERT), and used telomerase activity inhibitor AZT. **Results:** Selective infusion of AZT into bilateral adult hippocampus detent gyrus (DG) by stereotaxic surgery or 28 d osmotic minipump induced depression-like and anxiety-like behavior in mice. TERT knockout mice displayed extremely high aggression behavior level, depression-like phenotype, and anxiety-like symptoms, which were reversed by overexpression of TERT through infusion of AD-TERT into the DGs. Knock down of TERT gene in adult hippocampus by microinjection of LV-TERT-shRNA into the DGs also induced depression-like and anxiety-like behavior in mice. Twenty-one days chronic mild stress reduced TERT protein level and telomerase activity in adult hippocampus and led to depressive behaviors in mice but reversed by infusion of AD-TERT into the DGs. In addition, selective infusion of AD-TERT into the DGs induced antidepressive-like and antianxiety-like behavior changes in normal mice. **Conclusion:** Our study suggest implication of telomerase function in the pathology of mood disorders, implying adult hippocampal telomerase may be a unique target for treating affective disorders.

Keywords: telomerase; hippocampus; depression; anxiety; aggression
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S11.25

The role of telomerase in neuronal stem cell

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This overview is to comprehensively understand the role of telomerase in neuronal stem cell (NSC). We reviewed the papers concerning about the function of telomerase in the self-renew, proliferation, differentiation, survival and fate-decision of embryonic and adult NSC. It is revealed that telomerase is essential for the ability of self-renew and proliferation of NSC. Moreover, telomerase promote the ability of neuronal differentiation of NSC. Importantly, telomerase also exists in the new born neurons and play a critical role in the morphological and functional maturation. Telomerase also can protect neurons from apoptosis and excitoxicity and promote survival. Increasing data suggest that adult hippocampal telomerase involves in the modulation of depression-related behaviors by regulating hippcampal neurogenesis. Understanding the role of telomerase in NSC is not only important for gaining further insight into the mystery of NSC, but also helpful for searching a novel therapeutic target for the treatment of depression and other neurodegenerative diseases.

Keywords: telomerase; neuronal stem cell
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S11.26

Biosynthesis and antidiabetic activity of oral cyclic glucagon-like peptide-1 analogues

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Aim: Glucagon-like peptide-1 (GLP-1) attracts interest for notable benefits in T2DM patients, but the short half-life limits its clinical use. Anti-enzymatic and oral cyclic GLP-1 analogues were biosynthesized, and *in vitro* and *in vivo* bioactivity was evaluated. **Methods:** The inhibitory domains of DPP-IV and/or neutral endopeptidase 24.11 (NEP24.11) were introduced in *glp*-1. The fusion protein containing hGLP-1-*Mxe* GyrA intein-CBD (C-terminal binding domain) was induced to express in *E coli* BL21 (DE3), and purified by affinity chromatography on chitin beads and on-column splicing by induction of 2-mercaptoethanesulfonic acid (MESNA) at pH 8.5. The anti-enzymatic and GLP-1 receptor agonistic activities were tested. The hypoglycemic effects were investigated in ICR mice. **Results:** Three series of peptides named ***61***, ***31C*** and ***31*** were gotten, and each contains linear or cyclic molecular structures. The analysis of RP-HPLC showed that the residual area of $61N_2$ incubated with DPP-IV for 24 h was 49.9%, and those of 31CK and 31K incubated with NEP24.11 for 4 h were nearly 50% and 20%, respectively. 61K, 31CK and 31K could significantly increase the GLP-1R specific luciferase reporter gene expression transfected in NIT-1 cells from 0.5-5 μmol/L. 61K and 31CK single administration (0.5 mg/kg, sc) could markedly lower the blood glucose in ICR mice after oral glucose loading (P<0.05). **Conclusion:** The cyclic GLP-1 analogues can increase the stability against DPP-IV and NEP24.11, and possess notable GLP-1R agonistic and hypoglycemic activity.

Keywords: GLP-1; anti-diabetes; oral cyclic peptide
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S11.27

Protective effects of YBBGEP on experimental psoriasis

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Aim: YBBGEP is a compound iodine preparation is developed by Yantai Lanxue Pharmaceutical Co Ltd. The purpose of the present study was to investigate the potential protective effects of YBBGEP on the experimental psoriasis. **Methods:** The estrus cycle model with vaginal epithelium mitosis was used to evaluate the activity of YBBGEP on the epithelial cell mitosis in mice. The formation of granular layers in the tail scaled epidermis was measured. The mouse itching assay induced by dextran was observed to analyze its anti-pruritus. **Results:** The mitosis of vaginal epithelial cells was obviously inhibited in mice treated with YBBGEP 0.06 and 0.12 mL/kg (P<0.01). Significantly increase of the formation of granular layers in the tail scaled epidermis was detected after YBBGEP 0.06 and 0.12 mL/kg was treated (P<0.01). In the anti-itching test, YBBGEP also markedly decreased paroxysm frequency and the duration of the itch induced by dextran (P<0.05 or P<0.01). **Conclusion:** These results demonstrate that YBBGEP has protective effect on experimental psoriasis, which may be related to its inhibition of the epidermal cell hyperplasia, promotion on granular layer cell differentiation and relief itching.

Keywords: YBBGEP; psoriasis; protective effects

S11.28

Experimental studies of rLj-RGD3, a novel recombinant toxin protein containing three RGD motif from *Lampetra japonica*, on anti-OGD/RF effects in PC12 cells

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Aim: The aim of this paper is to investigate the protective effects of recombinant *Lampetra Japonica* RGD-peptide (rLj-RGD3) on PC12 cells oxygen-glucose deprivation and reperfusion (OGD/RF) injury, and discuss the protection mechanism. **Methods:** Normal cultured PC12 cells were randomly assigned to 6 groups: normal cell control group, model group, rLj-RGD3 high (25 μg/mL), medium (12.5 μg/mL) and low (6.25 μg/mL) groups and Edaravone positive control group (4 μg/mL). By using DMEM medium containing deoxidation agent (Sodium dithionite $Na_2S_2O_4$) merged deficiency sugar, PC12 cells were incubated for 2 h under oxygen-glucose deprivation and refperfusion for 24 h. Cell vitality, Lactate dehydrogenase (LDH) activity in cell supernatant fluid and glutathione (GSH) concentration in cell were measured after 24 h. Cell apoptosis situation was observed by AO/EB fluorescence color and Annexin V-PI flow cell technology. The protein expression levels of FAK, p-FAK, Caspase-3, Bcl-2 were detected by the Western blotting method. **Results:** The cell vitality of the model group was markedly decreased after OGD/RF. The activity of LDH in cell supernatant fluid was increased significantly and the content of GSH in cell was reduced significantly. In rLj-RGD3 administration group the cell vitality and the content of GSH were significantly increased. The activity of LDH in cell supernatant fluid decreased obviously and had certain dose dependence. The model group increased cell apoptosis significantly by the AO/EB dyeing and flow cytometry detection technology. By contrast, cells apoptosis was significantly reduced in rLj-RGD3

dosage group. In the model group the expression of FAK, p-FAK, Bcl-2 protein was significantly inhibited in PC12 cell, and the expression level of Caspase-3 was increased significantly. In rLj-RGD3 group FAK, p-FAK, and Bcl-2 were raised, and Caspase-3 expression was restrained. **Conclusion:** rLj-RGD3 has the protective effects on OGD/RF injury in PC12 cell. In addition, the mechanism of the protective effects can relate to the activation of Integrin-PI3K/Akt pathway.

Keywords: rLj-RGD3; oxygen-glucose; deprivation and reperfusion; PC12; integrin; antiapoptotic

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S11.29

Clonidine improve the performance of learning and memory via increased glutamic acid decarboxylase after chronic cerebral ischemia in rats

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Aim: Clonidine, an α_2-adrenergic receptor agonist, has been demonstrated to be neuroprotective when administered during ischemia. It is not known whether clonidine can improve the learning and memory after brain ischemic injury. We examined this possibility using a permanent forebrain ischemia model. **Methods:** We used bilateral common carotid arteries ligation (2VO) to induce chronic cerebral ischemia model. Four weeks after 2VO, clonidine was given (0.05 mg/kg, ip) for 7 d. Behavioral assessment of operant conditioning was performed by Morris water maze. Mortality rate of neuron was assayed by immunohistochemistry with confocal and the expression of glutamic acid decarboxylase (GAD) protein was measured by Western blot. **Results:** Our results showed that chronic cerebral ischemic injury could lead to disorder of congnitive function in rats and the expression of GAD is decreased. Giving clonidine could reverse these change, and the expression of glutamic acid decarboxylase (GAD) is increased. **Conclusion:** GAD is responsible for synthesizing GABA, which could plays a crucial role in brain ischemic injury and result in congnitive disfunction. α_2-adrenergic receptor agonist can increase the expression of GAD, which promote synthesizing GABA.

Keywords: clonidine; GAD; ischemia injury; spatial learning and memory

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S11.30

HPLC assay validation for KCNQ/M-channel opener QO58-lysin in rat plasma and its application to pharmacokinetics studies

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The voltage-gated KCNQ/M-potassium channels play a principal role in controlling neuronal excitability. Mutations or inhibition of KCNQ/M-channels can cause neurological diseases, such as myokymia and benign familial neonatal convulsions. Therefore, development of specific KCNQ2/3 channels openers may provide a therapeutic strategy for treatment of neuronal excitability disorders. We recently designed and synthesized a novel series of KCNQ/M-channel openers pyrazolo[1,5-a]pyrimidin-7(4H)-ones (PPOs). QO58-lysin, one lead compound of PPOs, has been shown to have antiepileptic and antinociceptive effects. To achieve accurate evaluation of QO58-lysin *in vivo*, in this study, we established a reliable and efficient HPLC assay for determination of pharmacokinetics of QO58-lysin in rat plasma. Separation was achieved using a reverse-phase C18 column with a mobile phase of 0.2 mol/L ammonium acetate in H_2O-acetonitrile (40:60, *v*/*v*). Nitrendipine was used as an internal standard (IS). The retention times of QO58-lysin and the IS in rat plasma were 3.8 and 5.4 min, respectively. Calibration curve was linear ranging from 0.1 to 120 μg/mL with a correlation coefficient (r^2) of 0.9996. The lower limit of quantification was 0.1 μg/mL. Accuracy, precision, recovery and stability were all within acceptable criteria according to FDA guidelines. The validated assay was successfully applied to determine the pharmacokinetics of QO58-lysin administered intravenously (10 mg/kg) and orally (12.5, 25, and 50 mg/kg) in SD rats.

Keywords: KCNQ opener; QO58-lysin; HPLC; pharmacokinetics

S11.31

Endoplasmic reticulum stress aggravation as the mechanism underlying Guttiferone K-induced apoptosis in colorectal cancer cell line HCT116

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Aim: Guttiferone K (GutK) is a novel cytotoxic compound able to selectively induce apoptosis in colorectal cancer cells. The study proposes endoplasmic reticulum (ER) stress aggravation as the underlying mechanism of action in GutK-induced apoptosis and suggests such mechanism as the rationale of its cancer selectivity. **Methods:** Treatment of various concentrations of GutK with or without specific inhibitor for JNK SP600125 and subsequent GutK treatment for 24 h with or without pre-treatment of chemical chaperone 4-PBA in colon cell line HCT116 confirmed that GutK acts through ER stress-induced apoptotic pathways. Immunoblotting of PARP cleavage and annexin-V flow cytometry were adopted to probe propensity to apoptosis whereby real-time PCR and immunoblotting were adopted to identify changes in ER stress marker CHOP and GRP78 protein and gene expression, respectively. **Results:** SP600125 markedly blocked GutK-induced apoptosis in HCT116. GutK induced up-regulation of CHOP and GRP78 in mRNA and protein expression in a dose-dependent manner. 4-PBA effectively reverted GutK-induced apoptosis. **Conclusion:** The study confirmed ER stress aggravation as the underlying mechanism of action in GutK-induced apoptosis and suggested that such mechanism might be one of the reasons for its cancer selectivity. These altogether rectifies further development of GutK as a potential selective anti-cancer drug.

Keywords: Guttiferone K; endoplasmic reticulum aggravation; cancer selectivity; apoptosis

S11.32

Inhibitory effects of osteopontin on human mast cells

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Aim: The concentration of the multifunctional extracellular matrix glycoprotein, osteopontin (OPN) is increased in inflammatory tissues and is believed to modulate the functions of inflammatory cells. In our study, the effects of OPN on inflammatory responses of human mast cells are investigated. **Methods:** OPN extracted from human milk and human mast cells cultured from $CD34^+$ monocytes isolated from peripheral blood were employed in the current study. Mast cell activation was induced by anti-IgE. Histamine release and cytokines (IL-8 and TNF-α) synthesis were determined by spectrofluorometric and ELISA assay, respectively. Adhesion and chemotaxis of human mast cells in response to OPN was determined by fluorescent assay and transwell assay, respectively. **Results:** In solution, human milk OPN but not recombinant OPN was found to inhibit anti-IgE induced histamine release only in the presence of manganese. In addition, OPN could suppress the chemotaxis of immature mast cells induced by CCL11 and CXCL12. When OPN is coated to culture surface, it was found to mediate adhesion of mast cells through RGD domain. Upon adhesion of the mast cells to OPN, anti-IgE induced IL-8 and TNF-α synthesis was reduced dose dependently but not histamine release. **Conclusion:** These studies suggest that OPN may be able to alleviate the inflammatory responses of human mast cell toward antigen, therefore affecting pathological conditions related to mast cell.

Keywords: osteopontin; mast Cells; inflammation

S11.33

Quantum dots as tools for monitoring HSV-TK/ganciclovir cancer suicide gene therapy

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Aim: To be able to label a gene and monitor its migration are key important approaches for the clinical application of cancer suicide gene therapy. Photonic nanomaterials are introduced in this work. **Methods:** One of the most promised suicide genes-herpes simplex virus thymidine kinase (HSV-TK) gene-is successfully linked with CdTe/CdS core/shell quantum dots (QDs) via EDC/NHS coupling

method. TK gene delivery and anti-cancer activity were assayed by confocal laser scanning microscopy and MTT methods. **Results:** From confocal microscopy it was demonstrated that plasmid TK intracellular trafficking can be effectively and distinctly traced via monitoring the luminescence of the QDs up to 96 h after transfection of QDs-TK conjugates into HeLa cells. MTT results show that the QDs-TK conjugates have a high efficient cytotoxicity after adding ganciclovir (GCV) into HeLa cells, whereas the QDs exert no detectable deleterious effects on the cellular processes. The apoptosis induced by QDs-TK conjugates with GCV is distinctly traced partly due to the strong luminescence of the QDs. **Conclusion:** These results provide useful insight towards using the QDs-TK conjugates in future studies to gain a better understanding of the efficiencies of the various processes involved early cancer theranostic even cancer therapy *in vivo*.

Keywords: quantum dots; herpes simplex virus thymidine kinase; ganciclovir; gene therapy

S11.34

The research method on carcinogenesis mechanisms of cigarette smoke condensate based on network toxicology

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Aim: To create a research method on carcinogenesis mechanisms of cigarette smoke condensate (CSC) based on network toxicology, mainly discuss the carcinogenesis of CSC, make carcinogenic network, provide a method for the research on carcinogenesis mechanisms. **Methods:** Make the network about carcinogenesis mechanisms of CSC by collating the literature in recent years. (1) Make the network about main compositions in CSC: CSC is a complex compound consists of hydrocarbon and hydrocarbon oxide compounds of B(a)P, amine, nitrosoamines and so on. Although these compounds are less, they play a cumulative role in the body. (2) Make the network about toxicological action of CSC. (3) Research the cancer network data on CSC-toxicological action in the OMIM database, there are 10 kinds of toxicological action are related to 8 kinds of cancer. (4) Set up the CSC-toxicological action network by the Cytoscape. **Results:** (1) Under analyzing the network, after being activated by metabolic enzymes, B(a)P can lead to cancer mediated by AhR and assisted by ARNT. (2) The HIF-1 and AhR pathway can be activated by hypoxia and B(a)P, due to ARNT is also HIF-1β, the two pathway may refrain each other. **Conclusion:** Applying the knowledge and technology of network toxicology to research the carcinogenesis of main composition of CSC. It can play a key role to analyze cancer etiology, discuss the carcinogenesis mechanisms and find the new anti-cancer drugs.

Keywords: network; toxicology; cigarette smoke condensate; cancer

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S11.35

Elevated 20-HETE contributes to the improved endothelial function in lipocalin-2 deficient mice

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Lipocalin-2 is a glycoprotein constitutively secreted by adipocytes. In obese human subjects, the circulating lipocalin-2 level is elevated and positively correlated with systolic arterial blood pressure, dyslipidemia and insulin resistance. In mice, deficiency of lipocalin-2 protects against aging- and obesity-induced endothelial dysfunction. High pressure liquid chromatography combined with enzyme-linked immunosorbent analysis revealed that the 20-HETE content was up-regulatedsignificantly in the aorta of lipocalin-2 knockout mice, whereas the amount of 11,12-diHETE decreased in the same tissues. Incubation with 20-HETE (10^{-7} mol/L) significantly attenuated the contractions induced by the TP receptor agonist U46619 in aortic rings and by acetylcholine in carotid arteries. In summary, increased production of 20-HETE contributes to the improved endothelial function in lipocalin-2 deficient mice.

Keywords: lipocalin-2; 20-HETE; endothelial dysfunction

S11.36

Measuring emotional and non-emotional behaviours in mice: a triple integrated test

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Aim: Current classical anxiety tests do not provide, individually, a pure and complete picture of an animal's emotional profile. The objective of this study was to investigate the utility of a triple integrated apparatus (TIA) that combines the three most widely used behavioral tests, namely the open field test (OFT), the modified elevated zero maze (mEZM) and the light/dark box (LDB). **Methods:** TIA was composed of OFT, mEZM and LDB, which were connected with each other side-by-side at the same height, respectively, with square openings on the center of the connecting walls of OFT and the dark compartment of LDB. Mice from two different strains were used and response to emotion-modulating drugs were evaluated for 15 min in TIA. **Results:** Factor analysis showed that emotional and non-emotional behaviors were dissociable. Kunming and C57BL/6J mice showed markedly different behavioral profiles in TIA, largely consistent with three individual tests. The anxiolytics diazepam increased the exploration of the aversive areas (OFT center, mEPM open arms, LDB light compartment), while the antidepressant fluoxetine had no effects.**Conclusion:** The results suggest that TIA may represent an alternative to the use of test batteries, which is sensitive to genetic/pharmacological influences on emotional and non-emotional behaviors.

Keywords: anxiety; animal model; open field test; modified elevated zero maze; light/dark box

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S11.37

A novel inhibitor of human La protein with anti-HBV activity discovered by structure-based virtual screening and *in vitro* evaluation

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Aim: The human La (hLa) protein, which forms a stabilizing complex with HBV RNA ribonucleoprotein to promote HBV replication, is a promising target of molecular therapy. This study aimed to discover novel inhibitors of hLa that could inhibit HBV replication and expression. **Methods:** A multistage molecular docking approach was used to screen a Specs database and an in-house library against hLa binding sites. Sequential *in vitro* evaluations were performed to detect potential compounds with high scores in HepG2.2.15 cells. **Results:** Of the 26 potential compounds with high scores chosen for experimental verification, 12 had HBV DNA inhibition ratios of less than 50% with $P<0.05$. Six had significant inhibition of HBV e antigen (HBeAg) levels, and 13 had significant inhibition of HBV surface antigen (HBsAg) levels by *in vitro* assays. Compounds HBSC-11, HBSC-15 and HBSC-34 (HBSC is system prefix for active compounds screened by the library) were selected for evaluation. HBSC-11 was found to have an obvious inhibitory effect on hLa transcription and expression. **Conclusion:** Our findings suggest that anti-HBV activity of HBSC-11 may be mediated by a reduction in hLa levels. In addition, our data suggest the potential clinical use of hLa inhibitors, such as HBSC-11, for treating HBV infection.

Keywords: hepatitis B virus; human La protein; structure-based virtual screening; docking; inhibitor

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S11.38

Phosphorylation of human La protein at Ser^{366} by CK2 contributes to HBV replication and expression *in vitro*

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Aim: Human La protein (hLa) is a multifunctional RNA-binding protein involved in the regulation of hepatitis B virus (HBV) expression. Casein kinase II (CK2), a protein kinase, is known to activate hLa by phosphorylating Ser^{366}. Tetrabromobenzimidazole (TBBz) has been shown to be a specific inhibitor of CK2 activity, which suggests that TBBz may be useful for reducing HBV gene expression. The aim of our study was to determine if inhibition of CK2 by TBBz and decreased phosphorylation of hLa Ser^{366} (pLa) would reduce HBV gene expression. **Methods:** pLa and total La expression levels were evaluated by immunohistochemistry in human liver tissues with or without HBV infection. HepG2.2.15 cells (an HBV-expressing cell line) were treated with TBBz, and cell viability and pLa levels were evaluated. Knockdown of hLa and CK2 levels by specific siRNA and mutant hLa Ala366 were utilized to establish the roles of pLa and CK2 in HBV gene expression. HBV DNA replication and HBsAg and HBeAg levels were analyzed in HepG2.2.15 cell supernatants by standard methods. **Results:** pLa was significantly overexpressed in HBV-infected human liver samples. TBBz decreased the phosphorylation of hLa, which coincided with decreased HBV expression. Mutant hLa Ala366 had reduced viral expression compared with hLa Ser^{366} treatment in hLa siRNA knockdown cells. Knockdown of CK2 also decreased the HBV parameters. **Conclusion:** hLa plays a key role in the regulation of HBV gene expression in a CK2-dependent mechanism via phosphorylation of hLa at Ser^{366}.

Keywords: human La protein; casein kinase II; hepatitis B virus; phosphorylation
Acknowledgements: This study was supported by the National Natural Science Foundation of China (81100289 and 30873105).

S11.39

Oleanolic acid regulates LOX-1/NADPH/ROS pathway inhibiting ox-LDL induced injury in HUVECs cells

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Aim: To investigate the effect and possible mechanisms of oleanolic acid (OA) in preventing endothelial cell oxidative injury induced by human high oxidized low-density lipoprotein (h ox-LDL). **Methods:** MTT assay was used to access suitable concentration of both h ox-LDL and OA. A model of human umbilical vein endothelial cell (HUVEC) oxidative injury was established by incubating the HUVECS with 20 μg/mL human high ox-LDL. In this study, we set up six groups: control group: the normal culture HUVECS; ox-LDL group: HUVECs were incubated with 20 μg/mL h ox-LDL for 24 h; low concentration OA group: HUVEC were pretreated with 5 μmol/L OA for 0.5 h, then as same as ox-LDL group; middle concentration OA group: HUVEC were pretreated with 10 μmol/L OA for 0.5 h, then as same as ox-LDL group; high concentration OA group: HUVEC were pretreated with 20 μmol/L OA for 0.5 h, then as same as ox-LDL group; vitamin E group: HUVEC were pretreated with 200 μmol/L VE for 0.5 h, then as same as ox-LDL group. LOX-1 and NADPH oxidase subunits p22phox, p47phox mRNA expression levels were evaluated by RT-PCR. LOX-1 and NADPH oxidase subunit gp91phox protein expression levels were evaluated by Western blotting. ESR was used to determine the time dependent release of NO and ROS, as well as the effect of ox-LDL, OA and VE on the release of NO and ROS. To generate LOX-1-siRNA-expressing plasmids, then use lipefectime™ 2000 method to transfect into HUVEC. The transfection rate was measured using flow cytometry and cell viability was measured by MTT assay. We set up four groups: control group; ox-LDL group; LOX-1-siRNA and ox-LDL group; and pCon group. Analysis LOX-1, P47phox and gp91phox expression of the above four groups. **Results:** The results show that treating with 20 μg/mL h ox-LDL increased the viability of HUVEC, while OA could converse the effect. Ox-LDL increased LOX-1, P47phox, gp91phox expression. But ox-LDL had no influence on P22phox expression. OA raised the cell survival rate obviously in a dose-dependent manner. OA down regulated LOX-1 mRNA and p47phox mRNA expression. Expressions of LOX-1 and gp91phox protein were regulated down by OA. Compared with ox-LDL group, pre-treating with different concentrations of OA groups and VE group down regulated LOX-1, p47phox and gp91phox expression, released less of both NO and ROS. In comparison, LOX-1-siRNA and ox-LDL group had less LOX-1, P47phox and gp91phox expression and less release of NO and ROS. **Conclusion:** Oleanolic acid has protective effect on HUVECs against injury induced by ox-LDL. The mechanism of its protective effect is to inhibit the signal pathway LOX-1/NADPH/ROS.

Keywords: oleanolic acid; LOX-1; NADPH oxidase; oxidized low density lipoprotein; HUVEC

S11.40

Novel hydrogen sulfide releasing derivatives of 3-*n*-Butylphthalide attenuate ischemic brain damage induced by transient middle cerebral artery occlusion in rats via inhibition of NOX4

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Aim: NBP was employed as the lead compound to obtain a novel class of H_2S-releasing derivatives. We investigated their antiplatelet activity *in vitro* and anti-ischemic stroke activity *in vivo*, and addressed the mechanisms that contributed to the neuroprotective effects. **Methods:** The effects of target compounds on adenosine diphosphate (ADP)-induced platelet aggregation were assayed using Born's turbidimetric method. Data were calculated and expressed as the IC_{50} values. We also examined the protective effects of the most potent compound I-a on cerebral ischemia induced by middle cerebral artery occlusion (MCAO). At 24 h post MCAO, neurological deficit and infarct size of brain were evaluated, NADPH oxidase (NOX) 4 protein expression in brain were determined by Western blotting. **Results:** The IC_{50} value of I-a (0.19 mmol/L) on ADP-induced platelet aggregation was 3.8- and 1.9-fold less than that of NBP (0.73 mmol/L) and Ticlopidine (0.36 mmol/L). And the pretreatment of I-a reversed the elevation of neurological deficit score and brain infarct size caused by cerebral ischemia. I-a treated groups obviously reduced the infarct size to 13.22% (193 mg/kg) and 19.17% (80 mg/kg) ($P<0.001$ *vs* vehicle group). Additionally, the increase of Nox4 protein expression after MCAO was attenuated by I-a. **Conclusion:** Our findings suggest that I-a may be a promising agent against cerebral ischemic injury via inhibition of NOX4 in brain.

Keywords: 3-n-butylphthalide derivative; antiplatelet activity; cerebral ischemia; MCAO; NOX4

S11.41

High level expression of anti-TNF-α monoclonal antibody in CHO/DHFR$^-$ cells

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Aim: TNF-α has been proved as a therapeutic target for rheumatoid arthritis. However, the cost of anti-TNF-α biologic agents is considerable and unacceptable at present. Here, a high level expression system of anti-TNF-α monoclonal antibody (mcAb) was constructed in dihydrofolate reductase-negative Chinese hamster ovary (CHO/DHFR$^-$) cells. **Methods:** The gene encoding LC and HC with the kozak sequence in the start codon was optimized for CHO cells, and an internal ribosome entry site (IRES) was linked between LC and HC. The fused LC-IRES-HC gene was then inserted into expression vector pcDNA3.1(+) and the selectable markers neomycin resistance gene (Neo^r) of the vector was replaced by DHFR gene. The recombinant plasmid was linearized and transfected into CHO/DHFR$^-$ cells. The plasmid was stepwise amplified by 0, 10, 20, 50, 100, 200, 500, and 800 nmol/L methotrexate (MTX) for 5–6 months. Stably transfected cells were selected, and then cloned using the limiting dilution method. The extraneous DNA integrated into the genomic DNA was detected by PCR, the transcription of LC or HC was examined by RT-PCR, and the production of anti-TNF-α mcAb in cellular supernatant was quantified using ELISA assay. **Results:** By the limiting dilution method, and intergration or RT-PCR analyses, 8 positive clones of the antibody were obtained. The fluorescence intensity of the antibody-overexprssed cells in the ELISA assay were dozens of times in compared to the control cells. **Conclusion:** These works provided a high level expression system of anti-TNF-α mcAb, and might establish a significant foundation for the following industrial production.

Keywords: rheumatoid arthritis; anti-TNF-α mcAb; CHO/DHFR$^-$cell; bicistronic expression
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S11.42

PF11 inhibits microglia-mediated neuroinflammation by inactivating NF-κB, Akt, MAPKs pathways

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Aim: Pseudoginsenoside-F11 (PF11), an ocotillol-type ginsenoside isolated from leaves of *Panax quinquefolium* (American ginseng), has been shown to improve the scopolamine-induced memory impairments in mice and possess significant neuroprotective activity in APP/PS1 mice. Although PF11 plays a lot of beneficial effects on disorders of the central nervous system (CNS), its cellular mechanism in microglia is unknown. In the present study, we assessed the anti-inflammatory effect of PF11 on the production of pro-inflammatory mediators in lipopolysaccharide (LPS)-stimulated N9 microglia. **Methods:** The expressions of iNOS, TNF-α, IL-6, and IL-1β in LPS-stimulated N9 microglial cells were determined by Western blotting and/or RT-PCR, respectively. NF-κB activation was investigated by High-Content assay and Western blotting. **Results:** PF11 suppressed production of nitric oxide (NO), prostaglandin E_2 (PGE_2) and the proinflammatory cytokines, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) in a concentration dependent manner. In addition, PF11 down-regulated the TLR4 protein expression, resulted in inhibiting NF-κB-dependent inflammatory responses by modulating a series of intracellular events of TAK1-IKK-IκBa-NF-κB signaling in N9 microglial cells. Moreover, PF11 inhibited Akt and MAPK signaling pathways. Importantly, PF11 significantly alleviated the death SH-SY5Y neuroblastoma cells, rat primary cortical and hippocampal neurons induced by the conditioned-medium from activated microglia. **Conclusion:** PF11 downregulated inflammatory iNOS, COX-2, TNF-α, IL-6, and IL-1β genes and proteins expression in N9 microglia interfering with the upregulation of TLR4, activation of NF-κB, MAPKs, and Akt pathways, thus, preventing neuron cells death. PF11 is a promising candidate for the development of neuroprotective drugs.

Keywords: PF11; microglia; inflammation; NF-κB; MAPK

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S11.43

Chaperone heat shock protein 70 modulators dual-regulate the development of behavioral sensitization induced by a single morphine exposure in rats

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Aim: Our previous studies have revealed behavioral sensitization, closely relating to drug addiction, mediated by chaperone heat shock protein 70 (Hsp70) in mice. This study aimed to investigate the effects of chaperone modulators on behavioral sensitization and Hsp70 in Nucleus Accumbens (NAc) core in rats. **Methods:** Rats received a single dose of morphine (1–10 mg/kg, sc) to induce a long-term behavioral sensitization. The time- and dose-effect of morphine on Hsp70 expression were detected by Western blot. Chaperone modulators including the inhibitors KNK437, methylene blue (MB), pifithrin-μ (PES), and inducer geranylgeranylacetone (GGA) were used to modulate Hsp70 and behavioral sensitization. **Results:** Morphine (3 and 10 mg/kg) could not only induce behavioral sensitization, but also increase Hsp70 expression in NAc core of rats. Pearson analysis indicated a significant positive correlation between magnitudes of behavioral sensitization and levels of Hsp70 expression (r=0.997). Intra-NAc core microinjection of KNK437 (2–10 μg/rat) attenuated Hsp70 expression and behavioral sensitization, while GGA (20–80 μg/rat) promoted them. Functionally, MB (10–90 μg/rat) and PES (1–4 μg/rat) impaired the development of behavioral sensitization. **Conclusion:** The critical involvement of chaperone Hsp70 in behavioral sensitization suggests a promising target for treatment of opioid addiction.

Keywords: chaperone; addiction; morphine

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S11.44

Angelica sinensis polysaccharides promote glycosaminoglycan synthesis: Benefiting osteoarthritis

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Aim: Loss of glycosaminoglycan (GAG) chains isa typical pathological change of osteoarthritis (OA). Here, effects of *Angelica sinensis* polysaccharides (ASPs) on GAG synthesis in chondrocytes and the mechanism were studied. **Methods:** OA Wistar rats orally treated with ASPs (25 mg/kg per day) for 5 weeks were sacrificed for the knee to detect GAG content by Safranin O stain, histopathological changes by Mankin's scoring, and protein expression of UDP-sugar synthetases and glycosyl transferases (GTs) involved in GAG synthesis by immunohistochemistry. Meanwhile, human primary chondrocytes were exposed to ASPs in presence or absence of IL-1 to evaluate ultrastructure changes by transmission electron microscope, content of GAG by DMB assay, mRNA and protein level of the enzymes by RT-qPCR and Western blotting, and content of UDP-sugar by High-Performance Anion-Exchange Chromatography. **Results:** *In vivo*, ASPs increased GAG content, decreased the Mankin's scores and up-regulated protein level of the enzymes. *In vitro*, ASPs set ultrastructural damages by IL-1 back, promoted UDP-sugars and GAG synthesis and reversed decline of mRNA and protein level of all the enzymes in presence or absence of IL-1. **Conclusion:** APSs presented anti-osteoarthritis activity by improving GAG synthesis *in vivo* and *in vitro*, due to up-regulation of UDP-sugar synthetases and GTs.

Keywords: ASPs; chondrocyte; GAG; UDP-sugar synthetase; glycosyltransferase

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S11.45

A network analysis based on the anti-inflammatory and anti-proliferative effect of EGCG in NSCLC

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Aim: As a major risk factor for lung cancer, chronic inflammation is a component of tumor microenviroment. In this study, the differentially protein network of EGCG treated NSCLC model (Lewis lung cancer C57A/B mice model) was analyzed based on protein expression profiling of tumor tissues. The potential drug target protein was discovered and demonstrated. **Methods:** 1) Lewis lung cancer exogenous C57A/B mice model was established and treated with EGCG in concentrations of 12.5, 25, 50 mg/ kg perday, ig) for 21 d. 2) Inflammatory microenvironment of tumor tissues was observed with MPO Assay Kit and immunohistochemical assay. 3) 2D-MS methods were used to detect the protein expression profiling (compare of control group tumor tissue and EGCG 50 mg/kg per day treated tumor tissue). 4) The differentially expressed proteins were demonstrated in Western blot method. 5) Tumor related differentially protein network was established based on BUND gene database and analyzed with Cytoscape software. 6) The binding of EGCG and potential drug target protein neutrophil elastase (NE) was forecasted with CDOCKER protocol of Discovery Studio software and was demonstrated in SPR method. **Results:** 1) EGCG significantly inhibits the tumor growth (tumor volume of EGCG 50 mg/kg per day group was decreased to 41.48% compared to control group) and metastasis (tumor metastasis number of EGCG 25 mg/kg per day group was decreased to 33.3% compared to control group) of NSCLC in C57 A/B mice. 2) Inflammatory microenvironment of tumor tissue is inhibited by EGCG. 3) Ten significant expression changed proteins are detected and demonstrated. 4) Result of CDOCKER analysis shows that EGCG binds to inflammation related protein NE, the –CDOCKER interaction energy is 29.7089 (–CDOCKER interaction energy value larger than 25 means legend can bind to receptor). 5) EGCG combines to NE chained SPR chip in concentration manner. **Conclusion:** EGCG inhibits the tumor growth, tumor metastasis and development of inflammatory microenvironment of NSCLC *in vivo*. Pivotal proteins which may involve in tumor proliferation, tumor metastasis and genesis and development of tumor inflammatory microenvironment are discovered by using 2D-MS method, differentially protein network analysis. EGCG binds to NE, which regulates both of tumor development and tumor related inflammation.

Keywords: EGCG; NSCLC; differentially protein network analysis; NE; SPR

S11.46

Development and application of adversedrug reaction monitoring software

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Aim: Adverse drug reaction (ADR) means a reaction which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function, definition excludes accidental or deliberate excessive dosage or maladministration. It is a risk which usually accompany with the normal drug therapy. Adverse drug reactions monitoring is not only a very important content of drug post-marketing studies but also a kind of effective method to strengthen the post-marketing drug safety regulation and to ensure a safe and effective human medication. In our country, spontaneous reporting system (SRS) is used for monitoring of adverse drug reactions, which is the most important measures for adverse drug reaction monitoring all over the world. In the implementation of spontaneous reporting system, those self-reported ADR reports is the main source for discovering the new adverse drug reactions signals of post-marketing drugs. This study is going to develop a practical software package including data collecting function, data processing function and monitoring & warning function, in order to achieve the automation of the ADR data extraction and adverse drug reaction signal detection and make data pre-processing faster and more convenient. **Methods:** This software package was developed under Microsoft Visual Studio 2005 development environment using Microsoft Office Access and Excel as supports with Microsoft Visual Basic 6.0 and C++ programming languages. It made references to the domestic and international major ADR signal quantitative detection methods such as PRR, ROR and MHRA in detecting suspicious adverse drug reaction signals from the spontaneous reporting system. This study established the database according to the fields of ADR reports and used adverse drug reaction reports reported by hospital as analysis data to do the analysis. **Results:** Bceause of the latest Adverse Drug Reaction Reporting and Monitoring Management Approach (Ministry of Health Order No 81) was promulgated on May 4, 2011; we can only analyze the records of ADR reports which were reported in the whole of 2012. This study obtained 3466 ADR reports which were provided respectively by Anhui Provincial Hospital and Yijishan Hospital of Wannan Medical College. Each ADR signal quantitative detection methods were able to show the top 20 drug-ADR combinations of highest frequency of occurrence in the records. **Conclusion:** After comparison, the results show that the adverse drug reaction monitoring software is quite practical. And to a certain extent it can make adverse drug reaction monitoring more systematic, make the methods more standard, make the indicators more quantitative and make the results more scientific. At the same time, the software application not only can be used as a scientific basis for Drug Administration to strengthen the management of drug but also can provide the basis for guiding the clinical rational drug use.

Keywords: adverse drug reaction ; ADR signal ; signal detection ; spontaneous reporting system

S11.47

Neuroprotective effects of paeoniflorin and albiflorin, two major constituents of JD-30, on $A\beta_{25-35}$-induced PC12 cells

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Aim: JD-30 is an active fraction extracted from Danggui-Shaoyao-San under guidance of bioactive evaluation in our previous studies. It has been reported that JD-30 could ameliorate cognition disorders and improve synaptic plasticity reduction in several Alzheimer's disease (AD) animal models. In this study, the neuroprotective effects of JD-30 and its two major constituents, paeoniflorin and albiflorin, were investigated. **Methods:** PC12 cells were seeded onto 6- or 96-well dishes and were pretreated with β-amyloid protein fragment 25–35 ($A\beta_{25-35}$) for 2 h before subjecting to 24 h treatment with JD-30, paeoniflorin or albiflorin. Cells were then collected and the survival, apoptosis and synaptophysin expression were detected with flow cytometry and immunofluorescence. **Results:** Neither JD-30 nor paeoniflorin and albiflorin had effects on the survival of PC12 cells, while JD-30 and paeoniflorin inhibited the apoptosis of PC12 cells and decreased the reduction of synaptophysin expression induced by $A\beta_{25-35}$, and the reaction intensity of JD-30 were higher than that of paeoniflorin. **Conclusion:** Our results suggest that paeoniflorin maybe one of the chief active constituents of JD-30, while it cannot completely cover the neuroprotective effect of JD-30.

Keywords: D-30; paeoniflorin; albiflorin; β-amyloid protein; PC12 cells

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S11.48

Simultaneous determination of long acting LHRH antagonists and its application to high-throughput pharmacokinetics

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Aim: Long acting luteinizing hormone-releasing hormone (LHRH) antagonists designed to be protease-resistant were a series of novel decapeptides structurally similar to LHRH. In this study, a high-throughput method based on a LC-MS/MS has been developed for the simultaneous determination of pharmacokinetics of five LHRH antagonists in rat via cassette dosing. **Methods:** The method was performed under selected reaction monitoring (SRM) in the positive ion mode. The analytes were extracted from 50 μL rat plasma by liquid-liquid extraction with acetonitrile. Chromatographic separation of the analytes was successfully achieved on a Hypersil column using a mobile phase composed of acetonitrilewater (30:70) containing 0.1% (*v*/*v*) formic acid. **Results:** The result showed good linearity and selectivity were obtained for all antagonists. The limits of quantification of the five LHRH antagonists were from 5 to 10 ng/mL. The average extract recoveries in the rat plasma were all over 72%. The intra-day and inter-day precisions (RSD%) were all within 10% and the accuracy was ranged from 92.54 to 109.05%. This method has been successfully applied to the pharmacokinetic studies of the five LHRH antagonists. The results indicated that the plasma drug concentration versus time curves after intravenous injection of these five antagonists via cassette dosing was all fitted to a two-compartment model. The results provided abundant information on the metabolic properties of the five novel LHRH antagonists. **Conclusion:** Based on the *in vivo* and *in vitro* stabilities of these peptides, it was concluded that LY616 and LY 608 could be the more stable candidate drug.

Keywords: LHRH; antagonists; pharmacokinetics; LC-MS/MS; cassette dosing

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S11.49

Rats exposed to traumatic stress wake up with a start afterwards during deep sleep: implications for uncovering PTSD nightmare

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Aim: Posttraumatic nightmares are a prevalent and distressing symptom of posttraumatic stress disorder (PTSD). In this study, we found some rats wake up with a start after traumatic stress and investigated the underlying neuroanatomical pathway of this behavior. **Methods:** Female SD rats exposed to inescapable footshock in proestrous. Twenty-one days after the shock, rats were used for observing whether wake up with a start by using infrared camera and recording EEG/EMG simultaneously. The activation of brain regions was evaluated via the level of c-Fos expression. All results were compared with normal awakening. **Results:** EEG delta power density was significantly increased in one-minute sleep prior to awake with a start, which suggested that the startle awakening was happened during deep sleep. Among the sleep/wake regulating nuclei the results showed that c-Fos expression decreased in orexinergic neurons of perifornical nucleus (PeF), and increased in noradrenergic neurons of locus coeruleus (LC). In addition, c-Fos expression of fear emotion regulating regions was decreased in infralimbic cortex (IL), and increased in both basolateral and central amygdala. **Conclusion:** These results indicate that rats exposed to trauma wake up with a

start afterwards during deep sleep, and this "symptom" may be mediated via the alteration of fear emotion and sleep/wake regulating regions. From a clinical perspective, the present results might provide evidences for uncovering PTSD nightmare.

Keywords: traumatic stress; wake up with a start; LC; PeF; fear

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S11.50

Evaluation of human monoclonal antibody toxicity, toxicokinetics and immunogenicity in cynomolgus monkeys after 4-week repeat IV dose with 4-week recovery

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Aim: A human monoclonal antibody was administered intravenously for 4-week in cynomolgus monkeys. The aim of this study was to characterize the toxicity of the antibody and in particular, the exposure-response relationship of the immune-mediated toxicity between these two dose regimens. **Methods:** Thirty-six cynomolgus monkeys (6 monkeys/sex/group, 3 groups) were given intravenous doses of the antibody at either 15 mg/kg QW or 30 mg/kg Q2W for 4 weeks followed by 4 weeks recovery. The following were assessed for all animals: clinical observations (cage side observations, detailed clinical observations, physical examinations/clinical assessments and injection site observations), body weight, food consumption, ophthalmology, ECG, blood pressure haematology, coagulation, blood chemistry, urine analysis, levels of concentration of the antibody, toxicokinetics (TK), immunogenicity (anti-drug antibody, ADA) in serum and gross and microscopic pathology. **Results and conclusion:** The antibody caused histopathological changes in the liver and clinical pathological changes were seen including decreases in albumin/globulin ratios and in white blood cell counts. However, the changes in histopathology and clinical pathological parameters were all recovered at the end of the recovery period. The monkeys given multiple intravenous doses of 15 mg/kg QW or 30 mg/kg Q2W of the antibody showed comparable serum exposures, assessed by AUC and the serum CL was similar between these two dose regimens. About 16.7% of monkeys developed ADA while receiving 15 mg/kg QW antibody, whereas 25% of monkeys developed ADA after receiving 30 mg/kg Q2W. All ADA positive animals were female and the presence of ADA in these animals was associated with reduced TK exposures. Given the non adverse nature of the findings, both dose regimens, 15 mg/kg QW and 30 mg/kg Q2W, were considered no-observed-adverse-effect levels (NOAEL).

Keywords: toxicokinetics; immunogenicity; cynomolgus; monoclonal antibody; ADA

S11.51

Cardiovascular protection effect of a novel Rho-kinase inhibitor, DL0805-2, and the potential druggability evaluation

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Aim: Rho/Rho-kinase is an important new target for the treatment of cardiovascular diseases. The aim of this study was to investigate the cardiovascular protection effect of DL0805-2, a novel Rho-kinase inhibitor, which was discovered in our previous work, and evaluate its potential druggability in cardiovascular diseases. **Methods:** We characterized the effects of DL0805-2 in biochemical, cellular and tissue based assays. The inhibitory effect of DL0805-2 on ROCK1 was tested by enzyme linked immunosorbent assay (ELISA). Cytotoxicity trail was carried on primary cultured rat thoracic vessel smooth muscle cells (VSMCs) and human umbilical vein endothelial cells (HUVECs), separately. Proliferation inhibition effect was also demonstrated on VSMCs stimulated by PDGF-BB (40 ng/mL). We used thoracic vessels and mesentery arteries as *in vitro* organ model to study the vasorelaxation effect of DL0805-2, and also discussed the mechanism under it. **Results:** DL0805-2 has a potent inhibitory effect on ROCK1 with IC_{50} of 194.09 nmol/L. It has little cytotoxicity on VSMCs and HUVECs at the concentration of 30 μmol/L while performed proliferation inhibition effect on VSMCs at 10 μmol/L. In the isolated thoracic vascular study, DL0805-2 exerted vasorelaxation in KCl (60 mmol/L) or norepinephrine (10^{-6} mol/L) induced contraction in a concentration-dependent manner, with pEC_{50} (pEC_{50}=-log(EC_{50}), mol/L) of 5.32 and 5.39, respectively. DL0805-2 of 1 μmol/L was also observed to suppress the vasoconstriction induced by angiotensinII (10^{-7} mol/L). Meanwhile in the study of isolated mesentery small arteries, DL0805-2 relaxed the vessel pre-contracted with KCl (60 mmol/L) with pEC_{50} of 5.36, and suppressed the vasoconstriction induced by angiotensin II (10^{-7} mol/L) at 3 μmol/L. The elementary mechanism research suggested that DL0805-2 may perform its vasorelaxation function through Rho/ROCK signal pathway and other pathways related to Ca^{2+} and this effect acted in an endothelium-independent manner. **Conclusion:** Rho-kinase inhibitor DL0805-2 as a new chemical entity has high efficiency and low toxicity which has been observed to have cardiovascular protection effect. The efficacy and mechanism on cardiovascular diseasesof DL0805-2 needs further investigation both *in vitro* and *in vivo* models in order to clarify the druggability comprehensively.

Keywords: ROCK1; inhibitor; VSMC; HUVEC; isolated vessel; druggability

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S11.52

Upregulation of MRTF-A pathway is involved in 17β-estradiol-stimulated migration of MCF-7 breast cancer cells

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Aim: Contrast to its proliferation activity on breast cancer, the effects of estrogen on breast tumor cell motility or invasion are more poorly understood. Here, the roles of MRTF-A pathway, the key downstream pathway of the Rho signaling, played in the 17β-estradiol (E_2)-stimulated migration of MCF-7 breast cancer cells was investigated. **Methods:** The influence of E_2 on the expression of MRTF-A was detected in MCF-7 cells with RT-PCR and Western blotting. The effects of MRTF-A overexpression on the migration of cells was analyzed using the wound healing assay and the transwell chamber assay. The synergic effects of MRTF-A and estrogen receptor α coactivator SMYD3 on the transactivation of MYL9 and CYR61, two well-known metastasis associated genes, was investigated with RT-qPCR, Western blotting, Immunocytochemistry and luciferase reporter assay. **Results:** Higher physiological levels of E_2 could significantly upregulate the expression of MRTF-A in MCF-7 cells, and overexpression of MRTF-A could significantly promote the migration of MCF-7 cells through its transactivation effects on MYL9 and CYR61 genes. SMYD3 and its histone methylation activity might be important in this event, and these effects could be suppressed by histone methylation inhibitor D5011. **Conclusion:** MRTF-A might be a switch for estrogen pathway to changing its proliferation-promoting roles to migration-stimulating roles in breast cancer. Either MRTF-A pathway blockers or histone methylation inhibitors might be considered as novel candidates for the therapy of breast cancer metastasis.

Keywords: 17β-estradiol; migration; breast cancer cells; MRTF-A; SMYD3

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S11.53

Tetrahydroxystilbene glucoside protects LPS-induced dopaminergic neurodegeneration via the inhibition of microglial activation

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Aim: Parkinson disease (PD) is one of the most common neurodegenerative diseases characterized by progressive dopaminergic (DA) neuronal loss in the substantia nigra (SN). Tetrahydroxystilbene glucoside (TSG) is known to possess anti-inflammatory, anti-oxidant and anti-aging activities. In this study, TSG-

mediated protection against LPS-induced DA neurodegeneration was evaluated. **Methods:** For *in vivo* studies, stereotaxic injection of LPS into rat SN was used to investigate TSG-mediated neuroprotection. For *in vitro* studies, rat primary midbrain neuron-glia co-cultures were applied to explore the mechanisms underlying this neuroprotection. **Results:** TSG protected DA neurons against LPS-induced neurotoxicity. *In vitro* studies revealed microglia were responsible for TSG-mediated neuroprotection and this effect was mediated by 1) inhibiting microglial activation and subsequent production of various pro-inflammatory factors, 2) suppressing NADPH oxidase activity and further decreasing reactive oxygen species (ROS) production, and 3) attenuating NF-κB cascade signaling pathways activation. **Conclusion:** TSG produces protection against LPS-induced DA neurodegeneration and might be of potential benefit for PD.

Keywords: Parkinson disease; neuroinflammation; microglial activation; tetrahydroxystilbene glucoside; neuroprotection

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S11.54

miRNA mimics — a novel approach for gene-specific interference of nicotine induced atrial fibrosis

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Aim: This present study was designed to decipher molecular mechanisms underlying nicotine's promoting atrial fibrillation (AF) by inducing atrial structural remodeling (ASR) after cigarette smoking/nicotine exposure. **Methods:** The rat model of AF was established by nicotine administration. Luciferase was used to detect the regulation of miR-133 on TGF-β1 and CTGF. The role of miRNAs on the expression and regulation of CTGF, TGF-β1 and collagen production was evaluated *in vivo* and *in vitro*. **Results:** The rats treated with nicotine were more prone to AF induction in response to a premature extrastimulus following a train of rapid pacing. Collagen content was significantly higher with treatment of nicotine. Western blot analysis revealed significant upregulation of CTGF and TGF-β1 genes at the protein level in nicotine-treated atrial fibroblasts. miR-133 was significantly downregulated in the presence of nicotine in rat atrial fibroblasts. Transfection of miR-133 into the cultured atrial fibroblasts remarkably reduced the protein level of CTGF and TGF-β1, and co-transfection of AMO-133 abolished the effect. Transfection of the synthesized miRNA mimics (miR-CTGF) significantly diminished collagen content and the effects were antagonized by their respective AMOs. **Conclusion:** Downregulation of miR-133 by nicotine triggers generation of atrial fibrosis and fibrillation via removal of post-transcriptional repression of CTGF and TGF-β1 and consequent production of collagens.

Keywords: nicotine; atrial fibosis; CTGF; miR-133; miRNA mimics

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S11.55

Changes in genes expression and aberrant promoter methylation during polypeptide from Chlamys farreri inhibits the transformation of human HaCaT keratinocytes induced by UVB irradiation

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Aim: To establish a model that human HaCaT keratinocytes is transformed by multiple doses of UVB and investigate the mechanism of polypeptide from chlamys farreri (PCF) can inhibite the transformation of HaCaT cell induced by UVB irradiation. **Methods:** Human HaCaT keratinocytes were devided into four groups: control group, UVB model group, UVB+2.84 mmol/L vitamin C positivecontrol group, UVB+2.84 mmol/L PCF group. Confluent layes of human HaCaT keratinocytes grown in a culture dish were exposed to UVB (15 mJ/cm^2). The procedure was repeated 12 times. After each exposure, cells were observed with Wrigt Giemsa staining under optical microscope. Malignant transformation was confirmed by formation of colonies on soft agar. Flow cytometry was applied to analysis cell cycle. Protein expression levels of p16, RASSF1A, Gadd45a and MGMT were determined by Western blot and RT-PCR. The methylation status in the promoter regions of RASSF1A, MGMT and P16 genes were observed by methylation-special PCR. **Results:** UVB (15 mJ/cm^2×12) could induce the malignant transformation of HaCaT cell. Cells treated multiple doses of UVB show a heterogeneous morphology characterized by the presence of giant cells with multiple nuclei. The transformed cells could grow in soft agar and the frequency of transformation of cell exposed to PCF was decreased significantly. Multiple genes expression changed in four groups during the malignant transformation by exposure to UVB. The hypermethlation of p16, RASSF1A genes had been successfully reversed after disposal of PCF. **Conclusion:** The results of RT-PCR, Western blot and DNA methylation assay indicated that PCF can regulatethe methylation of p16, RASSF1A genes in malignant transformation of human HaCaT keratinocytes induced by UVB irradiation.

Keywords: PCF (polypeptide from chlamys farreri); UVB; transformation; human HaCaT keratinocytes; DNA methylation

S11.56

The effects of p38 MAP kinase inhibitors on sulfur mustard-induced skin injure

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Aim: p38 mitogen activated protein kinase (MAPK) plays an important role in the mechanisms of sulfur mustard (SM)-induced injure. Inhibition of p38 suppressed SM-induced inflammation and tissue injury *in vitro*. Our study was to evaluate the effect of two sorts of p38 MAPK inhibitor SB203580 and BIRB796 on protection of SM-induced skin injure. **Methods:** SB203580 and BIRB796 were administered intraperitoneal injection to the SM-induced mouse ear vesicant model 24 h, 30 min before and during 12 h SM period. Ear edema was detected at different time points after SM challenge. To examine the impact of SB203580 and BIRB796 on activation of MAPK on SM-induced injury model, the phosphorylation of p38 and c-jun-N-terminal kinase (JNK) were analyzed with Luminex assay in immortalized non-tumorigenic human keratinocytes cells HaCaT. **Results:** On SM-induced mouse ear vesicant model, BIRB796 was shown to reduce approximate 17% ear edema at 24 h and 48 h after SM challenge. While SB203580 had marginal effect on ear edema. *In vitro* study revealed that compared to SM-challenged controls, the levels of p-p38 and p-JNK were both decreased significantly by BIRB796, whereas only p-p38 decreased by SB203580. Interestingly, p-JNK increased modestly by SB203580. **Conclusion:** In our study, p38 inhibitor BIRB796 could alleviate SM-induced skin injure, which might concerned with suppressing the activation of p38 and JNK simultaneously. SB203580 had little effect on SM-induced skin injury. It might due to the compensatory activation of JNK. The underlying mechanisms are worth of further investigation.

Keywords: sulfur mustard; mitogen activated protein kinase; p38; SB203580; BIRB796.

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S11.57

Effects of novel triazole-dithiocarbamate based LSD1 selective inhibitors on gastric cancer cell growth, invasion and migration *in vitro*

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The post-translational modifications of histone, *eg* methylation and demythalation, regulate chromatin structure as well as gene activation and repression. Lysine-specific demythelase 1 (LSD1), the first identified histone demythalase since 2004, has been reported to be up-regulated in numbers of cancers, including gastric cancer. Targeting LSD1 by specific inhibitors has shown to be a potential strategy to prevent cancer. Here, we generated and screened a series of novel 1, 2, 3-triazole-dithiocarbamate hybrids, which can specifically inhibit the LSD1 activity with IC_{50} less than 10 μmol/L. Interestingly, these LSD1 inhibitors exhibit FAD competitive, with selective and potent cytotoxicity against human gastric cancer cell line MGC-803, while they do not show any toxic effects on several normal cell lines. In addition, *in vitro* study suggested that in MGC-803 cells, the cell cycle was arrested and cell apoptosis was induced by these inhibitors. On the other hand, the cell migration and invasion were also markedly inhibited. Our findings indicate that these LSD1 inhibitors may be potential candidates to target gastric cancer cells.

Keywords: LSD1; inhibitor; epigenetics; gastric cancer

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Relationship between methylation of RASSF1A and p16 genes and lung cancer

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Aim: To explore the transcriptional expression and the promoter methylation of Ras association domain family 1A gene (RASSF1A) and p16 gene, and the major mechanisms for silencing of these two genes in non-small cell lung cancer (NSCLC) in Chinese. The p16, RASSF1A tumor suppressor genes are frequently inactivated by promoter methylation in lung cancers. **Methods:** The methylation level might occur at the early stage of lung cancer. RASSF1A, p16 gene is gaining popularity in the early diagnosis and treatment of lung cancer. Methylation of the promoters of p16 gene and RASSF1A gene was evaluated by methylation-specificPCR (MS-PCR) in lung cancer tissues and blood. **Results:** By contrast,the mean levels of p16 or RASSF1A promoter methylation was decreased in lung cancer samples compared to that in baseline samples(*P*-value=0.015 for p16; and *P*-value<0.001 for RASSF1A).The positive rates of promoter methylation of p16, RASSF1A genes in pathological tissues from patients with lung cancers were 31.9% (15/47), 21.3% (10/47) respectively. **Conclusion:** The methylation of tumor suppressor gene p16 and RASSF1A is possible important in human lung carcinoma. The methylation level and frequency of CpG islands were found to be closely related to the genesis and progression of lung cancer and it could be used in the early diagnosis and treatment of lung cancer.

Keywords: RASSF1A, p16, methylation, lung cancer

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S11.59

L-type Ca^{2+} channel mediates orexin-A-induced AMPK activation in hypothalamic ARC neurons

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Aim: Orexin-A, a neuropeptide secreted by the neurons in lateral hypothalamic area (LHA), is one of the key factors for the regulation of feeding behavior. Here, we investigated the involvement of calcium-dependent hypothalamic AMPK activation in the feeding behaviour regulation by orexin-A. **Methods:** We evaluated feeding behavior by using icv cannulation. The expression of AMPK and LKB1 protein were measured by Western blotting. The effect of orexin-A on high voltage activated calcium channel in NPY neuron was investigated by whole-cell patch clamp techniques. The immunocytochemical method was adopted to identify ARC NPY neurons in the rat hypothalamus. AMP/ATP ratio in ARC was detected by HPLC analysis. NPY content in ARC was detected by ELISA kit. **Results:** It was shown that calcium-dependent activation of hypothalamic AMPK was required for orexigenic effects of orexin-A. Orexin-A increased the activity of L-type Ca^{2+} channel in the isolated neropeptide Y (NPY)-containing neurons from arcuate nucleus (ARC) via OX1R-PLC-PKC signaling pathway, which was involved in orexin-A-induced hypothalamic AMPK activation. Furthermore, icv injection of L-type Ca^{2+} channel blocker, nifedipine, significantly inhibited orexin-A- and fasting-induced hyperphagia. **Conclusion:** Collectively, our findings demonstrate that orexin-A stimulates feeding behavior via L-type Ca^{2+} channel-dependent hypothalamic AMPK signal pathway.

Keywords: orexin-A; L-type calcium channel; AMPK; neurpeptide Y; food intake

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S11.60

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