S8.1

Therapeutic effect of Sedum Lineare Thunb on adjuvant arthritis rat and its mechanism

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Aim: To investigate the therapeutic effect of Sedum Lineare Thunb (SLT) on adjuvant arthritis (AA) and the possible mechanisim. **Methods:** Male SD rats were randomly divided into control group, model group, Tripterygium glycosides (TPT) group (10 mg/kg), SLT low and high dose groups (4 and 8 g/kg), 9 per group. Except the normal group, other groups were modeled to form adjuvant-induced arthritis with Freund's complete adjuvant and were given by intragastric administration. Each group rats were measured with primary and secondary paw swelling foot volume method. The changes of thymus or spleen index were observed. The level of serum inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were detected by Enzyme-linked immunosorbent assay (ELISA). Biochemical method were used to detect the activity of glutathione peroxidase (GSH-PX), nitric oxide (NO) and the prostaglandin E_2 (PGE_2) content in the joint fluid. **Results:** Compared with the control group, the model group serum TNF-α, IL-6, NO, and the PGE_2 in the joint fluid were increased significantly, and the activity of serum GSH-PX was down-regulated. After treated with SLT (4 and 8 g/kg), the primary and secondary paw swelling, the thymus and spleen index, and the level of serum TNF-α, IL-6, NO, and the PGE_2 in the joint fluid were decreased obviously, but the activity of GSH-PX in serum was increased. **Conclusion:** SLT had certain therapeutic effect on AA rats, the possible mechanism correlates with the regulation of immune function by reduce the inflammatory cytokines and inflammatory mediators, and improve the anti-oxidative capability.

Keywords: Sedum Lineare Thunb; adjuvant arthritis; inflammatory factor; antioxidant

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S8.2

Antioxidant, analgesic, anti-inflammatory, and hepatoprotective effects of the ethanol extract of *Mahoniaoiwakensis* stem

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The aim of this study was to evaluate pharmacological properties of ethanol extracted from *Mahoniaoiwakensis* Hayata stems (MOS_{EtOH}). The pharmacological properties included antioxidant, analgesic, anti-inflammatory and hepatoprotective effects. The protoberberine alkaloid content of the MOS_{EtOH} was analyzed by high-performance liquid chromatography (HPLC). The results revealed that three alkaloids, berberine, palmatine and jatrorrhizine, could be identified. Moreover, the MOS_{EtOH} exhibited antioxidative activity using the DPPH assay (IC_{50}, 0.743 mg/mL). The DPPH radicalscavenging activity of MOS_{EtOH} was five times higher that that of vitamin C. MOS_{EtOH} was also found to inhibit pain induced by acetic acid, formalin, and carrageenan inflammation. Treatment with MOS_{EtOH} (100 and 500 mg/kg) or silymarin (200 mg/kg) decreased theserum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels compared with the CCl_4-treated group. Histological evaluation showed that MOS_{EtOH} reduced the degree of liver injury, including vacuolization, inflammation and necrosis of hepatocytes. The anti-inflammatory and hepatoprotective effect of MOS_{EtOH} were found tobe related to the modulation of antioxidant enzyme activity in the liver and decreases in malondialdehyde (MDA) level and nitric oxide (NO) contents. Our findings suggest that MOS_{EtOH} has analgesic, anti-inflammatory and hepatoprotective effects. These effects support the use of MOS_{EtOH} for relieving pain and inflammation in folk medicine.

Keywords: *Mahoniaoiwakensis* Hayata; high-performance liquid chromatography; hepatoprotective effect; malondialdehyde

S8.3

Metabolomic assessment of the protective effect of CJ-1 in high fat diet-induced hepatosteatosis in mice

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CJ-1, a naturally abundant plant phenolic compound in vegetables and fruits, has been shown to have potent anti-oxidative and anti-obesity activity. In this study, we first investigated the beneficial effects of CJ-1 administration on nutritional hepatosteatosis model by a more "holistic view" approach, ^{1}H NMR-based metabolomics, to proof efficacy and to obtain information that lead to understanding the mode of CJ-1 action. The male C57BL/6 mice were placed for 16 weeks on a normal chow diet, a high fat diet (HFD, 60%), and a high fat diet supplemented with CJ-1 (50 and 100 mg/kg/day, orally). The liver histopathological and serum biochemical examinations indicated that daily CJ-1 administration protects against hepatic steatosis, obesity, hypercholesterolemia, and insulin resistance in HFD-induced NAFLD mice. In addition, partial least squares discriminant analysis scores plots demonstrated that the cluster of HFD feeding mice is clearly separated from that of normal group mice, indicating that the metabolic characteristics of these two groups are distinctively different, while CJ-1-treated mice are located close to normal group mice, indicating that HFD-induced disturbed metabolic profiles was reversed by CJ-1 treatment. Our results illustrate that the hepatoprotective effect of CJ-1 in part through reversing the HFD caused disturbed metabolic pathways, including lipid metabolism, glucose metabolism (glycolysis and gluconeogenesis), amino acids metabolism, choline and gut-microbiota-associated metabolism. Taken together, the selected metabolites could probably be the potential therapeutic biomarkers for understanding of the effect of CJ-1 in hepatosteatosis animal model.

S8.4

Inhibitory mechanisms of T7, a novel histone deacetylase inhibitor, on lipopolysaccharide-induced cyclooxygenase-2 expression in RAW 264.7 macrophages

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Aim: Gram-negative bacterial sepsis remains a common life-threatening event. Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria has been implicated in the pathological process of sepsis. Cyclooxygenase (COX)-2 catalyzes the conversion of arachidonic acid to prostaglandins, which play important roles in various homeostatic and inflammatory processes throughout the body. COX-2 expression has thus been linked with several diseases associated with inflammation. Histone deacetylase (HDAC) inhibitors have emerged as a new class of antitumor agents. Recently, HDAC inhibitors were also shown to exhibit anti-inflammatory properties. However, the underlying mechanism by which HDAC inhibitor suppresses inflammatory responses remains unclear. **Methods:** In this study, we explored the inhibitory actions of T7, a novel HDAC inhibitor, on LPS-induced COX-2 expression in RAW264.7 macrophages. **Results:** T7 concentration-dependently inhibited LPS-induced COX-2 expression in RAW264.7 macrophages. Results from reporter assay showed that LPS-induced COX-2 promoter- and kB-luciferase activities were inhibited after T7 exposure. In addition, LPS-induced p38MAPK, c-jun and p65 phosphorylation were suppressed by T7. Systemic administration of T7 also improve survival in endotoxaemic animals. **Conclusion:** In conclusion, T7 may cause p38MAPK and c-jun dephosphorylation, leading to the downregulation of COX-2 in RAW264.7 macrophages stimulated by LPS.

Keywords: COX-2; RAW 264.7 macrophages; LPS; HDAC

S8.5

Roles of DDAH/ADMA pathway in rheumatoid inflammation and its correlation with cortistatin

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Endothelial dysfunction represents the early stage of atherosclerosis, which is typically associated with rheumatoid arthritis (RA), a chronic inflammatory autoimmune disorder. Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, is not only an independent predictor for endothelial dysfunction but also a proinflammatory mediator. Elevated ADMA in patients with RA has been reported. In the present study, we investigated the potential effect of ADMA on inflammation process in collagen-induced arthritis (CIA) animal model and primary cultured fibroblast-like synoviocytes (FLS) exposed to tumor necrosis factor-α (TNF-α). In CIA rats, plasma levels of inflammatory cytokines TNF-α, interleukin-1β (IL-1β) and IL-6 were markedly increased, while plasma levels of ADMA did not increase. The expression of dimethylarginine dimethylohydrolase2 (DDAH2), the key enzyme for ADMA degradation, was markedly reduced in inflamed joint synovium of CIA rats. Moreover, the expression of anti-inflammatory factor cortistatin (CST) was markedly decreased in joint synovium of CIA rats. Treatment with TNF-α in FLS significantly increased the levels of ADMA, and decreased the mRNA and protein expression of DDAH2 accompany with an increase in production of IL-1β and IL-6 and a reduction in mRNA and protein expression of CST, while overexpression DDAH2 could block. Likewise, treatment with ADMA in FLS also significantly increased the levels of IL-1β and IL-6, and reduced the expression of CST. These findings suggest that DDAH/ADMA might be a potential target for treatment of RA, which may be related to CST.

Keywords: asymmetric dimethylarginine; dimethylarginine dimethylohydrolase; rheumatoid arthritis; inflammation; cortistatin

S8.6

Methylglyoxal, an endogenous aldehyde formed in diabetes, induces nociception by activation of peripheral TRPA1 receptors

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Aim: Methylglyoxal (MG), an endogenous aldehyde formed in the glycolytic pathway, is markedly increased in diabetes states and has been suggested to be involved in diabetic pain. The present examined whether MG produces pain and whether its nociceptive effect is due to activation of peripheral TRPA1 receptors. **Methods:** A variety of concentrations of MG and formalin was subcutaneously injected into rat hindpaw. The possible blockade effects of the TRIPA1 receptor blocker A967079 and MG scavenger D-arginine on MG-induced nociception were also examined. **Results:** By sharing the same pattern as formalin-induced acute nociception and tonic pain, MG dose-dependently produced a characteristic bi-phasic flinching response consisting of an initial, rapidly decaying acute phase (within 5 min after MG injection) followed by a slowly rising and long-lived (5–60 min after MG injection) tonic phase, with the ED_{50} value of 2.0% (13.5 μmol). Co-subcutaneous injection of the selective TRIPA1 receptor blocker A967079 (1.5 μmol) remarkably inhibited MG-induced acute and tonic pain by 40.0% and 71.0%, respectively. The MG scavenger D-arginine also inhibited MG induced chronic pain. **Conclusion:** Similar to the widely-used formalin test, MG pain test is simple, sensitive and reliable, which may represent an assay for diabetic pain testing. MG-induced pain may be via, at least partially, activation of peripheral TRIPA1 receptors.

Keywords: Methylglyoxal; TRIPA1 receptors; D-arginine; diabetic pain

S8.7

To compare the gastric ulcer induced by different non-steroidal anti-inflammatory drugs in mice

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Aim: We try to establish a reliable and quantitative ulcer model to evaluate mucosa protecting drug. **Methods:** The Kunming mice was used, 10 mice in each group, take aspirin 32, 64, and 128 mg/kg by gavage, indomethacin 30 mg/kg by subcutaneous injection, diclofenac sodium 7, 14, and 28 mg/kg by gavage for 7 d. **Results:** The aspirin 32 mg/kg group had 7 mice forming ulcers, the other 3 mice had no obvious injury; 64 mg/kg group had 10 mice forming ulcers, but some ulcers' border were too unclear to determine.128 mg/kg group had 6 mice forming ulcers, the other 4 mice were died. Indomethacin group had 5 mice forming ulcers, 1 mice died, the other 4 mice had no obvious injury. Diclofenac sodium 7 mg/kg group had 7 mice forming ulcers, the other 3 mice had no obvious injury; 14 mg/kg group had 10 mice forming ulcers; group 28 mg/kg had 8 mice forming ulcers, the other 2 mice were died. **Conclusion:** Diclofenac sodium 14 mg/kg can establish the best ulcer model in this study.

Keywords: non-steroidal anti-inflammatory drugs; gastric ulcer; aspirin; indomethacin; diclofenac sodium

S8.8

Hydrogen sulfide attenuates diabetic cardiomyopathy by normalizing ET -NOX-PKCε pathway in streptozotocin-injected rats

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Aim: Hydrogen sulfide (H_2S) plays important roles in mammalian cardiovascular system. The study was to investigate whether NaHS, a donor of H_2S, can improve diabetic cardiomyopathy through suppressing pro-inflammatory biomarkers such as endothelin A receptor (ET_AR), NADPH oxidase (NOX) and protein kinase Cε (PKCε). **Methods:** Male Sprague-Dawley rats were randomly divided into four groups. Except for the normal group, rats were injected with streptozotocin (STZ) (60 mg/kg, ip) once. During 5 to 8 weeks following STZ injection, animals were treated with aminoguanidine (100 mg/kg, *po*) or NaHS (5 mg/kg, *po*). **Results:** Compared with normal group, cardiac systolic function (LVSP, LV+d_p/d_{tmax}) and diastolic function (LVEDP, LV-d_p/d_{tmin}) in diabetic model (DM) group were deteriorated (P<0.01). mRNA and protein expression of ET_AR, PKCε, NADPH oxidase $p22^{phox}$ were upregulated in DM group (P<0.05 or P<0.01). NaHS significantly inhibited abnormal expressions of these molecules and improved cardiac function (P<0.05 or P<0.01). **Conclusion:** Exogenous H_2S administered at an appropriate dose plays protective effects to diabetic cardiomyopathy. As the donor of H_2S, NaHS attenuated diabetic cardiomyopathy by normalizing ET-NOX-PKCε pathway in rats.

Keywords: diabetic cardiomyopathy; ET_AR; NADPH oxidase; PKCε

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S8.9

Inhibitory effects of cassia oil on LPS-stimulated murine macrophage J774A.1 cells

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Aim: *Cinnamomum cassia* (*C cassia*) has long been traditionally used to treat cold, influenza and some inflammatory diseases such as gastritis, arthritis and rheumatism. This study aimed to investigate the inhibitory effects of essential oil from leaves of *C cassia* on lipopolysaccharide (LPS)-activated macrophage J774A.1 cells. **Methods:** LPS-activated J774A.1 cells were treated with 1–20 μg/mL cassia oil. Phagocytic activity, NO production, expression of pro-inflammatory cytokines, chemokines, iNOS, COX-2, and mPGES-1 of the treated cells were determined. **Results:** Cassia oil had inhibitory effects on LPS-activated J774A.1 cells. It markedly inhibited phagocytic activity at both 10 and 20 μg/mL. It inhibited NO production with IC_{50} value at 6.7 μg/mL. It down regulated the expression of IL-1β, IL-6, TNF-α, MCP-1, MIP-1α, iNOS, COX-2, and mPGES-1 in a concentration- dependent manner. **Conclusion:** The results from this study demonstrated that essential oil from leaves of *C cassia* has inhibitory effects on LPS-activated macrophages. It is possible to develop this oil as an anti-inflammatory agent in the future.

Keywords: *Cinnamomum cassia*; macrophage J774A.1 cells; lipopolysaccharide

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S8.10

Effects of BC1, a saponin of medicinal plant *Beesia calthaefolia* (Maxim) Ulbr on immunoregulation in mice

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Aim: BC1, as a kind of natural bioactive substance extracted from *Beesia calthaefolia* (Maxim) Ulbr, has medicinal applications to treat many inflammatory diseases. This study was designed to evaluate the effects of BC1 on cytokine secretion, such as TNF-α and IL-1β, which is beneficial for understanding the mechanism of BC1 on immunoregulation, and also will benefit our further research. **Methods:** The proliferation of splenic lymphocyte induced by mitogen (ConA or LPS) was detected by CCK assay. The neutral red phagocytize test of macrophages was determined by colorimetric method. The gene and protein expressions of TNF-α and IL-1β were measured by real time RT-PCR and ELISA in serum, spleen and lymphocytes respectively. **Results:** Our present study has shown that LPS could increase the gene and protein expressions of TNF-α and IL-1β, respectively. *In vitro*, BC1 (31.25–250 μg/mL) could inhibit the proliferation of splenic lymphocyte and inhibit the increased production of TNF-α and IL-1β in protein and gene levels, but could promote the proliferation of macrophages. *In vivo*, BC1 (12.5–50 μg/kg) could recover the increased expressions of TNF-α and IL-1β in the spleen of mice, but increase the decreased expression of TNF-α and IL-1β in serum. **Conclusion:** The function of immunoregulation of BC1 may be accomplished through modulating the gene and protein expressions of TNF-α and IL-1β.

Keywords: BC1; TNF-α; IL-1β; immunoregulation

S8.11

Propective effect of PCF against UVB-induced appptosis by NO/TGF-β/smad2, smad3/smad4 pathway

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Aim: To investigate the mechanism of UVB inducing apoptosis in HaCaT cells via iNOS/NO/TGF-β/Smads pathway and Polypeptide from *Chlamys farreri* (PCF) protecting HaCaT cells from UVB by 20 mJ/cm^2 UVB-induced apoptotic model of HaCaT cells and iNOS transient transfected HaCaT cells. **Methods:** UVB-induced apoptotic model of HaCaT cells was replicated and iNOS transient transfected model of HaCaT cells was established by Lipefectime^{TM} 2000. Cells were randomly divided into 7 groups: control group, UVB model group, UVB+10 μmol/L SB431542 group, UVB+1 mmol/L SMT group, UVB+100 μmol/L Carboxy-PTIO group, UVB+1.42–5.69 mmol/L PCF group and iNOS transient transfection group. Apoptotic rate of cells was determined by Hoechst33258 staining. The mRNA expression of TGF-β, smad2, smad3, smad4, smad7 were assayed by RT-PCR. Protein expression levels of TGF-β, p-smad2/smad3 and iNOS were determined by Western blot analysis. **Results:** The apoptosis rate of UVB group was 50%. Western blot results showed TGF-β protein reached a peak at 3 h (P<0.05) and 15 h (P<0.01) after 20 mJ/cm^2 UVB radiation, and protein reached a peak at 18 h (P<0.05). The mRNA expression of smad2 and smad3 had no statistical significance *vs* the control group, but the smad7 mRNA reached a peak at 6 h (P<0.01). The protein expression was obviously raised in iNOS transfected HaCaT cells (P<0.01). SB431542, SMT, PTIO, and 1.42–5.69 mmol/L PCF significantly inhibited UVB-induced apoptosis (P<0.05). 1.42–5.69 mmol/L PCF dose-dependently inhibited iNOS activation, NO release and down-regulate expression of TGF-β and p-Smad2/Smad3 (P<0.01), and up-regulate expression of smad7 in HaCaT cells irradiated by UVB. **Conclusion:** PCF could protect HaCaT cells from UVB-induced apoptosis. Its inhibitory effect on apoptosis may attributes to iNOS/NO/TGF-β/smads pathway.

Keywords: polypeptides from *Chlamys farreri* (PCF); ultraviolet; iNOS/NO; TGF-β; smads

S8.12

Bone marrow tolerogenic dendritic cell modulate the balance of Tregs and Th17 cells *in vitro*

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Aim: To induce bone marrow tolerogenic dendritic cells (TDCs) and to investigate the mechanism by which bone marrow TDCs modulate the balance between regulatory T cells (Tregs) and Th17 cells *in vitro*. **Methods:** Bone marrow TDCs were identified by the expression of CD11b, F4/80, IDO, IL-10 and TGF-β. Bone marrow TDCs modulate the differentiation of CD4$^+$CD25$^+$Foxp3$^+$ Tregs in the co-culture system were detected by CTLA-4, Foxp3 mRNA, Helios mRNA, IL-10 and TGF-β. Bone marrow TDCs modulate the differentiation of CD4$^+$IL-17$^+$Th17 cells in the co-culture system were detected by RORγt mRNA, IL-17 mRNA, and T cell lymphocyte proliferation. The percentages of CD11b$^+$F4/80$^+$, CD4$^+$CD25$^+$Foxp3$^+$ and CD4$^+$IL-17$^+$ were detected by flow cytometry, the expression of IDO and CTLA-4 were detected by Western-blot, the levels of IL-10 and TGF-β were detected by enzyme-linked immunosorbent assay (ELISA), T cell lymphocyte proliferative response was tested by MTT, the expressions of Foxp3 mRNA, Helios mRNA, IL-17mRNA and RORγt mRNA were detected by Quantitative Real-Time PCR (qPCR) or Real-Time PCR (RT-PCR). **Results:** Bone marrow TDCs expressed high levels of CD11b, F4/80, IDO, IL-10, and TGF-β. Bone marrow TDCs enhanced the percentages of CD4$^+$CD25$^+$ Foxp3$^+$ Tregs, increased the expressions of Foxp3 mRNA and Helios mRNA in both the thymus and spleen, raised the expressions of CTLA-4 and increased the levels of IL-10 and TGF-β in co-culture system. Bone marrow TDCs inhibited T lymphocyte proliferations, suppressed the percentages of CD4$^+$IL-17$^+$Th17 cells, and restrained the expressions of RORγt mRNA and IL-17 mRNA. **Conclusion:** Bone marrow TDCs were induced successfully. TDCs modulate the balance of Tregs and Th17 cells by promoting CD4$^+$CD25$^+$Foxp3$^+$ Tregs differentiation and suppressing IL-17 expressing Th 17 cells immune differentiation *in vitro*.

Keywords: dendritic cells; Tregs; Th17 cells; tolerogenic; balance
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S8.13

Silencing tribbles homolog 3 attenuates high fat intake induced nonalcoholic fatty liver disease

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Aim: Nonalcoholic fatty liver disease (NAFLD) is characterized by macrovesicular hepatic steatosis and chronic inflammation, leading to increasing morbidity and mortality. Tribbles homolog 3 (TRB3) was indentified to mediate diabetic cardiomyopathy. As the tightly crosslink between NAFLD and diabetic cardiomyopathy, in this study, we set out to examine the role of TRB3 in preventing NAFLD. **Methods:** TRB3 siRNA was used for silencing TRB3 in free fatty acid (FFA, 50 mmol/L)-treated AML-12 (mouse hepatocyte cell line) for 48 h *in vitro*. For study *in vivo*, double gender of C57BL/6NIA mice (one-year-old) were fed with standard diet (SD), or high-fat diet (HC), or high-fat diet with silencing TRB3 (HCST) for 12 weeks. SA-β Gal staining was used to examine cellular senescence, red oil O staining was used to examine lipid content and Western blot analysis was used to examine the expression of p-NF-κB p65, NF-κB p65, IL-1α, IL-6, and CXCL-8. **Results:** *In vivo* and *in vitro*, silencing TRB3 not only prevented high calorie intake-increased senescense, but also restricted lipid accumulation and inflammation. **Conclusion:** Silencing TRB3 attenuates high fat intake induced NAFLD, the mechanisms were likely due to repressing liver senescence and inflammation.

Keywords: NAFLD; TRB3; inflammation; senescence; high calorie
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S8.14

The effects and mechanisms of Akebia saponin D on the NAFLD livers

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Non-alcoholic fatty liver disease (NAFLD) has became a global public health issue, however the exact pathogenesis has not been elucidated and there is no ideal drugs for NAFLD. Akebia saponin D is a typical bioactive triterpenoid saponin isolated the rhizome of Dipsacus asper Wall. This study was aimed to evaluate the protective effects and mechanisms of ASD in NAFLD. C57BL/6-Lep$^{ob/ob}$ mice were used as the fatty liver animal models. *Ob/ob* mice received daily intraperitoneal injections of ASD for 4 weeks. *Ob/ob* mice showed a significant increase in the levels of LDL, TG, AST, ALT and a significant decrease in the activities of HDL in plasma and liver. Histopathological examination of the liver in *ob/ob* mice contained larger vesicular structures consistent with macrovesicular steatosis. The liver of ASD-

treated animals showed less lipid deposition. Treatment with ASD prevented the accumulation of reactive oxidant species and mitochondrial membrane potential dissipation in the liver. In the cellular level, ASD reduces oleic induced steatosis and enhances survivability in hepatocytes. These researches might provide a new target, a potential drug for the treatment of NAFLD, and provide new ideas for prevention and treatment of NAFLD.

Keywords: Akebia saponin D; Non-alcoholic fatty liver disease

S8.15

Valproic acid suppresses lipopolysaccharide-induced cyclooxygenase-2 expression via MAPK phosphatase-1 in murine brain microvascular endothelial cells

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Aim: Inflammation and vascular perturbations are increasingly implicated in the pathogenesis of neurodegenerative diseases. Prevailing evidence suggests that the antiepileptic and mood stabilizer, valproic acid (VPA), exhibits anti-inflammatory, neuro-protective and axon remodeling effects in neurodegenerative diseases. However, the underlying mechanism contributing to the suppression of inflammatory responses by VPA remains to be fully defined. **Methods:** In the present study, we explored the actions of VPA on lipopolysaccharide (LPS)-induced cyclooxygenase (COX)-2 expression in bEnd.3 mouse brain endothelial cells. **Results:** The LPS-induced COX-2 protein level was significantly suppressed by VPA. VPA inhibited p38MAPK and JNK phosphorylation in bEnd.3 cells exposed to LPS. Treatment of cells with a p38MAPK inhibitor (p38MAPK inhibitor III) or a JNK signaling inhibitor (JNK inhibitor II) markedly inhibited LPS-induced COX-2 expression. VPA suppression of JNK and p38MAPK phosphorylation and subsequent COX-2 expression were restored in cells transfected with MKP-1 dominant negative (DN) mutant. In addition, VPA caused an increase in MKP-1 phosphatase activity in bEnd.3 cells. **Conclusion:** In conclusion, VPA may cause MKP-1 activation to dephosphorylate p38MAPK and JNK, leading to the down-regulation of COX-2 in bEnd.3 brain microvascular endothelial cells stimulated by LPS, a proinflammatory stimulus. The present study also supports the therapeutic value of VPA in alleviating brain inflammatory diseases.

Keywords: valproic acid; COX-2; MKP-1; endothelial cells

S8.16

The disturbance of hippocampal CaMKII/PKA/PKC phosphorylation in early experimental diabetes mellitus

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Aim: To studyhowhippocampalserine/threonine kinases signaling changes inthe early phase ofdiabetic rats (both type 1 and type 2). **Methods:** Injection of streptozotocin or streptozotocin/high fatwas adopted to induce type 1 and type 2 diabetes mellitus rats. Immunoblotting and immunohistochemistryare used to determine changes in the phosphorylation of proteins. **Results:** In both type 1 and type 2 diabetic rats, the phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in the hippocampi decreased markedly, opposite to a significant increase in the phosphorylation of synapsin I (Ser 603) and GluR1 (Ser 831) in the same experiment. And hippocampal protein kinase C (PKC) and protein kinase A (PKA) signaling in type 1 and type 2 diabetic rats also changed. Moreover, hippocampal PP1alpha and PP2A protein down-regulated in type 1 diabetic rats, but significantly up-regulated in type 2 diabetics. **Conclusion:** The disturbance of CaMKII/PKA/PKC phosphorylation in the hippocampus is an early change that may be associated with the development and progression of diabetes-related cognitive dysfunction.

Keywords: diabetes; brain; hippocampus; CaMKII; phosphorylation; PKC; PKA

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S8.17

P2X7 receptor is required for inflammasome-mediated hepatic stellate cell activation

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Aim: The ATP-gated P2X7 receptor (P2X7r) is a promising therapeutic target in chronic inflammatory diseases including liver fibrosis. The underlying molecular mechanisms remain to be elucidated. **Methods:** We studied the effects of proinflammatory cytokines derived from LPS-stimulated RAW 264.7 mouse macrophage cell on Human hepatic stellate cell (LX-2). LX-2 cells were primed with LPS and subsequently stimulated for 30 min with 3 mmol/L ATP absence or presence of a selective P2X7 antagonist, A438079. **Results:** When LX-2 was cultured in the presence of LPS, mRNA expression of α-SMA and collagen-I were increased, as well as IL-1β, IL-18, and IL-6. And LPS also increased mRNA expression of caspase-1, P2X7r and ASC that the apoptosis-associated speck like CARD-domain containing protein. These mRNA expressions were higher in conditioned medium of RAW 264.7 cells. However, the expression of capase-1 and P2X7r was increased by the treatment of conditioned medium of RAW 264.7 cells with LPS, which also induced the expression of various proinflammatory cytokines, including, IL-18 and IL-6. Unprimed LX-2 cells failed to stimulate IL-1β mRNA expression in response to extracellular ATP. Furthermore A438079 reduced IL-1β mRNA expression in response to extracellular ATP. Also ATP-mediated IL-1β release blocked by A438079. **Conclusion:** Our results suggest P2X7r activation may play a direct role in HSC activation through release of proinflammatory cytokines of its proinflammatory actions via cytokines.

Keywords: hepatic stellate cells; inflammation; liver fibrosis; P2X7 receptor

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S8.18

Treatment of diabetic nephropathy by Chinese medicine from B2B

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Two herbal medicine formulae were developed for the treatment of Diabetic nephropathy. Diabetic nephropathy (DN) has surpassed other major diseases to become the most common cause of the end-stage renal disease. The major cause of DN is due to microvascular disease in the glomerular and eventually develops glomerulosclerosis. The therapeutical methods for DN are quite limited in Western medicine, therefore it is necessary to hunt for new treatment for DN from Chinese medicine. Two herbal medicine formulae were developed on the combination of the modern research with senior doctors' experiences of in China. One is Chaihuang-Yishen Granule (CHYS) composed of seven Chinese herbs, which was originally used for treating chronic kidney disease. The other is Tangshen Formula (TSF) composed of seven Chinese herbs, which was widely used for DN in Beijing.

Animal Experiments to verify the efficiency of the two herbal Medicine. Diabetic rats' models were established by receiving a right uninephrectomy plus a single intraperitoneally injection of STZ, then the effect of CHYS and TSF were examined. Then the action mechanisms were investigated by RT-PCR, Western blot, immunohistochemical staining and so on. OLETF rats model was also used to verify the effect of TSF. The potential proteins were demonstrated in OLETF rats compared with LETO rats by proteomic analysis.

Clinical trial of Tangshen formula on DN. A multicentric, randomized, placebo-controlled trial was carried out to observe the effect of TSF on 181 patients with type 2 diabetic kidney diseases. There were significant reduction of urinary albumin excretion rate in patients with DN stage III and stage IV in TSF treatment group compared with placebo. We also find out that there were significant reduction of serum creatinine and elevation of eGFR level in the treated group. But, the effect was not related with blood pressure, age and gender. Meanwhile, nine phospolipids were examined by metabonomic study in DN patients. After the treatment of TSF, the level of plasma phospholipids was close to normal. **Conclusion:** TSF is a potential drug for the treatment of diabetic nephropathy.

S8.19

Effect of Mugua pill on the degranulation of mast cells and adjuvant arthritis induced by Freund's complete adjuvant in rat

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Aim: The Mugua pill has long been used relieve rheumatoid arthritis in humans in traditional Chinese medicine, but the precise mechanism underlying its efficacy is still unclear. In the present study, we investigated the direct effects of the Mugua pill on mast cell mediated inflammation using cultured mast cells and an animal model. **Methods:** Dinitrophenyl (DNP)-IgE-sensitized rat basophilic leukemia RBL-2H3 cells and mouse bone marrow-derived mast cells (BMMC) were treated with Mugua pill. Cells were stimulated by DNP-BSA to induce degranulation and released β-hexosaminedase was determined colorimetrically to measure the degree of degranulation. Adjuvant arthritis (AA) induced by Freund's complete adjuvant (FCA) in wistar rats were administrated orally with 4.0 g/kg Mugua pill for 13 d. **Results:** Mugua pill significantly inhibited the antigen-induced degranulation of RBL-2H3 cells and BMMC cells at 0.01-0.32 mg/mL for 1 h-treatment. Mugua pill also suppressed intracellular calcium mobilization. Mugua pill significantly suppressed the antigen-induced up-regulation of TNF-α and IL-4 cytokines by RBL-2H3 mast cells. Paw swelling in FCA-treated rat were significantly suppressed by oral administration of Mugua pill. **Conclusion:** Our results strongly suggest that Mugua pill have anti-inflammatory effects *in vivo* by suppressing the activation of mast cells.

Keywords: Mugua pill; mast cells; degranulation; rheumatoid arthritis.
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S8.20

The suppressive effect of DSS on obesity in ovariectomized rats and its molecular mechanism

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Aim: To explore the mechanism of suppressive effects of 3'-daidzein sulfonate (DSS) on obesity in ovariectomized (Ovx) rats. **Methods:** Forty healthy female SD rats with weight of 220-250 g were respectively divided into the five groups at random: sham-operated group (saline), ovariectomized group (Ovx+saline), estradiol valerate group (Ovx+0.8 mg/kg) and DSS groups (Ovx+1.0/0.3 mg/kg). The weight and stem length was measured once a week. All rats were treated with saline, estradiol valerate or DSS for 6 weeks, and serum samples were collected to determine the content of high density lipoprotein (HDL), low and very low density lipoprotein (LDL/VLDL), triglycerides (TG), cholesterol (CHO), leptin and visfatin. **Results:** It's showed that DSS significantly decrease Lee's index, and increase HDL, but reduced LDL/VLDL, TG and CHO in serum. These findings indicated that DSS is effective in suppressing the obesity in ovariectomized rats. More specifically, leptin is enhanced and visfatin is restrained in DSS treated rats's serum, but that of visfatin is just right adversed in estradiol valerate treated group. **Conclusion:** It is possible that DSS will maintain an efficient level of circulating leptin and visfatin, which regulate the lipid metabolism and weight gain. It may represent a potential alternative therapy in the treatment of obesity abnormality in ovarian hormone-deficient women.

Keywords: 3'-daidzein sulfonate; ovariectomized rats; leptin; visfatin
Acknowledgements: This work was supported by grants from Health of Jiangxi Province (20092087) and Guidance Science and Technology Projects of Ganzhou City (2009118).

S8.21

The study of Methyl salicylate 2-*O*-*β*-*D*–lactoside's anti-inflammatory and analgesic effect

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Aim: The aim of study was to evaluate the anti-inflammatory and analgesic effect of methyl salicylate lactoside (MSL). **Methods:** The swelling of ear induced by croton oil in mice, the swelling of foot induced by carrageenan and the granuloma induced by cotton ball in rats were used to study the anti-inflammatory effects of MSL. The analgesic activity of MSL was evaluated by acetic acid-inducing twisting symptom in mice and carrageenan-inducing pain in rats. **Results:** MSL and aspirin could decrease the rate of ear swelling induced by croton oil. In the other two inflammatory-models, MSL had obviously attenuated foot swelling and also decreased the weight of granuloma caused by cotton ball in rats. After administration of aspirin and high-dose MSL, the pain induced by intraperitoneal injection of acetic acid was diminished in mice. In addition, different-dose MSL and aspirin markedly increased the pain threshold in carrageenan-inducing pain model in rats. **Conclusion:** The present results suggested that Methyl salicylate lactoside has prominent anti-inflammatory and analgesic effects.

Keywords: methyl salicylate lactoside; aspirin; anti-inflammation; analgesic effect

S8.22

The efficacy of Zhenwu decoction on adriamycin-caused nephritic syndrome by osmotic pump

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Aim: Nephrotic syndrome (NS) is a chronic glomerulus disease characterized by proteinuria, hypoalbuminemia, edema and hyperlipidemia. The NS model was traditionaly established by a singletail intravenous injection of Adriamycin, in our study we carried out a new model of nephritic syndrome (NS) using osmotic pump and carrying out the theraputic intervention to evaluate the of Zhenwu decoction on NS. **Methods:** Osmotic pump filled with adriamycin was implanted into rats enterocoelia, then induced the NS on rats by constant release of adriamycin with osmotic pump, and after intervention of Zhenwu decoction, the urine protein, blood pressure, serum lipid parameters, plasmaprotein, renal function indicators, and IgG expression in glomerulus were detected, which was to assess the establishment of model of NS rat and the efficacy of Zhenwu decoction on it. **Results:** The survival rate of rats with adriamycin-caused NS using osmotic pump was improved significantly, no rats died during experiment. The NS occurred in the rats of model group at second week after giving driamycin, and compared with control group, the urine protein on 24 h and blood pressure were increased significantly after model establishment, and blood lipid, as well as markedly decreased serum protein and renal hypofunction were detected (P<0.01); Compared with model group, the mental state, activity, food intake and hair color of rats were observably improved after administrating lower and higher doses of Zhenwu decoction (24.0 and 12 $g \cdot kg^{-1} \cdot d^{-1}$), and also both doses were capable of significantly decreasing urine protein and blood pressure (P<0.01), recovering rats serum protein level (P<0.05), improving BUN and Scr levels, decreasing the content of TC and TG (P<0.01), and the notably decreased IgG deposition in rats mesangial area. **Conclusion:** The stable model of rat with NS could be successfully established using constant release function of osmotic pump and after intervention with Zhenwu decoction, it showed the efficacy on improving NS from the aspects of decreasing the urine protein, increasing plasma protein level, relieving hypercoagulable state, the decline in contents of BUN and Scr and restoring glomerular structure.

Keywords: nephrotic syndrome; osmotic pump; adriamycin; Zhenwu decoction
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S8.23

The roles of Syk in TLRs-mediated signaling and inflammatory response

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Aim: Toll-like receptors (TLRs) are major family of pattern-recognition receptors and play a crucial role in innate immunity. The spleen tyrosine kinase (Syk) plays a key role in ITAM-containing signaling. Recently, several reports have indicated that Syk is involved in TLRs signaling but the role of Syk in TLR signaling is still controversial. **Methods:** Using primary cultures of macrophages derived from wild type (WT) and Syk-deficient mice, we compared the signaling and inflammatory responses elicited by TLR ligands. **Results:** Syk not only is involved in TLR4 endocytosis but also plays a dual role in TLR4-mediated signaling. LPS can induce higher levels of inflammatory genes in Syk-deficient than WT macrophages. TAK1

downstream signaling pathway was more activated in LPS stimulated Syk-deficient macrophages. In contrast, less LPS-induced TBK1-IRF3 activation was observed in Syk-deficient than WT cells. Moreover, Syk is present in both MyD88/TRAF6/TAK1 and TRIF/TRAF3/TBK1 signalsomes of TLR4 activation. Notably, LPS-induced K63-ubiquitination of TRAF3/6 was oppositely regulated. In HEK293T-overexpressing system, interactive domains of Syk to TRAF3/6, TAK1, and TBK1 were further identified, strengthening Syk is a common regulator to timely control the various TLRs responses. **Conclusion:** Syk plays an opposite regulatory role in TLR4-mediated TRAF6 and TRAF3 signaling pathways, and such delicate actions of Syk could help tune the innate immune response to lessen inflammation in the late infective phase.

Keywords: Syk; TLRs; TRAFs; signal transduction

S8.24

Research of pharmacodynamics and mechanism of ChaiQingxiaopi Capsule on hyperplasia of mammary glands

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Aim: To study the therapentic effect of ChaiQingxiaopi Capsule (CQC) on the hyperplasia of mammary glands (HMG) in rats and analyze the possible mechanism in treating HMG. **Methods:** (1) Seventy rats were divided into control, model, tamoxifen, Xiaoru Sanjie Capsule, low-, mid-, and high-dose CQC (0.433, 0.855, and 1.712 g/kg) groups. After 5 weeks treatment, we measured the height and diameter of nipple, then determined viscera indexes and the levels of estrogen (E_2), progestogen (P), prolactin (PRL), testosterone (T), superoxide dismutase (SOD) and malondialdehyde (MDA) in serum; and the pathologic changes in morphology of mammary glands were observed; (2) The acute blood stasis rat model was established with subcutaneous injection of 0.1% adrenaline hydrochloride and ice water bath. After 1 week treatment, blood was taken from the abdominal artery, and then its blood viscosity and plasma viscosity were measured by blood rheometer; (3) The expression of ERa and PR in mammary gland epithelial cells was analyzed by immuno-histochemistry. **Results:** CQC could remarkably reduce the diameter and height of the nipple, increase the index of thymus and spleen, reduced the index of the uterus and ovaries. It could also increase E_2, P, PRL, and decrease T level in serum, and increase SOD activity and decrease MDA ($P<0.05$ or $P<0.01$); CQC can effectively improve the blood viscosity and increase the anti-stress ability ($P<0.05$ or $P<0.01$); The more the hyperplasia of mammary glands is, the higher is the contents of ER and PR. CQC group can significantly reduce the mammary gland epithelial cells of the ERa and PR expression ($P<0.01$). **Conclusion:** CQC has significant therapeutic effect on HMG, can adjust the sex hormones levels in serum; improve the immunity, oxidative stress and blood rheology; and reduce the expression of ERa and PR in mammary gland epithelial cells. Its mechanism may be associated with CQC has a certain effect of phytoestrogen, on pituitary-the hypothalamus-gonadal axis has certain influence, by lowering the content of ERa in mammary glands or reduce mammary glands sensitivity to the ERa, the race for competitive estrogen receptors.

Keywords: CQC; HMG; pharmacodynamics; mechanism

S8.25

RhTACI-Ig blocked the activation of T cells via restoring BAFFR and TACI signaling

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Aim: RhTACI-Ig is a recombinant fusion protein containing the extracellular ligand-binding portion of transmembrane activator and CAML interactor (TACI) and the Fc portion of human immunoglobulin G. This study explored the molecular mechanism of B cell activating factor (BAFF) in activating T cells, and the effect of rhTACI-Ig. **Methods:** T cells from C57BL/6 mice were purified by microbeads and then were cultured with recombinant human BAFF (rhBAFF) in the presence of different concentrations of rhTACI-Ig, rhTNFR:Fc or control human IgFc. **Results:** RhBAFF evoked the proliferation and survival of T cells, upregulated the production of interleukine-2 (IL-2), IL-4, interferon-γ (IFN-γ) and transforming growth factor β (TGF-β) in cell culture supernatants, substantially increased the population of early antigen activated T cells, activated T cells and effectory T cells, and decreased the population of naïve T cells. The function of rhBAFF was associated with an upregulation of BAFF receptor (BAFFR), nuclear factor κB (NF-κB) p50 (in cell nucleus) and inhibitor of κB (IκB) kinase α (IKKα) expressions, while with a downregulation of both TACI and IκBα expressions. Neither rhBAFF nor rhTACI-Ig altered the level of IKKγ. RhTACI-Ig significantly inhibited the proliferation, survival and differentiation of T cells, restored the changes of above receptors and signal molecules. **Conclusion:** The data confirmed that BAFF promotes the activation of T cells through stimulating BAFFR and inhibiting TACI signaling. RhTACI-Ig was demonstrated to be an effective blocker on T cells response. In addition, we presumed that TACI may play a negative role in T cell activation.

Keywords: B cell activating factor; B cell activating factor receptor; transmembrane activator and CAML interactor; fusion protein; immunotherapy; T lymphocytes

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S8.26

RAGE/TLR4-regulated PPAR-γ signaling pathway mediates advanced glycation end products-induced inflammatory responses in human chondrocytes

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Aim: Diabetes is a risk factor for osteoarthritis. Advanced glycation end products (AGEs) are important pathogenetic mediators of diabetic complications. AGEs are the ligands of receptor for AGEs (RAGE) and toll-like receptor (TLR). Activation of peroxisome proliferators activated receptor (PPAR)-γ may regulate the inflammatory responses in chondrocytes. The role of PPAR-γ in AGEs/RAGE/TLR4-induced inflammatory responses in chondrocytes remains unclear. Here, we investigated the involved signaling pathway in AGEs-induced inflammatory responses in human chondrocytes. **Methods:** The resected cartilage specimens obtained from patients undergoing primary total knee arthroplasty were used to isolate human articular chondrocytes. Glycated albumin was prepared from bovine plasma albumin by incubation with *D*-glucose for 8 weeks. **Results:** AGEs up-regulated the RAGE and TLR4 protein expressions and down-regulated the PPARγ expression in human chondrocytes. AGEs also enhanced the expressions of cyclooxygenase (COX)-2, high mobility group box 1 (HMGB1), MMP-13 and IL-6 in a dose and time-dependent manner. PPAR-γ agonist pioglitazone effectively suppressed AGEs-induced expression of COX-2, HMGB1, MMP-13, IL-6, and collagen II degradation. The neutralizing antibodies of RAGE and TLR-4 could reverse the decreased PPAR-γ and increased COX-2 and HMGB1 expressions induced by AGEs. **Conclusion:** These results suggest that RAGE/TLR4-regulated PPAR-γ signaling pathway is involved in the AGEs-induced inflammatory responses in human chondrocytes.

Keywords: PPAR-γ; advanced glycation end products; chondrocytes; inflammation

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S8.27

The effect of noradrenaline and α_1-adrenoceptors antagonists on the rat hepatic stellate cells

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Aim: To investigate the distribution and function of α_1-adenoreceptor subtypes (α_1-AR) in rat hepatic stellate cells (HSC-T6), and to investigate the effect of noradrenaline (NA) on the activation and proliferation of HSC-T6 and on their synthesis capacity of extracellular matrix proteins (ECM). **Methods:** Cell immunohistochemical and RT-PCR were used to evaluate the distribution and function of α_1-AR. Cultured HSC-T6 were divided in 6 groups: (1) control group; (2) NA (10^{-5}, 10^{-7}, and 10^{-9} mol/L) group; (3) Chloroethylclonidine dihydrochloride (CEC) (α_{1B}-AR antagonists) group; (4) BMY7378 (α_{1D}–AR antagonists) group. After NA and the antagonists of adrenoceptor subtypes were delivered to the DMEM containing cultured HSC-T6, MTT assay was used to evaluate the cell proliferation.

Meanwhile, the expressions of transforming growth fact beta 1 (TGF-β_1) and smooth muscle α-actin (α-SMA) in HSC-T6 were detected by Western blotting and RT-PCR. The expressions of tissue inhibitor of metalloproteinase 1 (TIMP-1) and collagen-I (ColI) in HSC were detected by Western blotting and RT-PCR. **Results:** α_{1B}-AR and α_{1D}-AR expressed more than α_{1A}-AR. They expressed more on the cell membrane than other tissues in HSC-T6. With the changes of concentration, NA gradually significantly induced HSC-T6 proliferation, NA significantly in a concentration-dependent manner, which were reduced by antagonists of α_{1B}-AR, α_{1B}-AR. NA induced the mRNA expressions of TGF-β_1, α-SMA, TIMP-1, ColI, which were reduced by antagonists of α_{1B}-AR, α_{1D}-AR. **Conclusion:** NA can promote the activation, proliferation and secretion of ECM of HSC-T6, mainly through α_{1B}- and α_{1D}-AR.

Keywords: hepatic stellate cells; noradrenaline; α_1-adrenoceptors; hepatic fibrosis
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S8.28

Protective effect of sequoyitol on renal injury in type 2 diabetic rats and the potential mechanisms

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Aim: To observe the protective effect of sequoyitol on renal injury in type 2 diabetic rats and to explore their conceivable mechanisms. **Methods:** Type 2 diabetic rats model was established by intraperitoneal injection with a single dose of STZ (35 mg/kg) and fed with a high-fat high-sucrose diet. The model rats were randomly divided into Model (distilled water 5 $mL \cdot kg^{-1} \cdot d^{-1}$) group, sequoyitol with high, medium and low dosages (50, 25, and 12.5 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively) groups, and Acarbose (20 $mg \cdot kg^{-1} \cdot d^{-1}$) group. In addition, the control (distilled water 5 $mL \cdot kg^{-1} \cdot d^{-1}$) group was established. All animals were given drugs or distilled water with successive intragastric administration for 6 weeks. Levels of OGTT, AUC, Scr, BUN, T-AOC, and H_2O_2 in serum or kidney were measured. Levels of TNF-α and IL-6 in kidney were determined by ELISA. The primary pathologic changes of kidney were observed by H&E staining, and collagen deposition was stained by Masson visually. The expression of TGF-β1 positive cells in kidney were measured by immunohistochemistry. The expression of collageI, collageIII, TNF-α, TGF-β1, p22phox, and p47phox mRNA in kidney were performed by real time PCR. Protein expression of TGF-β1, p22phox, and p47phox in kidney were detected by Western blot. **Results:** Sequoyitol could reduce the content of OGTT, AUC, Scr, BUN, H_2O_2, TNF-α, and IL-6, elevate kidney tissue T-AOC and NO content, reduce the collageI, collageIII, TNF-α, TGF-β1, p22phox, and p47phox mRNA and protein expression, as well as ameliorate kidney pathologic lesions. **Conclusion:** Sequoyitol has protective effect on renal injury in type 2 diabetic rats.

Keywords: sequoyitol; type 2 diabetic; diabetic nephropathy; oxidative stress

S8.29

Endothelin-converting enzyme-1 regulates the re-sensitisation of signalling by the glucagon-like peptide-1 receptor

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Aim: Endothelin-converting enzyme-1 (ECE-1) may regulate trafficking of internalized GPCRs by degrading peptide ligands in endosomes. We examined the impact of ECE-1 on re-sensitisation of the glucagon-like peptide-1 receptor (GLP-1R), a therapeutic target in type 2 diabetes. **Methods:** HEK-293 cells with stable expression of human GLP-1R (HEK-GLP-1R) or EGFP-tagged GLP-1R and the INS-1E β-cell line (natively expressing GLP-1R) were used. Cytoplasmic [Ca^{2+}] was measured in fluo-4-loaded HEK-GLP-1R by plate reader to indicate GLP-1R activity. Live imaging used confocal microscopy. HEK-GLP-1R cells were transfected with ECE-1 plasmids or siRNA and expression determined by immunoblotting. Responses in INS-1E were determined by cAMP assay. **Results:** Imaging tagged or untagged GLP-1Rs with unlabelled or rhodamine-labelled GLP-1 showed rapid internalisation of ligand and receptor that could contribute to desensitisation. Re-sensitisation may need receptor and ligand processing. GLP-1R Ca^{2+} responses were desensitised by GLP-1 7–36 amide (10 min, 10 nmol/L). Re-sensitisation required 3 h after removal of free ligand, was unaffected by inhibition of protein synthesis but was reduced by the ECE-1 inhibitor, SM19712 (10 μmol/L), or ECE-1 knockdown. Overexpression of ECE1a, b, c, or d isoforms promoted re-sensitisation. In INS-1E, SM19712 inhibited recovery of receptor-mediated cAMP responses. **Conclusion:** ECE-1 may degrade GLP-1 in endosomes, facilitating GLP-1R trafficking and re-sensitisation. Interestingly, high glucose enhances ECE-1 levels suggesting GLP-1R and ligand processing may alter in diabetes.

Keywords: GLP-1; ECE-1; receptor trafficking

S8.30

Oxidative stress contributes to cardiac dysfunction in type 1 diabetic mice with myocardial infarction through inhibition of sonic hedgehog pathway

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Aim: Our previous study showed that an impaired sonic hedgehog (Shh) pathway contributed to cardiac dysfunction in diabetic mice with myocardial infarction (MI). The present study hypothesized that oxidative stress is involved. **Methods:** STZ-induced type 1 diabetic mice and rat neonatal cardiomyocytes were used. The antioxidant Tempol (3 mmol/L) was administrated at the onset of diabetes and till the end of experiments. **Results:** Tempol significantly increased myocardial proteins of Shh and patched-1 (Ptc1) at 10–18 weeks, and then improved cardiac dysfunction at 18 weeks in diabetic mice. Moreover, Shh and Ptc1 proteins were significantly increased on d 7 after MI, and capillary density was enhanced, percentage myocardial infarct was reduced and then cardiac dysfunction was ameliorated on d 21 after MI in diabetic mice with Tempol. *In vitro*, both a superoxide-generating mixture of xanthine oxidase (XO, 1–2 U/L) and xanthine (X, 0.5 mmol/L) and the advanced glycation end-products (AGEs, 100-400 ug/mL) significantly enhanced the ROS level and concentration-dependently decreased Shh and Ptc1 proteins in rat neonatal cardiomyocytes, an effect that was blunted by Tempol (0.1 and 0.5 mmol/L). **Conclusion:** These findings indicate that oxidative stress contributes to impaired Shh pathway in diabetic mice leading to cardiac dysfunction. Antioxidative strategies that are aimed at restoring the endogenous Shh pathway may offer useful means for improving diabetic cardiac dysfunction.

Keywords: diabetes; shh pathway; oxidative stress; myocardial infarction; cardiac dysfunction

S8.31

Study the mechanism of abnormal glucose tolerance in high-fat diet mice

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Aim: High fat dietcan lead to abnormal glucose tolerance and insulin resistance,but little is known about the underlying mechanism. The purpose of this study is to determine whether insulin signaling pathway in skeletal muscle induce abnormal glucose tolerance. **Methods:** Divide mice into two groups randomly: Normal control group; High-fat diet group. Detect fast blood glucose and blood lipid in mice after eating normal feed and high fat feed respectively for 6 weeks. Then observe the expression of Akt and its substrate TBC1D1 in skeletal muscle by Western blot. **Results:** Fasting blood glucose and blood lipid in high-fat diet group was obviously increased ($P<0.05$). The blood glucose also increased significantly in 0.5, 1, and 2 h after intraperitoneal injection of glucose, while the expression of phosphorylated Akt, phosphorylated TBC1D1 and total TBC1D1 in skeletal muscle significantly decreased ($P<0.01$). **Conclusion:** Abnormal glucose tolerance exists in high-fat diet mice. The decreased expression of phosphorylated Akt and phosphorylated TBC1D1 in skeletal muscle may be one of mechanisms of abnormal glucose tolerance induced by high-fat diet.

Keywords: high-fat diet mice; skeletal muscle; glucose tolerance; Akt; TBC1D1

S8.32

Liraglutide ameliorates glycometabolism and insulin resistance via GLUT4 up-regulation in diabetic KKAy mice

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Aim: Liraglutide has been used for treatment of type 2 diabetes mellitus since 2009. In this study, the anti-diabetic effect and mechanisms of liraglutide were

investigated in spontaneous diabetic animal model-KK/Upj-Ay/J (KKAy) mice. **Methods:** KKAy mice were treated by liraglutide (250 μg/kg per day) for 6 weeks. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were used to measure glucose and insulin tolerance dysfunction. Insulin levels were detected by ELISA assay. Hexokinase (HK) and pyruvate kinase (PK) contents were measured with biochemical system. Pancreas' dysfunction was observed by electron microscope. Gene and protein expression of GLUT4 was detected by real time-PCR and immunohistochemical (IHC). **Results:** Liraglutide could decrease fasting blood glucose, improve the area under the curve of OGTT and ITT, fasting serum insulin, and homeostasis model assessment insulin resistance. Furthermore, liraglutide could mend glycometabolism dysfunction through increasing glycolysis via HK and glycogenesis via PK activation. Ultrastructural examination of the pancreas showed that liraglutide could improve the damage state of islets beta cells and increase the insulin secretion granules. Real time-PCR and IHC results showed that gene and protein expression of GLUT4 could be increased by liraglutide. **Conclusion:** Liraglutide produced a potent anti-diabetic effect through ameliorating glycometabolism and insulin resistance in KKAy mice via stimulating insulin secretion, increasing glycogenesis production and glycolysis, and up-regulating the expression of GLUT4.

Keywords: liraglutide; glycometabolism; insulin resistance; GLUT4; KKAy mice
Acknowledgements: This study was supported by the National Natural Science Youth Foundation of China (No 30901808) and the Technology and Innovation Project of Shaanxi Province (No 2011KTCL03-20).

S8.33

Effects of "shenyankang partical" on chronic pyelonephritis in rats

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Aim: To study the effects of "Shenyankang partical (SYK)" in the chronic pyelonephrits (CPN) rats, and to explore its potential mechanisms. **Methods:** Unilateral chronic nephropyelitis models were established by retroactively infection with colibacillus. SYK (0.125, 0.25, and 0.5 g/kg) was administered by gavage. After dosed 90 d, the rats were sacrificed. Bacterial culture of urine and kidney, renal pathology were observed, and the mRNA level of IL-1β and TGF-β of kidney were detected by real time RT-PCR. **Results:** Viable organisms in urine and renal had not be seen, SYK significantly reduced the pathological injuries of kidney, lightened the degree of fibration of kidney, and decreased the mRNA level of TGF-β1 and IL-1 of kidney. **Conclusion:** SYK had protective effects against CPN. The mechanisms appear to be due to the inhibited progression of fibrosis through suppression of TGF-β1 and IL-1 mRNA expression of kidney.

S8.34

Expression change of insulin receptor in liver cell of type 2 diabetic rats after duodenal jejunal bypass surgery

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Aim: To study the effects of duodenal jejunal bypass (DJB) surgery on the expression of insulin receptor (InsR) in the liver tissue of type 2 diabetic (T2DM) rats, and explore the relationship between InsR expression and blood glucose. **Methods:** Wistar rats were randomly divided into four groups: Wistar control group (W–C), T2DM control group (T2DM-C), T2DM Metformin treat group, T2DM DJB surgery group (T2DM-DJB), 8 rats in each group. Fasting and oral 30 min blood glucose were detected before DJB operation and 2, 4, and 8 weeks after DJB operation. Eight weeks after surgery, the animals were scarified, RT-PCR and Western blot were used to analysis the mRNA and protein levels of InsR in liver tissue. **Results:** The expression of InsR mRNA in T2DM-C group significantly decreased to 61% compare to wild control animal, while T2DM-DJB and T2DM-M group in liver was raised back to 94% and 88% compare to wild control group. Western blot results showed the similar to RT-PCR, that InsR expression in liver cells was decreased in T2DM rat model and increased back to normal after DJB surgery or treated by metformin. **Conclusion:** InsR mRNA and InsR protein expressed in liver cells was significantly decreased in T2DM rats, DJB surgery and Metformin treatment for 8 weekes of the diabetic rats can significantly increase the expression of InsR both in mRNA and InsR protein level. DJB surgery cured the T2DM by decreaseing and fasting blood glucose of T2DM rats. DJB surgery has significant effect in T2DM treatment.

Keywords: duodenal-jejunal bypass surgery; type-2 diabetes; insulin receptor; liver cell
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S8.35

Homocysteine induces C-reactive protein expression in rat smooth muscle cells via NMDAr-ROS-MAPK signal pathway

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Aim: As an inflammatory marker, C-reactive protein (CRP) plays a direct role in atherosclerotic process. Homocysteine (Hcy) is largely accepted as an independent risk factor for cardiovascular diseases and could contribute to the inflammatory process of atherosclerosis. But it is still unclear whether Hcy is able to induce the expression of CRP in vascular cells. Our study was to observe the effect of Hcy on CRP expression in vascular smooth muscle cells (VSMCs) and to elucidate a possible signal pathway. **Methods:** Cultured rat VSMCs were stimulated by Hcy (10–300 μmol/L) for theindicated time (3-24 h) to detect the expression of CRP. Further, MK801, MAPK inhibitors, TTFA, DPI and NAC were used during the signal transduction study. mRNA and protein expression was detected by RT-PCR and Western blot, respectively. ROS were detected by a fluorescent probe. **Results:** Hcy significantly increased mRNA and protein expression of CRP in VSMCs in time- and concentration-dependent manners. MK801 reduced Hcy-induced CRP expression in VSMCs. Pretreatment of the cell with MK801 or DPI decreased Hcy-stimulated ROS generation, and NAC largely abolished Hcy-induced CRP expression in VSMCs. The further study indicated that MK801, NAC, PD98059, SB230580 significantly inhibited ERK1/2 and p38 phosphorylation, and PD98059, SB230580, PDTC antagonized Hcy-induced CRP expression in VSMCs. **Conclusion:** Hcy is able to induce CRP expression in rat VSMCs via NMDAr-ROS-ERK1/2 and p38 signal pathway.

Keywords: homocysteine; C-reactive protein; vascular smooth muscle cells; inflammation; atherosclerosis

S8.36

Pioglitazone exacerbates the hepatic steatosis and suppresses serum cholesterol clearance in two animal models of type 2 diabetes mellitus

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Aim: Nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) frequently coexist as they share the pathogenic abnormalities of excess adiposity and insulin resistance. The insulin sensitizing agents thiazolidinediones (TZDs) have been used in the treatment of NAFLD. However, the preclinical and clinical evidences implicate that the benefit from TZDs treatment in T2DM with NAFLD is controversial. This study aimed to analyze the effect and mechanism of pioglitazone on hepatic lipids metabolism in two commonly used animal models of T2DM. **Methods:** Diabetic KKA^y mice and *db/db* mice were orally administered with pioglitazone (25 mg/kg/day) for 30 d, and hepatic lipids contents and distribution were analyzed biochemically and by histopathology. Serum lipids, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were also measured. Real-Time PCR were used to determine the expression of genes involved in fatty acid and cholesterol metabolism in liver. **Results:** Both of KKAy mice and *db/db* mice exhibited obvious hepatic steatosis and hyperlipidemia compared with the wild type mice. Treatment of the two animal models with pioglitazone resulted in deteriorated microvesicular steatosis and elevated hepatic triglyceride. Serum triglyceride levels, however, were decreased by the treatment. Furthermore, pioglitazone significantly increased serum ALT activities, total

cholesterol (TC) and LDL-C levels. Gene expression profiling results showed that pioglitazone up-regulated adipocyte-specific and lipogenesis-related genes, as well as the genes involved in the transportation of fatty acid into the hepatocytes in KKA^y mice and *db/db* mice liver. Of interest was that the expressions of the genes involved in hepatic cholesterol uptake and excretion were sharply suppressed by pioglitazone. **Conclusion:** These results suggested that pioglitazone could induce adipogenic transformation of hepatocytes with adipose tissue-specific gene expression and lipid accumulation, which aggravated the liver lesion in diabetic mice with NAFLD. Pioglitazone also suppressed the clearance of serum cholesterol from liver. Thus, the TZDs treatment should be warned in T2DM with NAFLD and large controlled trials of long duration are needed to assess the long-term clinical benefits of pioglitazone in NAFLD patients.

Keywords: pioglitazone; hepatic steatosis; lipids metabolism; T2DM

S8.37

Effects of metformin on the expression of GLP-1R in the enteric nervous system of type 2 diabetic rats

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Aim: To study the effects of metformin on the expression of Glucagon-like peptide-1receptor (GLP-1R) in the enteric nervous system of type 2 diabetic (T2DM) rats, and to explore the relationship between GLP-1R expression, and blood glucose. **Methods:** Wistar rats were randomly divided into three groups: Wistar control group (W-C) , T2DM control group (T2DM-C), T2DM metformin treat group (T2DM-M), 6 rats in each group. Fasting blood glucose and fasting glucagon-like peptide-1 (GLP-1) were detected before orally administration of metformin and 2 weeks, 8 weeks after orally administration of metformin. Fasting insulin in the plasma before and 8 weeks after metformin was also detected. After 8 weeks, the rats were sacrificed and the expression of GLP-1R in the enteric nervous was studied by RT-PCR and Western blot. **Results:** Fasting blood glucose and fasting insulin of T2DM-M rats decreased after metformin using (P<0.01). The fasting serum GLP-1 of T2DM-M rats increased significantly after metformin (P<0.01). The difference was significantly (P<0.01). The expression of GLP-1R mRNA in T2DM-M rats increased significantly compared to T2DM-C rats (P<0.01). The expression of GLP-1R protein in T2DM-M rats increased significantly compared to T2DM-C rats (P<0.05). **Conclusion:** Metformin significantly increased the expression level of GLP-1R in enteric nervous.

Keywords: metformin; type-2 diabetes; enteric nervous system; gucagon-like peptide-1 receptor

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S8.38

Understanding signaling at the glucagon-like peptide-1 receptor

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Glucagon-like peptide-1 (GLP-1) is a key incretin peptide that promotes insulin secretion in response to nutrient ingestion, but also has a range of other actions including preservation of β-cell mass, reduction in gastric emptying and reduction in appetite that make it a desirable target for treatment of type II diabetes. GLP-1 exerts its effects by binding to the GLP-1 receptor (GLP-1R), which belongs to the family B subclass of the G protein-coupled receptor (GPCR) superfamily. In recent years, it has become clear that individual GPCRs can existin multiple receptor conformations and can elicit numerous functional responses, both G protein- and non-G protein-mediated. This has led to the discovery that different ligands can stabilize distinct subsets of receptor conformations that can "traffic" stimulus to diverse functional outputs with varying prominence, a concept referred to as biased agonism (also known as functional selectivity, stimulus bias or ligand-directed signaling). We will discuss the concept of ligand-directed signal bias as it applies to the GLP-1 receptor and the implications of this for development of drugs that target the GLP-1 receptor.

Keywords: G protein-coupled receptor; Glucagon-like peptide-1; Glucagon-like peptide-1 receptor; ligand-directed signal bias

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S8.39

Identification of the bioactive constituent and its mechanisms of action in mediating anti-inflammatory effects of Dregea volubilis

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Aim: The ethanol extract from the stem parts of *Dregea volubilis* was reported to ameliorate concanavalin A-induced liver injury by facilitating apoptosis of activated T cells. In this study, the bioactive constituent was identified and its mechanisms of action were investigated. **Methods:** Bioactivity guided purification was performed by using partitioning extraction and high performance liquid chromatography. The molecule of the key bioactive constituent was identified with NMR spectroscopy. The effects of this compound on cell proliferation and apoptosis of activated T cells were assayed by MTT and flow cytometry methods. The related apoptosis pathway was determined by Western blotting. **Results:** One of the key bioactive constituents was identified to be newdrevogenin B (marsectohexol-*D*-3-*O*-cymaropyranoside). This compound dose-dependently inhibited cell proliferation and induced apoptosis, which was selective for activated, rather than nonactivated, T cells. Increased active fragments of caspase-3 and caspase-9 and decreased Bid expression were found in the newdrevogenin B-treated cells. **Conclusion:** Newdrevogenin B, the bioactive constituent of *Dregea volubilis*, can facilitate mitochondria-dependent apoptosis of activated T cells and may have great potential to treat T cell-mediated inflammatory diseases.

Keywords: *Dregea volubilis*; bioactive constituent; T lymphocyte; apoptosis; mitochondria

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S8.40

Time feeding improved non-obese insulin resistance

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Aim: Circadian rhythmicity is displayed in behavioral activities, hormonal secretion and the expression of genes and proteins in most organisms on earth. Circadian system is an evolutionarily conserved mechanism to help organisms anticipate predictable environmental changes and to synchronize these to the 24 h per day. Environmental cues including light, temperature, social activity, behavioral interactions *etc* enable to affect the circadian rhythmicity. Recently, couples of evidence suggest that metabolic disorders including the obesity, insulin resistance and diabetes are related to the rhythmic imbalance. In this study, whether the time feeding improved the non-obese insulin resistance in mice was investigated. **Methods:** Male neonates were given a single intraperitoneal injection of low-dose streptozotocin to induce non-obese insulin resistance. The animals received regular time feeding pattern as the experimental group. **Results:** The food intake, body weight, and total cholesterol and triglycerides in the blood of the experimental group were similar to that of the insulin resistance group. Higher fasting blood glucose levels in the insulin resistance group were reversed by the time feeding treatment. Serum insulin levels were similar between the experimental group and the insulin resistance group. The experimental group showed better performance in glucose and insulin tolerance tests compared to the insulin resistance group.

Keywords: metabolism; time; lean; insulin; blood glucose

S8.41

Liver proteomic response to hypertriglyceridemia in human-apolipoproteinC-III transgenic mice

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Aim: Hypertriglyceridemia (HTG) induced by over-expression of human-apolipoproteinC-III (HuApoC-III) was reported to disturb hepatocyte bioenergetics

in transgenic mice presenting higher liver oxygen consumption and body metabolic rate. These metabolic modifications remained poorly understood. In the present work we studied global proteomic response of HuApoC-III mouse hepatocyte to HTG. **Methods:** The 2D-DIGE proteomic approach has been performed on whole hepatocyte, mitochondria and sub-mitochondrial compartments (matrix and inner membrane), comparing proteomes of wild type (WT), HTG low dose (LD) and high dose (HD) mice. **Results:** We observed a dose dependence of the mitochondrial proteomic response to the level of TG in plasma and, surprisingly that most of the matrix and inner-membrane proteins were respectively down- and up-regulated in comparison with WT mitochondria. The opposite behavior of these two major protein sub-populations indicates that an unexpected large-scale phenomenon occurs in HTG mitochondria. The variation of protein expression distribution is larger in matrix compartment than in inner-membrane where it is not significant. **Conclusion:** Proteomic studies allowed us to evidence dramatic changes in metabolism organization: catabolic and anabolic pathways capacities were respectively higher and lower in HTG mitochondria in comparison with WT. In the cytosol an increase in lipogenesis may be sustained by mitochondrial catabolism and several causes may lead to an oxidative stress.

Keywords: hypertriglyceridemia; proteomics; liver mitochondria

S8.42

BLyS participate in the interaction between T and B cells in collagen-induced arthritis mice and the regulation of TACI-Ig

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Aim: To investigate the role of B lymphocyte stimulator (BLyS) on the interaction between T and B cells in collagen-induced arthritis (CIA) mice and the regulation of TACI-Ig. **Methods:** Emulsion of type II collagen (0.1 mL) was injected intradermally into DBA/1 mice to induced CIA. T and B cells were co-cultured in BLyS with or without anti-BLyS (0.1 μg/mL) or TACI-Ig (10 μg/mL) in DMEM supplemented with 10% fetal calf serum (FCS). To investigate the interaction between T and B cells and the function of TACI, we utilized the methods of enzyme-linked immunosorbent assay (ELISA) to detect the levels of IL-2, IL-17, and IgM in T/B cell co-cultrue supernatant, flow cytometry to test the percentages of the $CD4^+CD154^+$, $CD4^+CD28^+$ T cells and $CD19^+CD40^+$ and $CD19^+CD80^+$ B cells, Western-blot to check the expression of CD28, CD154, CD40, CD80, MTT to test the T and B lymphocytes proliferations, and Quantitative Real-Time PCR (qPCR) to detect the mRNA expressions of BLyS and TACI in the thymus and spleen and silenced TACI on B cells. **Results:** B cells activated by BLyS increased levels of IL-2 and IL-17 in T cells cultrue supernatant, the percentages of $CD4^+CD154^+$, $CD4^+CD28^+$ T lymphocytes, and T lymphocyte proliferations. Meanwhile, TACI-Ig reduced the data above all. T lymphocyte activated by BLyS increased levels of IgM in B cells cultrue supernatant, the percentages of $CD19^+CD40^+$, $CD19^+CD80^+$ B lymphocytes, and B lymphocyte proliferations. And TACI-Ig inhibited them. Moreover, the mRNA expressions of BLyS in both the thymus and spleen were increased significantly in CIA mice. However, the expression of TACI mRNA in both the thymus and spleen decreased in CIA mice. At last, the mRNA and protein expressions of CD40 and CD80 in BTACI- cells (activated by BLyS) were higher than that in normal B cells (activated by BLyS). BLyS could promote the expression of CD154 mRNA, CD28 mRNA and the proteins expression of CD154 and CD28 markedly raised in T/BTACI- cells co-cultrue group. **Conclusion:** BLyS played an important role in the interaction of T and B lymphocytes. However, BLyS might be involved in the interaction between T and B cells through BAFF-R and BCMA instead of TACI. TACI-Ig exerted its inhibited effects on interaction of T and B cells might through neutralizing BLyS, thus preventing them from binding to their receptors.

Keywords: T lymphocytes; B lymphocytes; B lymphocyte stimulator; co-stimulatory molecules; collagen-induced arthritis; TACI-Ig

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S8.43

MicroRNA-124 feedback regulates TNF-a production via decreasing protein stability in LPS-triggered macrophages

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Aim: It was recently found miRNA-124, one of the most abundantly expressed miRNAs in the nervous system, could promotes microglia (tissue-resident macrophages in CNS) quiescence and suppresses experimental autoimmune encephalomyelitis (EAE) by deactivating macrophages. These findings prompted us to ask whether miR-124 is involved in regulation of the TLR activation in periphery macrophages. **Methods:** q-PCR was used to evaluate the mRNA expression of miR-124 and TNF-α. ELISA was used to detect protein level of TNF-α in the cell supernatants. Western blot was used to detect protein expression. 3′-UTR luciferase reporter assays was used to analyze the targets of miR-124. **Results:** The stimulation of toll-like receptor 4 (TLR4) rapidly increased the levels of miR-124 in macrophages and mice. miR-124 knockdown significantly increased the production of pro-inflammatory cytokines TNF-α at post-transcriptional level in LPS-triggered macrophages. miR-124 knockdown increased while overexpression decreased the protein stability of TNF-α. Furthermore, ubiquitin-specific proteases 2 (USP2) and ubiquitin-specific proteases 14 (USP14), two components of deubiquitinating enzymes, were found to be directly targeted by miR-124. Knockdown of USP2 and USP14 attenuated the miR-124-mediated protein degradation of TNF-α. **Conclusion:** Our data identify miR-124 as a very important feedback negative regulator for LPS-induced production of TNF-α, thus outlining new mechanisms for fine-tuning the TLR-triggered inflammatory response.

Keywords: micorRNA-124; macrophages; TNF-α; USP2; USP14

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S8.44

Study on the Paeoniflorin-6-O'-benzenesulfonate synthesis and its anti-inflammatory& immunomodulatory activity

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Aim: Total glucosides of paeony (TGP), an active compound extracted from the roots of *Paeonia lactiflora* Pall, was a valuable herb used in the treatment of RA. However, TGP's slow effects seriously limit its application in clinic. Paeoniflorin (Pae), as the the main active ingredients in TGP, is assimilated poorly after oral administration, which causes the slowly effects of TGP. In the present study, a active derivative of Pae was synthesized, and its anti-inflammatory & immunomodulatory activities were studied as well. **Methods:** The derivative (code name: CP-25) was synthesized with conventional acylation reaction, and its structure was identified by MS and NMR. The anti-inflammatory & immunomodulatory activity of CP-25 was evaluated by acute and chronic inflammation responses induced by carrageenin and DNCB, respectively. **Results:** The stutruce of Paeoniflorin-6-O'-benzenesulfonate was determined by MS and NMR. The rats treated with carrageenin displayed significant Paw swelling, and topical application of DNCB to the ear of mice provoked obvious swelling and monocytes infiltration. Oral administration of CP-25 (45, 90, and 180 mg/kg per day) significantly inhibited the inflammation reaction and improved the histopathology as well. Further study suggested CP-25 increased the production of IL-4 and IL-10, but decreased that of IL-2 and IL-17 in the serum, thymocytes and splenocytes supernatant. Moreover, the inhibitory actions of CP-25 on acute inflammation in rats appeared earlier than those of TGP and Pae, and similarly, the inhibitions of CP-25 on chronic inflammation was more apparent than those of TGP and Pae, which reflected by smaller ED50 of CP-25. **Conclusion:** These results suggest that the anti-inflammatory action of CP-25 was more superior to those of TGP and Pae.

Keywords: paeoniflorin-6-O′-benzenesulfonate; total glucosides of paeony; paeoniflorin; inflammation; interleukin-2; interleukin-4; interleukin-10; interleukin-17

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S8.45

Angiotensin II type 2 receptor correlates to therapeutic effects of losartan on rats with adjuvant-induced arthritis

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Aim: Angiotensin II type 1 receptor (AT1R) blocker losartan ameliorates the rheumatoid arthritis (RA) experimental model adjuvant-induced arthritis (AIA) disease progression. Angiotensin II type 2 receptor (AT2R) mainly exerts counter-regulatory actions to AT1R still remains obscure in RA. In present study, we investigated the role of AT2R in losartan-induced treatment effects on rats with AIA. **Methods:** AIA rats were treated with losartan (5, 10, and 15 mg/kg) and methotrexate (MTX) (0.5 mg/kg) *in vivo* from d 14 to d 28. Arthritis was evaluated by polyarthritis index and histological examination. Angiotensin II, TNF-α, and VEGF levels were examined by ELISA. Expression of AT1R and AT2R were detected by Western blot and immunohistochemistry analysis. After stimulation with IL-1β *in vitro*, the effect of CGP42112 (10^{-8} to 10^{-5} mol/L) or losartan (10^{-6} mol/L) on AIA monocytes chemotaxis induced by 10% fetal calf serum (FCS) were analyzed using Transwell assay. **Results:** Treatment with losartan ameliorated AIA symptoms, also suppressed production of TNF-α, VEGF, and Angiotensin II. After treatment with losartan, down-regulation of AT1R expression and up-regulation of AT2R expression in spleen and synovium of AIA rats correlated positively with reduction of polyarthritis index, respectively. AT2R agonist CGP42112 inhibited chemotaxis of IL-1β-stimulated AIA monocytes *in vitro*, maybe relating to up-regulation of AT2R expression. **Conclusion:** In summary, the present study proved for the first time up-regulation of AT2R expression correlated with reduction of polyarthritis index *in vivo*, and also AT2R stimulation inhibited IL-1β-stimulated AIA monocytes *in vitro*, thus strongly suggesting up-regulation of AT2R might be an additional mechanism of therapeutic effect of losartan on AIA rats.

Keywords: rheumatoid arthritis; adjuvant-induced arthritis; losartan; angiotensin II type 2 receptor

Acknowledgements: This study was supported by grants from the National Natural Science Foundation of China (No 30973543 and 81173075).

S8.46

Renoprotective effects of berberine and its possible molecular mechanisms in combination of high-fat diet and low-dose streptozotocin-induced diabetic rats

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Aim: Berberine (BBR), an effective compound of Chinese traditional herbal medicine, has preventive effects on diabetes and its complications. In this study, we investigated the therapeutic effects and underlying molecular mechanisms of BBR in rats with high-fat diet and streptozotocin (STZ)-induced diabetic nephropathy model. **Methods:** BBR (50, 100, and 200 mg/kg/d) were orally administered to male Sprague-Dawley rats after STZ injection and conducted for 8 weeks. Renal damage was evaluated by kidney weight to body weight ratio (KW/BW), urine microalbumin (UMAlb), urine protein for 24 h (UP24h), urine creatinine (UCr), and histological examination. Type IV collagen and transforming growth factor-beta1 (TGF-β_1) were detected by immunohistochemistry and ultrastructure of glomeruli was observed. Fasting blood glucose (FBG) , serum creatinine (SCr), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c) in serum and G protein-coupled receptor kinases (GRKs), cAMP in kidney were measured. **Results:** Remarkable renal damage, hyperglycemia and hyperlipidemia were observed in DN rats. BBR could restore renal functional parameters, suppress alterations in histological and ultrastructural changes in the kidney tissues, improve glucose and lipid metabolism disorders, and increase cAMP levels compared with those of DN model group. Furthermore, BBR down-regulated total protein expression of GRK2, GRK3 and up-regulated expression of GRK6 of renal cortex in DN rats, but had slight effects on GRK4 and GRK5. **Conclusion:** These studies demonstrate, for the first time, that G protein-AC-cAMP signaling pathway might be related to the renoprotection of BBR in high-fat diet and STZ-induced DN rats.

Keywords: berberine; diabetic nephropathy; G protein-coupled receptor kinases; renoprotection

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S8.47

TLR4 homotolerance and regulatory T cells are involved in the protection against insulitis in non-obese diabetic mice by Lipopolysaccharide

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Aim: Lipopolysaccharide (LPS), a component of gram negative bacteria outer-membrane that binds Toll-like receptor (TLR)-4, was reported to prevent autoimmune diabetes occurrence at the basis of the "hygiene hypothesis". In this study, we tested the cellar and molecular mechanism of the protection of LPS on diabetes incidence in non-obese diabetic (NOD) mice. **Methods:** 7–8-week-old NOD mice were randomly divided into three groups: control group, single LPS injection (SLI), and repeated LPS injections (RLI) groups. NOD mice in RLI group were given with 10 μg LPS by intraperitoneal (ip) administration every week for 4 weeks; NOD mice in SLI group were given with 10 μg LPS by ip administration at 7–8-week age, then saline every week for 3 weeks. After 4 weeks of intervention, 8 mice at 11–12-weeks of age per group were randomly selected to be sacrificed to perform intraperitoneal glucose tolerance test, examine histopathological insulitis, cytokine levels in serum, spleen T lymphocyte proliferation, the percentage of $CD4^+CD25^+Foxp3^+$ T regulatory cell (Treg), dendritic cell (DC) surface molecules and TLR4 expression. The remaining 10 mice per group were fed until 26 weeks of age to assess the incidence of diabetes. **Results:** LPS neither prevented nor reverted insulitis, but delayed the onset and decreased the incidence of diabetes in NOD mice, which was more significant in RLI group. And LPS intervention did not influence Th2 cytokine response, bone marrow-derived dendritic cells (DC) phenotype, and TLR4 expression in spleen, but suppressed spleen T lymphocyte proliferation, reduced the synthesis of strong Th1 proinflammatory cytokines, increased the generation of $CD4^+CD25^+Foxp3^+$ Tregs and downregulated TLR4 mRNA and protein expression in thymus, pancreas and DC. **Conclusion:** LPS-induced TLR4 tolerance and Treg differentiation are involved in the protection of LPS on the progression from insulitis to diabetes in NOD mice

Keywords: autoimmune diabetes; lipopolysaccharide; hygiene hypothesis; TLR4; T regulatory cell

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S8.48

Anti-arthritic, analgesic and anti-inflammatory activities of total saponins extract from seeds of *Nigella glandulifera* Freyn

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Aim: To explore the efficacy of total saponins, a compound obtained from seeds of *Nigella glandulifera* Freyn (TSF) in treating arthritic, pain, and inflammation using experimental models. **Methods:** Complete Freund's adjuvant (CFA) was used to induce the primary, secondary paw edema model, and collagen-induced arthritis (CIA) was used to induce paw edema model to observe anti-arthritic activities of

TSF in rats, the alterations in paw volume were recorded. In addition, acetic acid-induced writhing and xylene-induced ear edema model were used to evaluate the analgesic, anti-inflammatory effect in mice. **Results:** In CFA-induced arthritic model, TSF ameliorate the primary (P<0.05) and secondary (P<0.01) paw edema in rats. In CIA-induced arthritis model, TSF reduced the severity of joint swelling (P<0.01). In the writhing test, TSF demonstrated a significant inhibition of writhing (P<0.01). Moreover, TSF significantly inhibited the xylene induced ear edema formation (P<0.01). **Conclusion:** TSF possesses significant anti-arthritic, analgesic, and anti-inflammatory activities.

Keywords: *Nigella glandulifera* Freyn; total saponins; anti-arthritic
Acknowledgements: This work was supported by the Grant from Major Science and Technology Projects of Xinjiang Uygur Autonomous Region of China (201130105-4).

S8.49

Anti-inflammatory effects of ethanol extract of *Viola tianshanica* Maxim in RAW264.7 macrophages

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Aim: *Viola tianshanica* Maxim had a long history of drug use in the southern region of Xinjiang, which had the ability of clear away heat and toxic materials. We investigated the anti-inflammatory effects of ethanol extract of *Viola tianshanica* Maxim (EEVT) *in vitro*. **Methods:** RAW264.7 cells were serum starved for 12 h and treated with EEVT (12.5–100 μg/mL) for a further 24 h to determine cell viability by CCK-8 assay; RAW264.7 cells were preincubated with EEVT (12.5–100 μg/mL) for 1 h, followed by a further 24 h treatment with 2 μg/mL LPS for measurement of pro-inflammatory cytokines in the cell culture medium, such as TNF-α and IL-1β by ELISA assays. The production of NO in the cell culture medium was examined by Griess reaction. **Results:** Exposure of RAW264.7 cells to EEVT (12.5–100 μg/mL) for 24 h caused no reduction of cell viability. Preincubated cells with EEVT at concentrations of 12.5, 25, 50, and 100 μg/mL dose-dependently inhibited the production of TNF-α by 13.0%, 21.8%, 36.9%, and 46.0%, respectively, and inhibited IL-1β production by 47.5%, 70.1%, 84.2% and 97.4%, respectively, also reduced NO level by 28.2%, 30.5%, 44.6%, and 52.4%, respectively. **Conclusion:** The ethanol extract of *Viola tianshanica* Maxim has significant anti-inflammatory effect in LPS-treated RAW264.7 cells.

Keywords: *Viola tianshanica* Maxim; ethanol extract; anti-inflammation
Acknowledgements: This study was supported by Tianshan Distract Science and Technology Project.

S8.50

Chronic administration of corticosterone induced depression-like sleep EEG changes via glucocorticoid receptor in locus coeruleus

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Aim: Depression-like sleep disorders are not only seen in depression patients, but also in those who underwent stress, exogenous administration of glucocorticoids or other diseases with elevated circulation glucocorticoids. In order to find potential treatments, the mechanisms of corticosterone (CORT) induced sleep disorders were investigated. **Methods:** Male SD rat was subcutaneously injected with CORT 40 mg/kg for continuous 7 d. Sleep parameters were examined by EEG and EMG recording. GR protein level in locus coeruleus (LC) was detected by Western blot. And GR antagonist RU486 was microinjected into LC to verify if it could block CORT's effects. **Results:** Administration of CORT significantly reduced the total sleep time, NREM sleep time and enhanced REM sleep time. In the same time, the ratio of REM sleep time was remarkably increased. After 7-d treatment, GR protein level in LC was significantly decreased both in cytoplasm and nucleus. And microinjection of RU486 (100 ng & 250 ng) into LC every day prior to CORT treatment notably reversed the depression-like sleep disorders. **Conclusion:** These findings demonstrate that the reduction of total sleep time and augmentation of REM sleep time and ratio, which resulted from incessant administration of CORT, were mediated by the GR in LC.

Keywords: corticosterone; depression-like sleep; glucocorticoid receptor; RU486
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S8.51

Angiotensin II type 2 receptor correlates to therapeutic effects of losartan on rats with adjuvant-induced arthritis

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Aim: Angiotensin II type 1 receptor (AT1R) blocker losartan ameliorates the rheumatoid arthritis (RA) experimental model adjuvant-induced arthritis (AIA) disease progression. Angiotensin II type 2 receptor (AT2R) mainly exerts counter-regulatory actions to AT1R still remains obscure in RA. In present study, we investigated the role of AT2R in losartan-induced treatment effects on rats with AIA. **Methods:** AIA rats were treated with losartan (5, 10, and 15 mg/kg) and methotrexate (MTX) (0.5 mg/kg) *in vivo* from d 14 to d 28. Arthritis was evaluated by polyarthritis index and histological examination. Angiotensin II, TNF-α, and VEGF levels were examined by ELISA. Expression of AT1R and AT2R were detected by Western blot and immunohistochemistry analysis. After stimulation with IL-1β *in vitro*, the effect of CGP42112 (10^{-8} to 10^{-5} mol/L) or losartan (10^{-6} mol/L) on AIA monocytes chemotaxis induced by 10% fetal calf serum (FCS) were analyzed using Transwell assay. **Results:** Treatment with losartan ameliorated AIA symptoms, also suppressed production of TNF-α, VEGF, and Angiotensin II. After treatment with losartan, down-regulation of AT1R expression and up-regulation of AT2R expression in spleen and synovium of AIA rats correlated positively with reduction of polyarthritis index, respectively. AT2R agonist CGP42112 inhibited chemotaxis of IL-1β-stimulated AIA monocytes *in vitro*, maybe relating to up-regulation of AT2R expression. **Conclusion:** In summary, the present study proved for the first time up-regulation of AT2R expression correlated with reduction of polyarthritis index *in vivo*, and also AT2R stimulation inhibited IL-1β-stimulated AIA monocytes *in vitro*, thus strongly suggesting up-regulation of AT2R might be an additional mechanism of therapeutic effect of losartan on AIA rats.

Keywords: rheumatoid arthritis; adjuvant-induced arthritis; losartan; angiotensin II type 2 receptor
Acknowledgements: This study was supported by grants from the National Natural Science Foundation of China (No 30973543 and 81173075).

S8.52

Utilization of glucose, blood pressure and lipid lowering medications among people with type 2 diabetes in the United States 1999–2010

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Aim: Changes in drug treatment of diabetes in the United States were studied using data from the National Health and Nutrition Examination Survey 1999-2010. **Methods:** Data on 3094 participants aged ≥20 with diagnosed type 2 diabetes were analyzed. The use of medications for lowering blood glucose, blood pressure (BP), and lipids in the past month was assessed by questionnaire. **Results:** Utilization of all 3 types of medications increased significantly from 1999–2002 to 2007–2010 (P<0.01). Usage of metformin increased from 34.8% to 53.8% during this period (P<0.001), and was the most common medication for diabetes in 2003–2010. Dipeptidyl peptidase-4 (DPP-4) inhibitors, approved in 2007, were used by 7.4% of the participants in 2007–2010. Usage of angiotensin receptor blockers (ARB) and beta-blockers increased from 7.4% to 21.4%, and from 15.3% to 31.8%, respectively, across the 12-year period (both P<0.001). In 2007–2010, 52.2% of the participants were on statins. **Conclusion:** There were significant increases in the use of glucose, BP and lipid lowering medications during 1999–2010, especially in the use of metformin, ARB and beta-blockers. Metformin is the recommended first line drug for diabetes, while DPP-4 inhibitors have started to be used in recent years. Statins were underutilized.

Keywords: diabetes; prescription medications; NHANES
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S8.53

The influence of adrenergic receptor agonists on the function of rat fibroblast-like synoviocytes

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Aim: Adrenergic receptor agonists were reported influencing the progress of rheumatoid arthritis models *in vivo*. In this study, the direct effect of adrene rgic receptor agonists on rat fibroblast-like synoviocytes (FLS), including cell proliferation and the secretion of cytokines, *in vitro* were investigated.

Methods: Rat FLS were stimulated by norepinephrine (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}), and isoproterenol (10^{-5}). The FLS proliferations were detected by MTT. The secretion of cytokines (IL-1β, TNF-α, OPG, and RANKL) was assayed by ELISA. **Results:** Norepinephrine (10^{-5}), isoproterenol (10^{-5}) markedly suppress the FLS proliferations, and norepinephrine (10^{-8}) markedly promoted the FLS proliferations, compared with normal FLS. Norepinephrine (10^{-5}), isoproterenol (10^{-5}) apparently reduced the level of IL-1β, TNF-α, norepinephrine (10^{-7}, 10^{-8}, and 10^{-9}) could up-regulate the level of IL-1β, TNF-α. Norepinephrine (10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}) and isoproterenol (10^{-5}) up-regulated the level of OPG. Isoproterenol (10^{-5}) markedly suppress the secretion of RANKL. **Conclusion:** Norepinephrine has dual-direction regulation on the function of FLS, which depend on the concentrations of norepinephrine, relating with the activation on α and β adrenergic receptor. Isoproterenol suppress the FLS proliferations and the secretion of cytokines, which were related with β adrenergic receptor.

Keywords: adrenergic receptor agonists; fibroblast-like synoviocytes; norepinephrine; isoproterenol

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S8.54

Evaluation of a novel model of chronic glomerulonephritis through osmotic pump and protective effect of Zhenwu decoction

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Aim: Chronic glomerulonephritis (CGN) is a clinical syndrome of glomerular disease which is main characterized by haematuria, proteinuria and edema, has become a global pandemic that severly diminishes the quality of life for millions. By now, treatment options and resources remain woefully antiquated main partly owing to maladaptive animal models and drugs. An osmotic pump controlled cationic bovine serum albumin (C-BSA) delivery to produce CGN is a novel model, which avoids the disadvantages of conventional models and provides a new sight to establish CGN model. Zhenwu decoction, which is a classical prescription of Wen Yang Li Shui from Treatise on Febrile Diseases, consists of five herbal remedies (*Radix Aconiti Lateralis Preparata, Poria, Rhizoma Atractylodis Macrocephalae, Paeonia lactiflora, Rhizoma Zingiberis Recens*, with a weight ratio of 3:3:2:3:3) that has been extensively used for the treatment of various kidney diseases. In the present study, we evaluate the novel model of chronic glomerulonephritis through osmotic pump deliveried C-BSA and explore the effects of Zhenwu decoction on the CGN rat model. **Methods:** Male Sprague Dawley rats were randomly divided into Normal, Conventional Model (CM, tail intravenous injection of C-BSA daily for 4 weeks), Osmotic pump Model (OPM, 28-d C-BSA pump with a flow rate of 0.25 μL/h), Prednisone acetate (PA, 8 mg/kg), and Zhenwu decoction with high-, middle- and low-dose (16.8, 8.4, and 4.2 g/kg·d) groups. Following the treatment once a day for 7 weeks, Urinary protein within 24 h were tested by using Coomassie brilliant blue method, blood biochemical parameters including serum total protein (TP), albumin (ALB), serum creatinine (SCr), blood urea nitrogen (BUN), total cholesterol (TC) and triglycerides (TG) were detected by automatic biochemistry analyzer and IgG expression in the glomerulus was determined by immunofluorescence method. **Results:** (1) In comparison to the Normal group, the levels of 24 h urinary protein, SCr, BUN, TC and TG were increased obviously (P<0.01), TP and ALB were reduced significantly (P<0.05 or P< 0.01) in MC and OPM groups; Interestingly, Zhenwu decoction decreased the levels of 24 h urinary protein, SCr, BUN, TC, and TG significantly and increased TP and ALB significantly (P<0.05 or P<0.01). (2) There was little deposition of IgG in the glomerular mesangial area of normal rats. The deposition of IgG in kidney was lower in Zhenwu decoction groups than that in MC and OPM group. (3) The blood biochemical parameters, such as SCr, BUN, TC, TG, TP, and ALB, and IgG deposition performance changes were similar in the two models of Osmotic pump model and Conventional model. **Conclusion:** Results of our present studies reveal that CGN rat induced by osmotic pump for controlled C-BSA delivery is a feasible and reliable method. Zhenwu decoction has a good protective effect on the CGN rats induced by C-BSA.

Keywords: chronic glomerulonephritis; Zhenwu decoction; osmotic pump; C-BSA

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S8.55

Effect of *all-trans* retinoic acid and β-carotene on MMP-9 production in murine macrophages

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Aim: Our previous study and preliminary data have shown that the carotenoid lutein regulates matrix metalloproteinase-9 (MMP-9) and phagocytosis in macrophages. In this study, we investigated the effects of *all-trans* retinoic acid (atRA), a bioactive vitamin A metabolite, on MMP-9 production of murine RAW264.7 and primary-cultured macrophages. **Methods:** MMP-9 was determined by Western blotting and RT-PCR. MMP-9 activity was assayed by gelatin zymography. MMP-9 promoter activity was examined by reporter luciferase activity assay. **Results:** Both atRA and β-carotene enhanced the MMP-9 production in macrophages. The induction was differentially regulated by these two substances. While MMP-9 induction by atRA was not affected by the MAPKs inhibitors, its induction by β-carotene was mainly repressed by the inhibitor targeting ERK1/2. In parallel, β-carotene caused an obvious ERK1/2 activation. Moreover, atRA- and β-carotene-induced MMP-9 production were inhibited by the RA receptor α (RARα) and/or RARβ antagonist, respectively. This was demonstrated by the observations that RARα and β agonists augmented MMP-9 production and atRA and β-carotene enhanced RA responsive element-mediated and MMP-9 promoter-constructed luciferase activity. **Conclusion:** We provide evidence here for the first time that atRA and β-carotene differentially regulate MMP-9 production in macrophages.

Keywords: carotenoid; MMP; retinoic acid receptor; RAR; nuclear receptor

S8.56

Theraputic effect of rhTNFR:Fc on rats with adjuvant induced arthritis via attenuating inflammatory response

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Aim: To examine the effects of rhTNFR:Fc on disease progression, joint inflammation, bone destruction and the production of inflammatory mediators in a rat model of adjuvant induced arthritis (AA). **Methods:** AA was induced by complete Freund adjuvant. AA rats with experimental arthritis were randomly separated into different groups and then treated with rhTNFR:Fc (1, 3, and 9 mg/kg), or IgG-Fc (9 mg/kg), subcutaneously after immunization every 3 d for a total of 15 d. Global assessment, polyarthritis index, hind paw swelling and swollen joint count, paw radiography, ankle joint histopathology, spleen histopathology examination and score were used for evaluating the drug effects on AA rats. Activities of Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in serous and supernate of peritoneal macrophage (PMφ) were assessed by ELISA. T lymphocyte function was assessed by measuring percentage of T lymphocytes in the peripheral blood (PB) and mesenteric lymph node (MLN). **Results:** In the AA rats, remarkable secondary inflammatory responses exhibited, administration of rhTNFR:Fc significantly reduced the global assessment, polyarthritis index, hind paw swelling and swollen joint count, ameliorated X-ray and histopathological manifestations, decreased the expression of IL-

1β, IL-6, and TNF-α in PB and PMφ. RhTNFR:Fc significantly inhibited T and B lymphocyte proliferation. In PB and MLN the percentages of $CD3^+CD4^+$ and $CD4^+CD25^+$ T lymphocytes were higher in the AA rats than in the normal rats, and $CD4^+CD62L^+$ and $CD4^+CD25^+FoxP3^+$ T lymphocytes were lower in the AA rats. RhTNFR:Fc decreased the percentage of $CD4^+CD25^+$ and restored the percentage of $CD4^+CD62L^+$ and $CD4^+CD25^+FoxP3^+$ T lymphocytes to normal levels, but had no obvious effect on the percentage of $CD3^+CD4^+$ T lymphocytes. **Conclusion:** Data presented here demonstrate that administration of rhTNFR:Fc significantly attenuates progression of experimental arthritis, with reductions in inflammatory response.

Keywords: adjuvant arthritis; inflammation; TNF-α; macrophage; cytokines; T lymphocytes

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S8.57

Hypoglycemic effect of co-administration of berberine and sodium caprate via AMPK in inhibiting hepatic gluconeogenesis

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Aim: Berberine (BER), a natural product and active ingredient of genera Berberis and Coptis, has been demonstrated to possess anti-diabetic activities. However, the poor bioavailability of this agent greatly limits its clinical application. In our previous study, we demonstrated that co-administration of sodium caprate, an absorption enhancer, with BER could significantly increase the bioavailability of BER without any serious mucosal damage. Here, we investigated the effects of BER on AMP-activated protein kinase (AMPK)/gluconeogenesis pathway and the effects of sodium caprate on hypoglycemic action of BER. **Methods:** The ability of BER co-administered with sodium caprate to reduce insulin resistance was investigated in diabetic rat model induced by high-fat diet and low dose STZ. Western blot was performed to evaluate effects of BER on AMPK signaling proteins involved in hepatic gluconeogenesis in diabetic rat and HepG2 hepatocytes. **Results:** BER reduced body weight and caused a significant improvement in glucose tolerance without altering food intake in diabetic rats. Similarly, BER reduced plasma triglycerides and improved insulin action in diabetic rats. BER down-regulated the elevated expressions of gluconeogenesis key enzymes PEPCK and G6Pase, inhibited the translocation of TORC2 from cytoplasm to nucleus and increased AMPK activity in liver tissues. The effect of BER was higher when co-administered with sodium caprate. BER treatment resulted in reduced glucose production in HepG2 hepatocytes. BER increased AMPK activity, reduced the expression of PEPCK, and the nuclear transcription factors PGC-1, HNF-4α and FOXO1. **Conclusion:** The effect of BER on gluconeogenesis could be partly blocked by AMPK inhibitor, Compound C. BER could suppress hepatic gluconeogenesis in rat model of diabetes at least in part via stimulation of AMPK activity and this action of BER is augmented by sodium caprate.

Keywords: Berberine; sodium caprate; gluconeogenesis; AMPK

S8.58

Effect of Compound K on enhancing insulin secretion in MIN6 pancreatic β-cells by upregulation of GLUT2

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Aim: Compound K (CK) is a final intestinal metabolite of protopanaxadiol-type ginsenosides from *Panax ginseng* and shows various bioactivities. Although it has also been found to have the property of anti-diabetes, the long-term effect of CK on insulin secretion in β-cells is still unclear. In this study, CK was prepared from ginsenoside Rd by snailase hydrolysis and its effect on the insulin secretion activity in MIN6 pancreatic β-cell lines *in vitro* was assessed. **Methods:** The expression of glucose transporter isoform-2 (GLUT2) and the cellular ATP content were also examined by Western blot and HPLC analysis, respectively. **Results:** The results showed that CK significantly enhanced insulin secretion, increased cellular ATP content, and upregulated the expression of GLUT2. **Conclusion:** These findings indicate that CK exerts prominent stimulatory effects on insulin secretion in the MIN6 cells partly via upregulating the expression of GLUT2.

Keywords: Compound K; insulin secretion; ATP; GLUT2

S8.59

High glucose, inflammation, and anti-diabetic tactics

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Aim: Diabetes demonstrates as high blood glucose (HG) and low-grade inflammatory responses. HG may cause inflammation. In turn, inflammation will cause high glucose. However, what is reason or cause required to be elucidated. In this study, we aimed to investigate how HG interacted with inflammation in immune cells. **Methods:** Primary mouse macrophages, Raw264.7, THP-1, and Hut102 cells were induced by adding high glucose (15, 25, and 45 mmol/L) or lipopolysaccharide (LPS) (0.02–2 μg/mL) for 48 h of incubation. Then, IL1a, IL1b, IL6 and TNFa protein levels were assayed by ELISA, respectively. Cell glucose metabolism was measured by biochemical method. **Results:** After HG stimulation, all cell lines showed no significant changes of inflammatory factors at the protein levels within 48 h. However, LPS immediately induced a significant increase in inflammatory factors at protein levels and also cause significant glucose metabolism dysfunction in those cells. **Conclusion:** These results indicate that HG might not be a key issue at all to cause the development of inflammation in diabetic populations. However, inflammation causes significant glucose metabolism dysfunction in diabetic individuals. Maybe, it is reasonable to conclude that key tactics of anti-diabetic drugs should aim not to lower blood glucose only but control the low-grade inflammatory stress.

Keywords: diabetes; inflammation; anti-diabetic drugs

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S8.60

Identification of L312 as a Novel Selective PPARγ Modulator

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Aim: Selective PPARγ modulator (sPPARγM), which retains potent insulin sensitizing activity and avoids the side effects induced by classical PPARγ agonism, is regarded as safer insulin sensitizer compared to full PPARγ agonists. This study reported a novel sPPARγM L312. **Methods:** First, SPR based PPARs-ligand binding and PPARs transactivation assays were used as primary screening to identify compounds with selective high affinity to PPARγ ligand binding domain (LBD) and lowoer PPARγ agonism. Next, selective PPARγ modulations were distinguished with the 3T3-L1 preadipocyte differentiation and PPARγ target genes expression assay. Finally, diet induced obesity mice and *db/db* T2DM mice were used to evaluate the *in vivo* insulin sensitizing activity. **Results:** Novel compound L312 displayed the equal affinity to PPARγ-LBD as rosiglitazone, but the weaker activity to PPARγ transactivation in the luciferase assay and in pre-adipocyte differentiation. In mature adipocytes, L312 up-regulates the gene expression of adiponectin (insulin sensitizing adipokine) more robustly than those genes related to lipid accumulation. Moreover, L312 alleviate insulin resistance with less weight gain, hepatic steatosis and adipose accumulation *in vivo* during treatment. **Conclusion:** L312 was identified as a novel sPPARγM, which can improve insulin resistance yet with fewer full PPARγ agonists side effects.

Keywords: L312; selective PPARγ modulator

S8.61

Changes of different sites of skeleton in Lewis rats with type II collagen-induced arthritis (CIA)

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Aim: It is evident for rheumatoid arthritis (RA) to induce osteoporosis during its development and treatment. However the mechanism of how RA induces osteoporosis is not yet clear. In order to know the characteristics and mechanism of bone loss induced by RA, changes of different sites of skeleton in Lewis rats with type II collagen-induced arthritis (CIA) was investigated by bone histomorphometry, bone biomechanics and bone mineral density (BMD). **Methods:** Eight-week-old Lewis rats were randomly divided into vehicle control and CIA groups. All rats were given sc injections of 7 mg/kg calcein on the 14th, 13th, 4th, and 3rd d before death, to determine dynamic changes in bone tissues. RAts from vehicle control and CIA groups were sacrificed by cardiac puncture under sodium pentobarbital anesthesia at 60 and 120 d after immunization. BMD of femur and

the forth lumbar vertebrae (LV4) were studied using dual X-ray absorptiometry. Biomechanical properties of femur were determined from the three-point bending test. The static and dynamic parameters of tibia and the fifth lumbar vertebrae (LV5) were examined with histomorphometrical analyses. **Results:** Compared with control group, BMD of femur and LV4 were significantly decreased in all CIA groups. Biomechanical properties (elastic load, maximum load, break load, stifffness, energy to break load) of femur were significantly decreased in all CIA groups. Compared with control group, trabecula bone volume, trabecular number (Tb.N) of PTM and LV5 were significantly decreased, while bone turnover rate(BFR/BV), osteoclast number per mm (Oc.N) and percent osteoclast surface perimeter (Oc.S.Pm) were increased all CIA group. Cortical area (Ct.Ar), percent cortical area (%Ct.Ar) and bone formation of Tx in periosteal surfaces were decreased in all CIA groups. **Conclusion:** CIA in rats at 60 and 120 d after immunization could lead to systemic bone loss. The mechanism may be related to the increase of bone resorption in cancellous bone and the decrease of formation in cortical bone.

Keywords: osteroporosis; rheumatoid arthritis; collagen-induced arthritis; histomorphometry; bone biomechanics; bone mineral density

S8.62

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

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

Keywords: ginsenoside Rg1; MSCs; proliferation; microenvironment

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S8.63

Nicotine enhances insulin sensitivity in vascular smooth muscle cells

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Aim: This study was to investigate the effect of nicotine on insulin sensitivity in vascular smooth muscle cells (VSMCs) and explore the underlying mechanisms. **Methods:** After six weeks nicotine treatment (3 mg/kg/day), Sprague-Dawley rats were sacrificed and the thoracic aortas without endothelium and adventitia were used for organ bath experiment and Western blot analysis. Insulin (10^{-9} mol/L to 10^{-5} mol/L) induced vasodilation was studied. Next, we studied the effect of nicotine on two insulin resistant models of cultured smooth muscle cells. Primary VSMCs were co-cultured with nicotine (6×10^{-6} mol/L) and dexamethasone (1×10^{-6} mol/L) or high concentration insulin (5×10^{-7} mol/L) for 48 h, and then insulin stimulation of glucose uptake was detected. **Results:** Aortic function test showed that insulin induced greater smooth muscle relaxation in nicotine treated group. Nicotine enhanced insulin stimulation of glucose uptake in both dexamethasone and high concentration insulin induced insulin resistant VSMC models. Western blot analysis showed that after chronic nicotine treatment, insulin sensitivity related protein (peroxisomal proliferators activated receptor γ, PPARγ) was significantly upregulated. **Conclusion:** Nicotine enhances insulin sensitivity in vascular smooth muscle cells. Nicotine induced PPARγ upregulation might be involved in the underlying mechanism.

Keywords: nicotine; insulin sensitivity; vascular smooth muscle cells; peroxisomal proliferators activated receptor γ

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S8.64

The proteasome inhibitor MG132 enhances LDL uptake in hepatocytes through regulating LDLR and PCSK9 expression: a novel role in cholesterol homeostasis

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Aim: The low-density lipoprotein receptor (LDLR), a determinant regulator in cholesterol homeostasis, is tightly regulated in liver cells. Previous studies demonstrated that the degradation of LDLR is lysosome dependent, whereas the evidence for the involvement of proteasome in LDLR regulation is relatively rare. Thus we aimed to study the role of proteasome in LDLR modulation by using a specific proteasome inhibitor MG132. **Methods:** HepG2 hepatocytes were treated with MG132 at various concentrations (0.1, 0.3, and 1 μmol/L) or time points (2, 4, 8, and 24 h). LDL uptake was detected by Dil-labelled LDL uptake assay. Gene and protein expression levels were measured by real-time PCR and Western blot, respectively. The involvement of proprotein convertase subtilisin/kexin type 9 (PCSK9, a major protein mediating LDLR degradation in lysosome) was proved by silencing PCSK9 with specific siRNAs. **Results:** MG132 treatment increased LDL uptake, LDLR gene and protein level in HepG2 cells, whereas decreased PCSK9 gene and protein expression. MG132 (0.3 μmol/L) enhanced LDLR gene and protein expression during short-time (0–8 h) treatment through PKC-dependent pathway, and increased LDLR protein level during long time treatment (24 h) through reducing PCSK9 mediated LDLR degradation in lysosome. Combined use of MG132 (0.3 μmol/L) and pravastatin (5 μmol/L) significantly increased LDL-uptake and LDLR expression, and the pravastatin's effect on PCSK9 up-regulation was inhibited. Using hamster as an *in vivo* model, we confirmed the beneficial effects of MG132 on LDLR enhancement. **Conclusion:** We herein report for the first time that the proteasome inhibitor MG132 bearing a dual function in modulating LDLR and PCSK9 expression may exert beneficial effects in cholesterol homeostasis.

Keywords: Low-density lipoprotein receptor; proprotein convertase subtilisin/kexin type 9; protein kinase C; hamster

S8.65

Glucocorticoid reversed the increased plasma cells induced by IL-21 in MRL/MpJ mice

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Aim: Some researches have indicated that IL-21 plays an important role in both human and murine B cells differentiation into memory cells and plasma cells, and glucocorticoid (GCS) administration can inhibit the process of B cell transformation into plasma cells. In this study, the effect and mechanism of GCS on the increased plasma cells induced by IL-21 in MRL/MpJ mice were investigated. **Methods:** Ten-week-aged MRL/MpJ mice were treated with prednisone (5 mg/kg) by intragastrically once per day, continuously for 8 weeks. Proteinuria was measured by the semi-quantitative Albustix paper. The levels of serumal anti-dsDNA, antinuclear antibody (ANA), IL-21, IgG, IgM were tested by enzyme-linked immunosorbent assays. Histopathological examination of kidney and spleen were using H&E staining. Splenic B cell subsets and plasma cells were analyzed by flow cytometry. The expression of B lymphocyte-induced maturation protein-1 (BLIMP-1), Bcl-6 and IL-21 mRNA were detected by real-time quantitative PCR. **Results:** After treatment with prednisone for 8 weeks, the level of proteinuria of MRL/MpJ mice was significantly decreased and the pathological lupus-like involvements in kidney and spleen were improved markedly, the level of serum ANA and IL-21 were also decreased. Prednisone treatment could decrease the percentage of $CD4^{+}CD69^{+}$ T cells, $CD19^{-}CD138^{+}$ plasma cells and $CD27^{+}CD38^{+}$ memory cells, while increase the decreased percentage of total B cells ($CD19^{+}$) in

spleen of MRL/MpJ mice significantly. And the expression of BLIMP-1 mRNA and Bcl-6 mRNA was up-regulated in MRL/MpJ mice spleen while was reversed after prednisone treatment. These results indicated that IL-21 was an important cytokine which involved in autoimmune disease, prednisone treatment could interrupt the process of B cells differentiation into memory cells and plasma cells, inhibit autoantibody production and ameliorate interstitial nephritis. **Conclusion:** Prednisone treatment for autoimmune disease is correlated with B cells differentiation into Ab-secreting plasma cells which induced by IL -21.

Keywords: prednisone; IL-21; BLIMP-1; Bcl-6; plasma cell

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S8.66

Salvianolic acid A protects against diabetic nephropathy in type 2 diabetes

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Aim: Salvianolic acid A (SalA) is one of the main water-soluble active components in *Salvia miltiorrhizae*. The aim of this study was to investigate the effects of SalA on prevention and treatment of diabetic nephropathy. **Methods:** Spontaneously type 2 diabetic mice (KK/Upj-Ay/J) were used in the experiment. The mice with blood glucose above 8 mmol/L were chosen and randomly divided into 4 groups: diabetic control; SalA treatment groups (0.4, 1.3, and 4 mg/kg); age matched C57BL/6 mice were empolyed as normal control. SalA was oral administrated daily for 4 months. **Results:** After 3-month of treatment, the blood-BUN level was lower in SalA treatment groups compared with diabetic control. However, the influence of SalA on Cr wasn't observed due to the lower blood-Cr level in diabetic mice model. After 4-month of SalA treatment, the urinary NAG/Cr and microalbumin was lower compared with diabetic control; SalA treatment also attenuated the expanded glomerular extracellular matrix and glomerular area, the proliferation of mesangial and basement membrane, and renal tubular vacuolar degeneration. Through all the experiment process, SalA showed a trend to decrease blood glucose and reduce TG, TC. **Conclusion:** The results suggested that SalA could protect against diabetic nephropathy in type 2 diabetes.

Keywords: salvianolic acid A; type 2 diabetes; diabetic nephropathy

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S8.67

Asiatic acid inhibits transforming growth factor-β1-induced collagen expression in keloid fibroblasts by PPAR-γ activation: implication for new therapeutic approach

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Aim: To investigate the effects of asiatic acid (AA) on cell proliferation, invasion and collagen expression in TGF-β1-stimulated keloid fibroblasts and the underlying mechanisms. **Methods:** 5-ethynyl-2-deoxyuridine (EdU) incorporation assay, transwell invasion assay, enzyme-linked immunosorbent assay, Western blot, quantitive polymerase chain reaction and RNA interference assay were performed. **Results:** AA could suppress TGF-β1-induced expressionsof collagen type I and plasminogen activator inhibitor-1 (PAI-1), inhibit phosphorylations of Smad 2/3, while elevate Smad 7 protein levelin keloid fibroblasts. Noteworthy, the above effects of AA were abrogated by either pretreatment of GW9662 (PPAR-γ antagonist) or PPAR-γsilencing. **Conclusion:** The present study demonstrated that AA inhibited TGF-β1-induced collagen and PAI-1 expression in keloid fibroblasts through PPAR-γ activation.

Keywords: asiatic acid; keloid fibroblasts; TGF-β/Smad pathway; PPAR-γ

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S8.68

The effect of HeiJiangDan Ointment intervention on cell apoptosis and vascular injury during radiation dermatitis induced by ^{60}Co γ-ray in mice

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Aim: HeiJiangDan is an in-hospital preparation which has the function of clearing toxic and activating blood. It derived from JiZiLuanFaGao which was first recorded in the book of ChuanXinShiYongFang edited by LiuYuXi in Tang Dynasty. In this study, the effect of Heijiangdan Ointment intervention on cells apoptosis and vascular injury during radiation dermatitis induced by ^{60}Co γ-ray in mice were investigated. **Methods:** Female Wistar mice with 4 grade radiation dermatitis induced by ^{60}Co γ-ray were randomly divided into 4 groups (n=12): model group (treated with saline), Heijiangdan Ointment group, recombinant human epidermal growth factor (rhEGF) group, and Trolox group. Drug efficacy and its mechanisms were tested by colorimetry, immunohistochemistry, Western blot, *etc*. **Results:** On 11th d, there was a significant increase on the expression of p53, Fas, FasL, Caspase-3, Caspase-9, Bax, and a significant decrease on the expression of Bcl-2 around the dermatitis tissue in rats (P<0.01). The expression of Fas, Caspase-3 and Bax in dermatitis tissue were significantly lower than those in model group (P<0.01), the expression of Caspase-9 was lower than those in model group (P<0.05). There was no difference in the expression of p53, FasL, and Bcl-2 between Heijiangdan Ointment group and model group. On 21th d, there was a significant decrease on the expression of α-SMA and VEGF around the dermatitis tissue in rats (P<0.01). The expression of α-SMA in dermatitis tissue were significantly higher than those in model group (P<0.01), the expression of VEGF was higher than those in model group (P<0.05). **Conclusion:** The results showed that the pathologic changes of radiation dermatitis was closely related with cell apoptosis and blood vessel repairs. Heijiangdan Ointment may speed up the healing of IV° radiation dermatitis, reduce the inflammatory response in wound, and promote the repair of elastic fibers and collagen. It can inhibit cell apoptosis and heal the microvascular injury in the dermatitis caused by oxidative stress.

Keywords: radiation dermatitis; cell apoptosis; Heijiangdan Ointment; vascular injury; traditional Chinese medicine external treatment

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S8.69

Hepatoprotetive effects of saponins from *Panax japonicus* against alcohol-induced liver injury via inhibition of toll-like receptor-4 mediated signaling pathway

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Aim: To investigate the protective effects of saponins from *Panax japonicus* (SPJ) on alcohol-induced hepatic injury and the potential mechanisms involved. **Methods:** SPJ (200 and 400 mg/kg) were given intragastrically to mice for one week. Alcohol (56% alcohol at 15 mL/kg) was administered orally every 12 h for three times after 1 h of the fourth dose of SPJ. Liver injury was evaluated by biochemical and histopathological examination. Lipid peroxidation and the activity of antioxidants were measured by spectrophotometric method. Expression of cytokines was determined by ELISA and RT-PCR. Nuclear factor κB (NF-κB) activation was measured by Western blotting. Toll-like receptor-4 (TLR4) and CD14 expression on F4/80 positive liver macrophages were assayed by flow cytometry. **Results:** SPJ pretreatment significantly protected against alcohol-induced liver injury as evidenced by decrease of serum alanine aminotransferase, aspartate aminotransferase and triglyceride levels and recover of liver histopathological injury in mice, as well as restoration of impaired anti-oxidative capacity. In addition, SPJ remarkably attenuated the over-production of proinflammatory cytokines including tumor necrosis factor-α and interleukin-6 and inhibited activation of NF-κB in liver tissue. Further investigation displayed that the activation of liver macrophges were suppressed via SPJ down-regulating TLR4/CD14 expression in alcohol treated mice. **Conclusion:** SPJ protected against alcohol-induced liver injury mainly due to its ability to inhibit the activation of Kupffer cells by decreasing TLR4 expression and then the subsequent formation of reactive oxygen species and inflammation insult.

Keywords: saponins from *Panax japonicus*; alcohol-induced liver injury; toll-like receptor-4; oxidative stress; inflammation
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S8.70

Effects and mechanisms of total glueosides of paeoniflorin derivatives (CP-25) on collagen-induced arthritis in mice

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Aim: According to the changes that secondary paw swelling, polyarthritis index, and histological morphological of collagen-induced arthritis (CIA) rats, the anti-inflammatory and immunoregulation activity between CP-25 and TGP or Pae was compared on the models of CIA. To study the effect of CP-25 on peritoneal macrophage (PM) (TNF-α, IL-1, PGE_2) from rats of adjuvant-induced CIA. To study the effect of CP-25 on proliferation of T, B lymphocyte and the secretion levels of interleukin2 (IL-2), IL-4, IL-10, and IL-17 from rats of adjuvant-induced CIA, and this study tends to clarify the therapeutic effects of CP-25 on CIA rats. **Methods:** CIA was used to induce type II collagen in rats. Therapeutic treatment of interagastric administration Pae (35, 70, and 140 mg/kg), CP-25 (8.725, 17.5, 22.5, 35, 45, 70, 90, 140, and 180 mg/kg), Prednisone (Pre), (d24-d36) were given after immunization. Hind paw volumes of rats were measured by volume meter. Arthritis index, spleen index were recorded. TNF-α, IL-1, PGE_2 produced by thymus PM was measured by ELISA. IL-2, IL-4, IL-17, IL-10, TGF-β level produced by thymus T and B cells was measured by ELISA. Pathological changes in synovium of joint were observed by light microscope. The proliferation of T and B lymphocytes was assayed using the MTT assay. **Results:** CP-25 (22.5, 35, 45, 70, 90, 140, and 180 mg/kg, d24-d36) significantly suppressed significantly the paw swelling as well as down-regulated the index of polyarthritis, spleen index by thymus T cells and proliferation of T and B lymphocytes in CIA rats. The articular histopathological changes were also improved. This suggested that CP-25 had therapeutic effects on CIA rats. CP-25 (10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} mol/L)significantly inhibited proliferation of T and B lymphocytes. The inhibition of CP-25 was more superior to those of TGP and Pae. Compared with Pae and TGP group, the concentrations of IL-4 and IL-10 elevated significantly in CP-25, whereas the concentrations of IL-2, IL-17, and TGF-β1 reduced significantly of CIA rats. CP-25 (22.5, 35, 45, 70, 90, 140, and 180 mg/kg) was shown to relieve swelling of joint to some extent. Meanwhile, CP-25 also suppressed the over production of TNF-α, IL-1 and PGE_2. **Conclusion:** CP-25 could alleviate the inflammation of joint at different degree and decrease the spleen index, proliferation of T, B lymphocyte and articular histopathological changes in rats with CIA. CP-25 has anti-inflammatory effect, which its mechanism may be related to the modification of the abnormal immunological function of peritoneal macrophage from CIA rats. CP-25 decreased the expression in T, B cell for the Buff on CIA rats. The therapeutic effects of CP-25 on models of CIA were more superior to those of TGP and Pae.

Keywords: collagen induced arthritis; CP-25; paeoniflorin; total glucosides of paeony; lymphocyte; macrophages

S8.71

Immunoregulation effects of polysaccharides isolated from *Panax japonicus* in immunosuppressed mice

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Aim: Rhizoma *Panax japonicus* (PJ), belonging to *Panax species* of Araliaceae, has been used to treat a variety of diseases as a folk medicine. *Panax japonicus* polysaccharides, an activity substance isolated from the water-soluble compounds of *Panax japonicus*, is a major effective component of *Panax japonicus*, besides its saponins. Several studies have demonstrated that *Panax japonicus* polysaccharides significantly exhibited anti-fatigation and anti-aging effects *in vivo*. However, immunoregulatory activity of *Panax japonicus* polysaccharides are far from clear. The present study is designed to elucidate the effects of *Panax japonicus* polysaccharides on immunological regulation in immunosuppressed mice induced by cyclophosphamide. **Methods:** In this study, we established an immunosuppressed animal model by intraperitoneal injection of cyclophosphamide in Balb/c mouse. The mice were administered with the polysaccharides at a dose of 125, 250 and 500 mg/kg, respectively in the experiment groups, and same volume PBS in the control group. After 18 d treatment the mice were killed, and the index of immune organ was evaluated by spleen weight; the subgroups of peripheral blood lymphocytes, including $CD3^+$, $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, and NK cells were detected by flow cytometry (FCM). **Results:** After cyclophosphamide injection the spleen weight in the model group were significantly lower than that of normal group, and *Panax japonicus* polysaccharides could be significantly antagonist the immune organ weight loss. Cyclophosphamides significantly reduced percentage of lymphocytes as compared to that of normal group ($P<0.01$). *Panax japonicus* polysaccharides enhanced the population of $CD3^+$, $CD4^+$, and $CD8^+$ T cell percentage, which displayed significant difference when the intermediate- and high-dose *Panax japonicus* polysaccharides groups compared to the model group ($P<0.05$). Cyclophosphamides significantly inhibited NK and B cells. In *Panax japonicus* polysaccharides treated groups, only high-dose group restored the B cell percentage to near the normal level. But all doses of *Panax japonicus* polysaccharides had marked effects on NK cells, which were elevated near the normal level. **Conclusion:** These results suggest *Panax japonicus* polysaccharides has potential role for enhancing lymphocyte percentage in the immunosuppressed mice.

Keywords: *Panax japonicus* polysaccharides; immunosuppression; lymphocyte; cyclophosphamide

S8.72

Preventive effects of andrographolide on the development of diabetes in autoimmune diabetic NOD mice via inducing immune tolerance

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Aim: Andrographolide, as an active component in traditional anti-diabetic herbal plants, is a diterpenoid lactone isolated from *Andrographis paniculata* promising for its potent anti-inflammatory and hypoglycemic effects. However, the effect of andrographolide on the development of diabetes in autoimmune diabetic NOD (non-obese diabetic) mice still remains unknown. This study aims to investigate the protective effects of andrographolide on the development of autoimmune diabetes and further clarify the underlying mechanism. **Methods:** NOD mice were randomly divided into four groups, which were administered with water, and 50, 150, and 500 mg andrographolide/kg body weight (BW) respectively for 4 weeks. ICR mice were also selected as the species control group. The oral glucose tolerance and histopathological insulitis were examined. The Th1/Th2/Th17 cytokine secretion was determined with ELISA. The transcriptional profiles of T-bet, GATA3 and RORγt in the pancreatic lymphatic node (PLN) samples derived from the NOD mice were detected by RT-PCR. **Results:** After 4 weeks of oral supplementation, andrographolide significantly inhibited insulitis, delayed the onset and suppressed the development of diabetes in 30-week-old NOD mice in a dose dependent manner. The protective status is correlated with the substantially decreased production of interferon (IFN)-γ and interleukin (IL)-2, increased IL-10 and transforming growth factor (TGF)-β, as well as reduced IL17. Andrographolide also increased the mRNA expression of GATA3, and decreased the T-bet and RORγt mRNA expression. **Conclusion:** Our results suggest that andrographolide prevented type 1 diabetes mainly via maintaining the Th1/Th2/Th17 homeostasis.

Keywords: andrographolide; autoimmune diabetes; Th1; Th2; Th17

S8.73

Effects of Salvianolic acid A on renal injury in rats with type 2 diabetes

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Aim: In this study, we investigated the effects of Salvianolic acid A (SAA) on the prevention of diabetic nephropathy in type 2 diabetic rats. **Methods:** An animal model of renal injury with type 2 diabetes was developed using a high-sucrose, high-fat diet and low-dose streptozotocin (STZ). The diabetic rats were orally given SAA for 16 weeks. Blood glucose, blood uric nitrogen, serum creatinine,

urine *N*-acetyl-β-glucosaminidase (NAG) and urine protein were measured. Pathologic examination observation was performed to analyze renal injury. **Results:** Hyperglycemia, evaluated UAE and NAG/Cr were observed in the model group. SAA could reduce the level of NAG/Cr. The urinary protein level in SAA group decreased significantly, compared with that of the model control group. The kidney was pathologically examined hypertrophy, mesangial expansion and glomerular sclerosis. Administration of SAA could attenuate histopathological injury. **Conclusion:** The results suggest that SAA might prevent nephropathy in type 2 diabetes.

Keywords: salvianolic acid A; type 2 diabetic rats; renal injury
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S8.74

BAFF/BAFF-R involved in B lymphocyte proliferation of rats with collagen-induced arthritis via PI3K/Akt/mTOR signaling and the regulation of paeoniflorin

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Aim: Rheumatoid arthritis (RA) is a systemic autoimmune disease. B lymphocyte proliferation and activation are essential for RA. B cell-activating factor belonging to the TNF family (BAFF) is a B cell survival and maturation factor. BAFF bind to its receptor BAFF-R (BAFF/BAFF-R) can promote B lymphocyte proliferation and activation. PI3K/Akt/mTOR signaling pathway plays a vital role in lymphocyte proliferation and survival. However, it is unknown whether BAFF/BAFF-R regulates B cell proliferation and survival in RA through PI3K/Akt/mTOR pathways. Paeoniflorin (Pae) is one of the principal bioactive components of total glucosides of paeony (TGP). Pae has anti-inflammatory effects on collagen-induced arthritis (CIA) rats by modulating G protein-coupled signaling. However, whether Pae regulate B cell activation via BAFF/BAFF-R and PI3K/Akt/mTOR signaling pathway to ameliorate RA need more study. This paper investigates the role of PI3K/Akt/mTOR signaling mediated by BAFF/BAFF-R in antibodies production and the regulation of Pae on the signaling pathway in rats with CIA. **Methods:** The rats with CIA were randomly separated into different groups and treated with Pae (25 and 100 mg/kg) once per day from d 18 to 38 after immunization. The effects of Pae on CIA rats were evaluated by arthritis scores, paw swelling, joints and spleens histopathology and indices of spleen and thymus. Concentrations of BAFF, anti-CII antibody, IgA, IgG, and IgM in CIA rats serum were measured by Enzyme-linked immunosorbent assay (ELISA) kits. Total protein expression of BAFF-R, P110δ, P-Akt1/2/3, and mTORC1 [Raptor (10E10)] were measured by immunohistochemistry and Western blotting. The effects of Pae to splenic lymphocytes by *in vitro* proliferation experiment and the rate of flow cell apoptosis. **Results:** From d 18 after immunization, CIA rats joint synovial hyperplasia were reached 4–5 layers, cell infiltration obviously, pannus and cartilage erosion seriously. In CIA rats spleens, white pulp proliferated, germinal centers expansion, red pulp hyperemia and spleens were infiltrated with inflammatory cells. The concentrations of IgA, IgM, IgG, BAFF, and anti-CII antibody in CIA rats obviously increased compared with normal rats. B lymphocyte function of CIA rats abnormal increased. The administration of Pae obviously decreased arthritis score, paw swelling, decreased spleen index and alleviated histopathological manifestation in CIA rats; inhibited the level of IgA, IgM, IgG, and anti-CII antibody in CIA rats. BAFF level in serum was increase compared with the normal group. BAFF-R was expressed in the mantle zone (MC) and marginal zone (MZ) in the spleen. Compared with mantle zone and marginal zone, the expression of BAFF-R was lower in the germinal center (GC). Pae could reduce BAFF and BAFF-R expression. The expressions of P110δ, P-Akt1/2/3, and mTORC1 in CIA rats significantly increased. Pae could decrease the level of P110δ, P-Akt1/2/3, and mTORC1. B cell proliferation stimulated by BAFF and IGF-1 obviously increased, the rate of apoptosis decreased, Pae could restrain B cell proliferation, increase cell apoptosis. **Conclusion:** PI3K/Akt/mTOR signal pathway activation mediated by BAFF/BAFF-R might regulate B lymphocyte proliferation and survival. Pae had therapeutic effects on rats with CIA. These effects might be relative to regulating PI3K/Akt/mTOR signal mediated by BAFF/BAFF-R, and down regulate B lymphocyte proliferation, survival and the antibodies production in further.

Keywords: RA; collagen-induced arthritis; B lymphocyte; BAFF; signaling pathway; PI3K/Akt/mTOR; Pae
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S8.75

Interlaboratory transferability study of the *Pig-a* mutation assay with immunomagnetic enrichment

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Aim: *N*-Ethyl-*N*-nitrosourea (ENU) is a potent mutagen that has been studied as part of an international trial that continues to evaluate the reproducibility and transferability of *Pig-a* mutation assay. The current report extends this line of investigation using an updated method that utilizes immunomagnetic separation prior to flow cytometric scoring. **Methods:** Groups of male Sprague-Dawley rats were given 3 daily doses of ENU at 0, 10, 20, or 40 mg/kg. Bloods collected on -d 1, 14, and 30 were evaluated for *Pig-a* mutantfrequencies in total peripheral blood erythrocytes and reticulocytes (RETs); d 4 samples were scored for micronucleated reticulocyte frequencies (%MN-RET). **Results:** While ENU-induced *Pig-a* mutant frequencies were consistent with previously reported results, analysis ratesand number of cells evaluated were dramatically increased. ENU also induced significant increases in %MN-RET. **Conclusion:** The results indicate that the new *Pig-a* scoring methodology is reliable and transferable, and support the concept that *Pig-a* mutation and MN analyses can be readily combined into one study.

Keywords: *Pig-a* mutation; immunomagnetic separation; micronuclei
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S8.76

(-)-Epigallocatechin gallate inhibits Angiotensin II-induced C-reactive protein production in hepatocytes

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Aim: Some researches have shown that (–)-epigallocatechin gallate (EGCG) has an anti-inflammatory effect. The aim of this study was to investigate the inhibitory effect of EGCG on Angiotensin II (Ang II)-induced C-reactive protein (CRP) in human liver cells. **Methods:** Ang II-induced CRP expression in liver cells (L-02) was determined using RT-PCR and Western blot. The cells were pretreated with EGCG at 1, 10, and 100 μmol/L for 1 h before stimulated with Ang II (10^{-6} mol/L) for 24 h. To examine if Ang II is capable to induce CRP accumulation in liver, a model of rat receiving subchronic administration of Ang II was used. After 7 d of Ang II infusion, rats were killed and liver and blood samples were harvested. CRP in plasma and liver was measured by enzyme-linked immunosorbent assay and immunohistochemical staining, respectively. **Results:** *In vitro* experiments showed that Ang II at 10^{-9} mol/L to 10^{-6} mol/L caused an apparent time- and concentration-dependent increase of CRP production in liver cells, and EGCG was able to inhibit this effect. Meanwhile, Ang II induced a significant increase of CRP in plasma and a higher expression of CRP in liver *in vivo*. However, EGCG markedly reduced CRP level in plasma and CRP expression in liver in comparison with control. **Conclusion:** EGCG may inhibit Ang II-stimulated CRP generation in liver.

Keywords: (–)-epigallocatechin gallate; C-reactive protein; angiotensin II; liver cell
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S8.77

Correlation effect change of internal environmental in Estrogen-induced SD rats prostatitis

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Aim: To explore the internal environment correlation estradiol-induced SD male rats with chronic prostatitis. **Methods:** 3% pentobarbital anesthesias, castrated surgery under the sterile conditions; animal recovery after five days, 30 male rats were randomly divided to 3 groups, each one contained 10 ones. From d 6, subcutaneously injected with 0.25 mg/kg estradiol and 1.25 mg/kg estradiol,

respectively, the solvent control group injected with olive oil, once a day, for 30 consecutive days. **Results:** WBC, RBC, HGB and HCT were lower in the estrogen groups than that in the control (P<0.01); PAP was decreased obviously (P<0.01); the testosterone were significantly increased in high-dose group (P<0.01), CRP (C-reactive protein) in the estrogen groups also showed an increasing trend, but no statistically difference; There was no significant difference in the surface of prostate tissue among with groups. Histopathological analyses indicated that there was inflammatory infiltrates in the estrogen groups prostate stromal and brown particles precipitate in spleen are visible, the rest of the organ is no significant change. Organ and organ coefficient in the thymus and spleen were significantly reduced. **Conclusion:** According to the experimental results, chronic non-bacterial prostatitis induced by estrogen can lead to a series of changes of the internal environment within the hematology, serum biochemistry, hormone levels, organs and organ coefficient.

Keywords: estrogen; prostatitis; rat

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S8.78

Targeting inflammatory diseases by macrophages -mediated drug delivery

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Aim: Macrophages are particularly attractive targets for inflammatory diseases because they promote pathogenic inflammatory responses in diseases such as rheumatoid arthritis and atherosclerosis. Yeast cells have been found able to target macrophages via β-1,3-*D*-glucan. In this study, we investigated the oral targeting capability of yeast shells in arthritis rats. **Methods:** Yeast shells (YS) were prepared from yeast cells by acid, alkali, and organic solvent treatment to remove their cellular contents. Indometacin (IND) was microencapsulated into YS by *in situ* self-assembly of polyethyleneimine (PEI) and IND, while quantum dots (QDs) were loaded by spontaneously deposition due to electrostatic interaction. *In vitro* release was performed in media simulating gastrointestinal conditions. The pharmacokinetic and pharmacodynamic studies were conducted at IND dose of 5 mg/kg in adult SD rats. The systematic distribution of YS was studied *in vivo* imaging in nude mice after QDs-YS were orally administered. **Results:** The IND YS and QDs YS were successfully prepared as demonstrated by measurements of zeta-potential, transmission electron microscopy, and fluorescence microscopy. IND loading capacity was high up to 30%, with an entrapment efficiency of 70%. Pharmacokinetic study showed that IND YS was rapidly released at pH 7.4, while almost no release occurred at pH 1.2. The bioavailability of IND in the case of the yeast system was highly improved when compared with raw IND. The anti-inflammatory efficacy was also highly improved after microencapsulation of IND in YS. **Conclusion:** The YS can highly load the IND and successfully target the inflammatory sites by phagocytosis of macrophages, which in turn significantly improved the IND bioavailability and anti-inflammatory efficacy.

Keywords: yeast cell; oral targeting; anti-inflammation; indomethacin; drug delivery

S8.79

Effect of matrine on Nogo-A/NgR of experimental autoimmune encephalomyetic rats

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Aim: We observed the effect of matrine on the expression of Nogo-A and NgR in experimental autoimmune encephalomyetic (EAE) rats and investigated the possible mechanism of matrine for the prevention and treatment of EAE rats. **Methods:** The EAE models were induced by immuned spinal cord extracts of guinea pig with complete Freunds' adjuvant. Then we divided 50 Wistar rats into five groups randomly: normal group, model group, two matrine-treated groups (150 and 200 mg/kg), dexamethasone-treated group. Rats of MAT-treated groups and DEX-treated group were injected intraperitoneally with MAT and DEX daily for 16 d, respectively, whereas rats of normal group and model group were injected intraperitoneally with normal saline. We also observed body weights and clinical signs daily. Histopathological evaluation of spinal cord was analyzed by hematoxylin-eosin (HE) and chromotrope 2R-brilliant green (C-2R-BG). Nogo-A and NgR in spinal cord were determined using immunohistochemistry and RT-PCR, respectively. **Results:** Matrine treatment significantly putted off the morbidity, decreased neurological scores and weight loss after EAE. MAT-treated rats showed a significant decrease in CNS infiltration of inflammatory cells and demyelination. Furthermore, the expression of Nogo-A and NgR in the spinal cord showed a marked reduction after MAT treatment, particularly in rats treated with higher doses of MAT. **Conclusion:** Matrine might be a new and safe therapy for EAE, as well as the mechanism is related to reduce the expression of Nogo-A and NgR.

Keywords: matrine; experimental autoimmune encephalomyelitis; Nogo-A; NgR

S8.80

The effects of Qianlieping capsule on acute and chronic prostatitis and hemorrheology

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Aim: The present study was to evaluate the effects of Qianlieping capsule on acute and chronic prostatitis and hemorrheology. **Methods:** The acute prostatitis induced by carrageenan and chronic prostatitis induced by heamorrhoidlysant were established for evaluating the anti-inflammatory effects of Qianlieping capsule. The rat model of blood stasis was established for evaluating the effects of Qianlieping capsule on hemorrheology. **Results:** Qianlieping capsule (1.2 and 0.6 g/kg) significantly reduced the rat prostate index in model of carrageenan-induced prostatitis, the pathological changes in prostate tissue such as degeneration or necrosis in gastric epithelium, glands dissolved, inflammatory cell infiltration, increase in the protein secretion and adhesion degree of surrounding tissue. Qianlieping capsule (1.2 g/kg) significantly reduced the rat prostate index in model of xiaozhiling-induced chronic prostatitis. Qianlieping capsule significantly reduced the quantity of chronic inflammatory cells in the prostate tissues and the retention of protein secretion in the glandular cavity. Most of the prostate tissue structure was basically normal (P<0.01). **Conclusion:** Qianlieping capsule can inhibit the acute and chronic prostatitis and improve the hemorrheology in the model of blood stasis.

Keywords: Qianlieping capsule; acute prostatitis; chronic prostatitis; hemorr-heology

S8.81

Hypoglycemic and vasculoprotective effects of tea extract

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Vascular complications are major causes of disability and death in patients with diabetes mellitus. Epidemiological studies indicate that tea consumption may reduce the risk of cardiovascular disease. To investigate the preventive effects of tea on hyperglycemia and vascular complications of diabetes, we report the extraction and composition as well as the hypoglycemic and vasculoprotective effects of black tea extract (BTE), green tea extract (GTE), and dark tea extract (DTE). High Performance Liquid Chromatography (HPLC) and the colorimetric methods were conducted to analysis of tea catechins, caffeine, polyphenols, amino acids and polysaccharides of BTE, GTE and DTE. The inhibitory effects of α-glucosidase, aldose reductase (AR), advanced glycation end-products (AGEs) and glucose uptake promotion effect in BTE, GTE, and DTE were explored *in vitro*. Contents of six major catechin forms and tatol catechin as well as polyphenols are higher in GTE and DTE than BTE. BTE, GTE, and DTE showed the inhibitory effects of α-glucosidase, AR, and AGEs, but only DTE exhibited the glucose uptake promotion effect in HepG2 cells. The results suggest that regular consumption of tea can help prevent the progression of hyperglycemia and the vascular complications of diabetes.

Keywords: black tea; green tea; dark tea; hypoglycemic effect; vasculoprotective effect