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## PLENARY LECTURE (PL01-PL08)

### PL01 Discovery of novel DNA alkylating agents with potent antitumor activity: the benzoacronycine series

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Originally isolated from the stem bark of a small Australian Rutaceous tree (*Acronychia baueri=Sarcomelicope simplicifolia*), acronycine is an antitumor alkaloid with low cytotoxic potency, both *in vitro* and *in vivo*. The isolation of the unstable, highly reactive acronycine epoxide from several New-Caledonian *Sarcomelicope* species led to the hypothesis of bioactivation of acronycine by transformation of the 1,2-double bond into the corresponding oxirane *in vivo*. Consequently, derivatives modified in the pyran ring and having a similar reactivity but an improved stability were synthesized. The optimization process led to the identification of S 23906-1, one of the most potent derivatives in the benzo[b]acronycine series. Gel retardation experiments showed that S 23906-1 formed highly stable, covalent adducts with purified DNA. The use of an oligonucleotide duplex containing inosine instead of guanosine identified the guanine-N2 group in the minor groove of DNA as the reactive entity. The inactive diol derivative failed to bind DNA, indicating that the C1-C2 functionality was the DNA reactive moiety. The tight correlation between alkylating properties and cytotoxicity observed in the whole series strongly suggests that DNA alkylation is a key step in the molecular mechanism of action of these derivatives. When tested on a panel of tumor cell lines, S 23906-1 was 50 fold more potent than acronycine in inhibiting cellular proliferation (average $IC_{50}$ 0.4 μmol/L versus 21 μmol/L). Tumor cells exposed to low concentrations of S 23906-1 (< μmol/L) were arrested in the G2+M phases of the cell cycle and underwent apoptosis. Among the four murine transplantable tumors used *in vivo*, S 23906-1 was markedly active only against the C38 colon adenocarcinoma, inducing tumor regression at the optimal dose (6.25 mg/kg). When evaluated in orthotopic models of human solid tumors, S 23906-1, administered intravenously or orally, demonstrated a marked antitumor activity, equal or superior to that of drugs currently used in the clinic. S 23906-1 increased the survival of animals bearing A549 and NCI-H460 lung tumors, being more active than vinorelbine, and induced complete tumor regression of H69 small-cell lung carcinoma lasting at least 250 days. An important survival benefit was also observed against two ovarian tumors, against which S 23906-1 was as active as paclitaxel, inducing 80 % long-term survivors in the NIH:OVCAR-3 model. Acronycine was either marginally active or inactive in these models.

S 23906-1, which exhibits a novel spectrum of antitumor activity compared with existing anticancer drugs, is currently in phase 1 clinical trials.

### PL02 Nootropic effects and mechanism of ginsenoside Rg1

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Ginsenoside Rg1 is a main active principle of ginseng which shares many activities of ginseng. In present paper we will take overview of the proven memory-enhancing effect of Rg1 and discuss all its possible mechanics in detail.

With passive avoidance step down and step-through tests and Morris water maze, Rg1 showed improving effects in impairment of memory induced by anisodine, cyclohoximide, alcohol, $NaNO_2$, β-amyloid and chronic stress, natural senescence and cerebral ischemia-reperfusion.

The nootropic mechanism of ginsenoside Rg1 is as follows:

Firstly, Rg1 accelerated acetylcholine (ACh) biosynthesis and up-regulation of M-cholinergic receptors. This finding that Rg1 simultaneously increases in M-receptors and ACh level could not be explained by existing theory. Obviously, a new theory is needed to be established for explanation of the effects of ginseng on cholinergic system.

Secondly, Rg1 increased synaptic plasticity. In anesthetized and freely moving rats, Rg1 enhanced basic synaptic transmission and magnitude of LTP induced by high frequency stimulation. The further study showed that Rg1 increased mossy fiber sprouting in adult rats and increased brain weight, thickness of cerebral cortex and synapses density in hippocampus of weaning mice, indicating that Rg1 increased synaptic plasticity in both efficacy and structure. This is morphological basis to understand the nootropic mechanism of Rg1.

Thirdly, Rg1 enhanced BDNF, NT3 and Bcl-2 expression leading to increase of neural activity and inhibition of apoptosis and necrosis.

Finally, Rg1 promoted proliferation of hippocampal progenitor cells *in vitro* and *in vivo* tests as well as normal adult rodents and cerebral ischemia in gerbils. Rg1 can also differentiate progenitor to neurons, mainly form granule cells in

hippocampus.

In conclusion, Rg1 showed memory-enhancing effects in more than 10 models of impairments of memory, improved all stages of memories (acquisition, consolidation, and retrieval of memory). Its nootropic mechanism involved regulation of neurotransmitters, receptors neurotrophic factors and gene, especially, Rg1 as a small molecular drug can increase hippocampal neurogenesis is the first report in the world, suggesting that Rg1 can be used for treatment of AD, stroke and various amnesia.

**PL03 The obligatory role of endothelial $SK_{Ca}$ and $IK_{Ca}$ channels in EDHF-mediated responses**

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The vascular endothelium controls vessel tone by releasing nitric oxide and prostacyclin as well as by a third pathway that involves the hyperpolarization of the vascular smooth muscle. Changes in vascular smooth muscle membrane potential are an important regulating mechanism for smooth muscle contractility. The hyperpolarization of the smooth muscle cell can contribute to the mechanisms involved in the relaxation elicited by endothelial-derived NO and prostacyclin and is the predominant mechanism involved in the relaxations elicited by EDHF. The mechanism of endothelium-dependent hyperpolarizations, once attributed to an elusive endothelium-derived hyperpolarizing factor, is better understood. In most of the blood vessels, the first step involves an increase in the intracellular calcium concentration of the endothelial cells followed by the opening of two population of potassium channels, the calcium-activated potassium channels of small and intermediate conductance ($SK_{Ca}$ and $IK_{Ca}$) which leads to the hyperpolarization of the endothelial cells. Then, the endothelium-dependent hyperpolarization of the smooth muscle cells can be evoked by direct electrical coupling through myo-endothelial junctions and/or the accumulation of potassium ions in the intercellular space between the endothelial and smooth muscle cells. Potassium ions evoke the hyperpolarization of the vascular smooth muscle cell by activating the smooth muscle inward rectifying potassium channel (Kir) and/or the $Na^+/K^+$-ATPase. Additionally, in response to bradykinin, the endothelial cells of some blood vessels, such as the porcine coronary artery, release arachidonic acid metabolites derived from the endothelial cytochrome P450 monooxygenase, epoxyeico-satrienoic acids. Epoxyeicosatrienoic acids hyperpolarizes the smooth muscle cells by activating large conductance calcium-activated potassium channels ($BK_{Ca}$) expressed on their plasma membrane. These independent mechanisms are not necessarily exclusive and can occur simultaneously and even in synergy, and are likely to play a role in the local control of blood flow especially in the coronary circulation and in the periphery.

**PL04 Pharmacology and modernization of traditional Chinese medicine (TCM)**

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Traditional TCM does not need modernization. What needs modernization are the commercial TCM products which have become the prevailing form of TCM. Traditional TCM treats patients individually and different practitioners prescribe their individual formulations. There is no way to modernize such a fine-tuned traditional approach. Commercial TCM is a marketable product for the treatment of the mass population. These are two different forms of medical practice. This form of TCM has many problems and needs modernization. What is TCM modernization? There are several issues. How to choose the formulation for product development and how to prove its mass effectiveness are two problems. The most serious issue with industrialized TCM is how to ensure batch to batch consistency or how to standardize. TCM formulations contain many substances and there is a scientific rationale for it. In view of its complexity, is it possible to standardize TCM chemically? Until today there is no information as to how many chemicals and what proportions are needed to produce the biological effect. It is therefore impossible to use chemical determinations or even fingerprinting as methods to standardize TCM products. However, it is possible to employ pharmacological assays to demonstrate consistent effectiveness of products. Such assays should be easy, reliable, repeatable, related to product therapeutic use and inexpensive. Such standardization has been used in some of our studies and proved feasible. Pharmacological standardization may not be perfect but it is probably the most logical and meaningful way of standardization.

**PL05 Neurotrophins and their receptors in inflammation**

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The neurotrophin family has recently been involved in inflammatory and remodelling processes occurring in chronic inflammatory diseases, in particular in asthma. Nerve growth factor (NGF) is a high molecular weight peptide that belongs to the neurotrophin family. It is synthesized by various structural and inflammatory cells and activates two types of receptors, the TrkA (tropomyosin-receptor kinase A) receptor and the $p75^{NTR}$ receptor, in the death receptor family. NGF was first studied for its essential role in neuronal growth and survival. Recent reports indicate that it may also help mediate inflammation, especially in the airways. Several studies in animals have reported that NGF may induce bronchial hyperresponsiveness, an important feature of asthma, by increasing sensory innervation. It may also

induce migration and activation of inflammatory cells, which infiltrate the bronchial mucosa, and of structural cells, including epithelial, smooth muscle cells and pulmonary fibroblasts. Increased NGF expression and release is observed in asthma patients after allergenic bronchial provocation. Taken together, the data from the literature suggest that NGF may play a role in inflammation, bronchial hyperresponsiveness and airway remodelling in asthma and may help us to understand the neuro-immune cross-talk involved in chronic inflammatory airway disease.

**PL06 Over-phosphorylation of FKBP12.6, phospholamban, relating to exacerbation of cardiac arrhythmias and failure**

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**KEY WORDS** calcium; arrhythmias; FKBP12.6; phospholamban; heart

**AIM:** Cardiac arrhythmias occur severely in diseased and failing hearts and remain an important cause of mortality in cardiovascular disorders. It was intended to explore mechanisms of abnormal ion channels underlying cardiac arrhythmias and failure and in responses to drug interventions. **METHODS:** Chronic infarction plus isoproterenol (ISO) medication or *L*-thyroxin (THY) repetitive medication promote cardiac remodeling and exaggerated arrhythmias via over-phosphorylation. Cardiac arrhythmias were assessed on ischemia/reperfusion by a score system and hemodynamics measured and the mRNA of RyR2, FKBP12.6, SERCA2a, PLB (phospholamban) and PKA were assayed. **RESULTS:** An exacerbated cardiac arrhythmias/hemodynamics were measured in infarcted hearts with medication of ISO or THY administration of 10 d. A down-regulated mRNA of RyR2 in ISO group but elevated in the THY groups were seen. The mRNA of FKBP12.6 , SERCA2a, PLB, and PKA was altered in the two models. Propranolol was effective to regress abnormal abundance of mRNA of the $Ca^{2+}$ regulating system, together with a reduction in the hypertrophy and arrhythmias. It was also effective with CPU 86017 a multi-channel blocker and a novel endothelin receptor antagonist. Dajisentan. **CONCLU-SION:** An over phosphorylation by ISO and THY of the RyR2, FKBP12.6, SERCA2a, and PLB by PKA resulted in the exaggerated cardiac arrhythmias and hemodynamics. It is suggested that de-phosphorylation can be achieved by multiple approaches.

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**PL07 Preventive neuroprotection: from experimental data to therapeutic**

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**KEY WORDS** cerebral ischemia; ischemic tolerance; neuroprotection; prevention; neurodegenerative diseases

The concept of preventive neuroprotection is based on experimental concept of brain ischemic tolerance in which a cerebral resistance against ischemia consequences is induced prior to its occurrence. Pharmacological agents mimicking the biological mechanisms observed in brain ischemic tolerance might increase the resistance of patients with high stroke risk to the deleterious effects of brain ischemia. Activation of cytoprotective proteins or regulation of deleterious molecular pathway could constitute the main pharmacological targets to induce preventive neuroprotection. Several pharmacological agents such as statins or fibrates have been demonstrated experimentally, and more recently clinically, to induce a preventive neuroprotection related to their pleïotropic anti-inflammatory and antioxidant properties. In future, the prevention treatment of stroke occurrence may be completed by preventive neuroprotective treatment. Moreover, some drugs could have potentially both preventive and neuroprotective properties, which are likely linked. Beyond pharmacological effects in stroke, the strategy of preventive neuroprotection is also clinically relevant in neurodegenerative diseases because of the occurrence of a progressive neuronal death. Experimental and clinical data support hypothesis that the same strategies of preventive neuroprotection useful in stroke could also be effective in neurodegenerative diseases such as Parkinson disease or Alzheimer disease.

**PL08 A novel antiarrhythmic target—M3-R/IKM3**

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The total efficient rate of antiarrhythmic agents is only 30 % to 60 %, and the compounding problem is the lack of an effective therapy for some serious arrhythmias. Muscarinic receptors have been cloned and subdivided into the five subtypes $M_1$, $M_2$, $M_3$, $M_4$ and $M_5$. And $M_2$ receptors have long been believed to be the only functional subtype of muscarinic acetylcholine receptor (mAChR) in the heart, although recent studies have provided evidence for the presence of other subtypes. The present study presents evidence for the presence of the $M_3$ receptor subtype in the heart, biophysics and physiological functions of the $M_3$ receptors, $M_3$ receptor pathological significance in ischemic arrhythmias and the antiarrhymic mechanism of $M_3$ receptor agonists by the methods of animal models *in vivo* and *in vitro*, competition binding of [*N*-methyl-$^3$H]-scopolamine methyl chloride, patch clamp, confocal microscopy, molecular biology and so on. The results show: (1)Competition binding of [*N*-methyl-$^3$H]-scopolamine methyl chloride with various mAChR antagonists, such as pirenzepine ($M_1$ antagonists), methoctramine ($M_2$ antagonists), 4-DAMP($M_3$ antagonists), tropicamide ($M_4$ antagonists) , yieldes data consistent with the presence of multiple subtypes ($M_1$/$M_2$/$M_3$) of mAChRs in both human's and

canine's atrial and ventricular tissues. Furthermore, pilocarpine and choline selectively bind with $M_3$ receptors, and the $K_d$ values are 2.2 μmol/L and 0.9 nmol/L, respectively. (2) Expression of mRNAs encoding all five subtypes was detected by reverse transcription-polymerase chain reaction(RT-PCR) in both atrial and ventricular samples. Immunoblotting with subtype-specific antibodies confirmed the presence of $M_1$, $M_2$, $M_3$, and $M_5$, but not $M_4$, proteins in membrane preparations from both atrial and ventricular tissues. $M_3$-R shows characteristic localization to the intercalated discs, whereas other subtypes are more evenly distributed throughout the surface membrane. (3) Patch-clamp recordings in dispersed myocytes from guinea-pig and canine atria reveal that pilocarpine(10 μmol/L) and choline (10 mmol/L), $M_3$-selective antagonists, induce a novel $K^+$ current with delayed rectifying properties. Potential contamination by other currents was minimized by including the following compounds in the bath solution: dofetilide (1 mmol/L, to inhibit $I_{Kr}$), 293B (20 mmol/L, to block $I_{Ks}$), glyburide (10 mmol/L, to prevent ATP-sensitive $K^+$ current) and 4-aminopyridine (4-AP, 200 mmol/L to block transient outward $K^+$ current and the ultra-rapid delayed rectifier $K^+$ current). The current was suppressed by low concentrations of $M_3$-selective antagonists 4DAMP (2 nmol/L) and *p*-F-HHSiD (20 nmol/L). Antagonists towards other subtypes ($M_1$, $M_2$ or $M_4$) all failed to alter the current. Our data indicate that the current is mediated by $M_3$ receptors and named as $IK_{M3}$. The result shows that $M_3$ receptors exist functionally. (4)To investigate the physiological functions of $M_3$ receptors and $IK_{M3}$, we perform standard microelectrode technique to observe the effects of pilocarpine and choline in a dose-dependent manner on the heart sinus rate (HR), action potential duration (APD), and rest membrane potential (RMP) in canine and guinea-pig atria. The results show pilocarpine and choline reversibly slow HR, shorten APD and hyperpolarize RMP dose-dependently. The effects can be inhibited by 4DAMP and *p*-F-HHSiD, $M_3$ selective antagonists, but cannot be inhibited by other mAChRs antagonists. In the rat and rabbit models, choline (10 mg/kg) strongly slows HR, decreases $dp/dt_{max}$ and left ventricular systolic pressure (LVSP), and the effects can be inhibited by 4DAMP, but cannot be inhibited by other mAChRs antagonists. The results indicate that $M_3$ receptor perform negative inotropic effects and negative frequency. (5)To investigate the relationship of $M_3$ receptors and $IK_{M3}$ with ischemic arrhythmias, we observe the effect of $M_3$ receptor agonists and antagonists on ventricular arrhythmias induced by the left coronary occlusion in rats. The results show that choline significantly decreases arrhythmia numbers. $M_3$ selective antagonists 4DAMP rather than other mAChRs antagonists block the effects. Furthermore, to investigate the relationship between $M_3$ receptors and arrhythmias in heart failure, we observe the long-term effects of $M_3$ receptor agonists and antagonists on ventricular arrhythmias induced by left coronary occlusion in hyperlipoproteinemic rats. The results shows choline can significantly improve rats' survival rate. $M_3$ selective antagonists 4DAMP can block the effects, but other mAChRs antagonists cannot. And the effect of choline is markedly different from other antiarrhythmic agents such as lidocaine, verapamil and soporcarpidine. (6)To further investigate the antiarrhythmic mechanism of $M_3$ receptor agonists, we observe the long-term effects of choline on APD, $I_{to}$ and $I_{k1}$ in heart failure rats (induced by the left coronary occlusion in hyperlipoproteinemic rats). The results show choline markedly ameliorates ventricular myocytes electric remodels in heart failure rats. The effects can be inhibited by 4DAMP, and it is strongly different from other antiarrhythmic agents. It indicates that the antiarrhythmic mechamism of $M_3$ receptor agonists might be relevant to myocytes electric remodels. And to investigate the other antiarrhythmic mechanism of $M_3$ receptor agonists, induced by the left coronary occlusion in rats we observe the effects of $M_3$ receptor agonists and antagonists on serum malondialdehyde (MDA), serum superoxide dismutase (SOD), intracellular $Ca^{2+}$, cardiomyocytes' apoptosis and the expression level of Bcl-2 and Fas. The results show choline decreases the level of MDA , increases the level of SOD and decreases intracellular $Ca^{2+}$. The test of TUNEL(terminal deoxynucleotid transferase directed d-UTP nick and end labeling) shows choline improves cardiomyocytes' apoptosis induced by the left coronary occlusion in rats. The immuohistochemistry results show Bcl-2 level increases and Fas level decreases. Those indicate that choline can prevent cardiomycytes' apoptosis. So these results indicate that (1)there is $M_3$ subtypes in cardiomyocytes, which mediate the current of $IK_{M3}$; (2) $M_3$ shows characteristic localization to atria and ventricule in human and canine; (3)$M_3$ receptor agonists can shorten APD, slow HR, increase RMP and perform negative inotropic effects and negative frequency; (4)$M_3$ receptor is correlated to the arrthythmias induced by ischemia and heart failure, and $M_3$ receptor agonists can decrease arrhythmic frequency; (5) The antiarrhythmic mechanism of $M_3$ receptor agonists might be relevant to ameliorate myocytes electric remodels, prevent oxidation and apoptosis, shorten APD and decrease intracellular $Ca^{2+}$. The present study provides a novel antiarrhythmic target, $IK_{M3}$.

## ORAL COUMMUNICATION (O01-O32)

### O01 Comparison of the effects of telmisartan and ramipril on cerebral arteriolar inward remodeling in spontaneously hypertensive rats

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**KEY WORDS** arteriolar eutrophic remodeling; AT1 receptor blocker; ACE inhibitor; cerebral arterioles

**AIM:** The goal of this study was to compare the effects of an angiotensin II receptor ($AT_1$) blocker (ARB), telmisartan, with those of an angiotensin-converting enzyme inhibitor (ACEI), ramipril, on cerebral arteriolar structure and mechanics in spontaneously hypertensive rats (SHR). **METHODS:** Experiments were performed in 6 month-old Wistar Kyoto rats (WKY, *n*=16) and age-matched SHR that were untreated (SHR, *n*=16) or treated for 3 months with telmisartan (SHR-TEL, $1.93 \pm 0.04$ mg·kg$^{-1}$·d$^{-1}$, *n*=21) or ramipril (SHR-RAM, $1.00 \pm 0.02$ mg·kg$^{-1}$·d$^{-1}$, *n*=15). We measured cerebral arteriolar pressures (CAP, mmHg, servo-

null), cerebral arteriolar external diameter (ED, μm), cross-sectional area of the vessel wall (CSA, $\mu m^2$, histology) and stress and strain values during a stepwise hypotension after deactivation of cerebral arterioles (EDTA). **RESULTS:** Treatment of SHR with telmisartan or ramipril normalized cerebral arteriolar mean pressure (42±2 [mean±SEM] and 45±2, respectively, *vs* 45±2 mmHg in WKY and 63±3 mmHg in untreated SHR; $P<0.05$) and CSA (369±11 and 401±14, respectively, *vs* 414±17 $\mu m^2$ in WKY and 575±19 $\mu m^2$ in untreated SHR; $P<0.05$). Treatment with telmisartan or ramipril increased ED (101±4 and 103±4 μm, respectively, *vs* 108±6 μm in WKY and 87±3 μm in untreated SHR). Finally, treatment with telmisartan or ramipril attenuated but did not normalize the rightward shift of the stress-strain curve in SHR suggesting that both treatments modified cerebral arteriolar mechanics. **CONCLUSION:** These findings indicate that treatment with telmisartan is as effective as treatment with ramipril in reversing inward remodeling and thus that $AT_1$ receptors are involved in cerebral arteriolar remodeling during chronic hypertension.

### O02 Proteomics analysis of human umbilical vein endothelial cells (HUVEC) after treatment with low molecular weight heparin

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**KEY WORDS** endothelium; human umbilical vein endothelial cells; low molecular weight heparin; proteomics

**AIM:** The endothelium is involved in the generation and the regulation of multiple physiological and pathological processes of blood vessels. Previously we confirmed low molecular weight heparin (LMWH) could inhibit tumor metastasis by protecting human umbilical vein endothelial cells (HUVEC). To understand the effects of LMWH on the protein expression of HUVEC, we performed a comprehensive proteomics to survey global changes in proteins after LMWH treatment in HUVEC cells. **METHODS:** Exposure of HUVEC cells to 0.01 mmol/L LMWH for 24 h. Two-dimensional electrophoresis (2-DE) with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) and database search were used to identify the difference proteins between LMWH-activating and quiescent HUVEC cells. RESULTS: Up to 43 various proteins have altered their expression levels following the treatment of LMWH. Four proteins were identified with (MALDI-TOF-MS). Two of the affected proteins were determined to be isoforms of cytoskeletal vimentin intermediate filaments. Another two proteins were serine/threonine protein phosphatase PP1-beta catalytic subunit (PP-1B) or Beta-actin and C-type lectin superfamily member 1 precursor (Cartilage-derived C-type lectin). **CONCLUSION:** In this study, we illustrated the protein basis for functions of LMWH on endothelium, and one of them was cytoskeleton protein vimentin.

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### O03 Role of toll-like receptor 2 and toll-like receptor 4 in post-ischemic coronary endothelial dysfunction in mice

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**KEY WORDS** ischemia; endothelium; toll-like receptor; inflammation

**AIM:** A growing body of evidence suggests a role of the toll-like receptors (TLR) in inflammatory processes. In addition to LPS, TLR are activated by many endogenous ligands such as heat shock proteins and oxygen-derived free radicals which are both produced during cardiac ischemia-reperfusion (I/R). Among TLR, TLR-2 and TLR-4 are expressed in endothelial and myocardial cells and appear to regulate neutrophil-endothelial interactions. Since neutrophil adhesion is a critical event in endothelial injury induced by I/R, we assessed whether TLR-2 and TLR-4 were involved in I/R induced coronary endothelial injury. **METHODS:** Wild type (WT) and TLR-2 or TLR-4 knock-out (TLR-2 KO, TLR-4 KO) mice were subjected to sham surgery or 30 min of left anterior descending coronary ligation followed by 60 min of reperfusion. Coronary segments (diameter, 170 to 200 μm) were then removed distal to the site of occlusion and mounted in a wire myograph. In parallel, neutrophil accumulation was assessed by measuring myeloperoxidase (MPO) activity in tissue previously subjected to I/R. **RESULTS:** I/R reduced the distal relaxation to acetylcholine in WT mice (maximal relaxation: sham WT 68+/-5%; I/R WT 34+/-4% $P$=0.006). In contrast, this reduced response to acetylcholine was absent in TLR-4 KO mice (sham KO4 62+/-6%; I/R KO4 65+/-6%) as well as in TLR-2 KO mice, in which I/R paradoxically improved the relaxation (sham KO2 63+/-9%; I/R KO2 81+/-12% $P$=0.001 *vs* I/R WT). The endothelium-independent response to the NO donor sodium nitroprusside was not affected in all groups. Moreover, the increased MPO activity observed after I/R (U/100mg tissue: sham WT 53+/-17; I/R WT 133+/-12 $P$=0.003) was significantly reduced in TLR-4 KO mice (I/R KO4 103+/-9 $P$=0.023 *vs* I/R WT) and absent in TLR-2 KO mice (I/R WT 133+/-12; I/R KO2 51+/-15 $P$=0.004 ) showing a decrease in neutrophil infiltration. **CONCLUSION:** This study is, to the best of our knowledge, the first to report post-ischemic coronary endothelial injury in mice. We also demonstrate for the first time that TLR-2 and TLR-4 signaling contribute importantly to the endothelial dysfunction that occurs following I/R, possibly by reducing neutrophil adhesion and thus neutrophil-mediated endothelial injury.

### O04 Synergism of atenolol and nitrendipine on hemodynamic amelioration and organ protection in hypertensive

**rats**

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**KEY WORDS** atenolol; nitrendipine; hypertension; end organ damage

**AIM:** This study was designed to investigate the possible synergism of atenolol and nitrendipine on blood pressure (BP) and blood pressure variability (BPV) reductions, baroreflex sensitivity (BRS) amelioration, and organ protection in hypertensive rats. **METHODS:**The dose is 20 mg/kg for atenolol, 10 mg/kg for nitrendipine and the combination of these two drugs. In acute study, a single dose was given via a catheter previously inserted into the stomach in spontaneously hypertensive rats (SHR). In subacute study, SHR, DOCA-salt rats, and 2K1C rats were used. They received the same dose by gavage daily for 10 d. BP was measured 24 h after drug administration. In chronic studies, these drugs at the aforementioned dose were mixed into rat chow. SHR were treated for 4 months. BP was then continuously recorded for 24 h. After the determination of BRS, rats were killed for organ-damage evaluation. **RESULTS:** In acute study, it was found that the combination of atenolol and nitrendipine had an obviously greater and longer BP reduction than treatment with each of these two drugs separately. In subacute study, an effective decrease in BP 24 h after administration was found only in the rats treated with the combination. In chronic studies, it was found that the combination possessed the obvious synergism on BP and BPV reduction, BRS amelioration and organ protection in SHR. Multiple-regression analysis showed that decrease in left ventricular hypertrophy was most significantly related to the decrease in systolic BPV and BP, decrease in aortic hypertrophy was most significantly related to the increase in BRS and decrease in systolic BPV, and amelioration in renal lesion was most significantly associated with the restoration of BRS. **CONCLU-SION:** Treatment with combination of atenolol and nitrendipine exhibited a rapid and persistent antihypertensive effect and possessed an obvious synergism on BP and BPV reduction, BRS restoration and organ protection in hypertensive rats. The decrease in BPV and the restoration of BRS may importantly contribute to organ protection in SHR with chronic treatment.

## O05 Effects of the NADPH oxidase inhibitor apocynin on the left ventricular dysfunction induced by cocaine administration

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**KEY WORDS** cardiac dysfunction; cocaine; NADPH oxidase; apocynin

**AIM:** In a previous study, we have shown the role of alpha1-adrenoceptor in the left ventricular (LV) dysfunction after chronic cocaine administration via the induction of NADPH oxidase. In this study we used the NADPH oxidase inhibitor apocynin, to further investigate the real involvement of this prooxidant system in this LV dysfunction. **METHODS:** Wistar rats were treated with saline solution (control) or cocaine (coc 2x7,5 $mg \cdot kg^{-1} \cdot d^{-1}$ ip) with or without apocynin (apo 50 $mg \cdot kg^{-1} \cdot d^{-1}$ in food). After 7 d, we evaluated the LV function by echocardiography (fractional shortening FS, cardiac index CI), hemodynamics (mean arterial pressure MAP, heart rate HR, total peripheral resistance TPR), LV hypertrophy (LV weight/Body weight) and LV NADPH oxidase activity by chemiluminescence. **RESULTS:** The NADPH oxidase inhibition prevented the cocaine-induced FS alteration and CI decrease. The TPR were slightly reduced without reaching statistical significance. There were none difference between the groups for the MAP and the HR. Apocynin treatment decreases the LV hypertrophy and the NADPH oxidase activity. **CONCULSION:** Our results showed that the NADPH oxidase participated in the LV dysfunction induced after 7 d of cocaine administration, precisely in cardiac contractility and hypertrophy.

## O06 The effect of acetylcholine on the ultrastructure of torpedo acetylcholinesterases

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**KEY WORDS** acetylcholinesterase; atomic force microscopy; molecular dynamics

**AIM:** To observe the effects of acetylcholine (ACh), the natural substrate of acetylcholinesterases (AChE), on the conformational state of the active gorge of AChE. **METHODS:** Atomic force microscopy (AFM). **RESULTS:** The surface of the enzyme particles was smooth. The boundary of them was clear and the shapes were ellipsoid. However, the morphology of the enzyme after reacted with ACh became almost utterly different. The most obvious change was a hole or a gorge emerged in the protein, which had a width of 6.5±0.2 nm and deepened into half of the enzyme molecules. The morphology of the active gorge and the enzyme molecules was quite similar with those found by X-ray in previous literature. The effects of ACh on the opening of the enzyme gorge were related to the concentration of ACh. **CONCLUSION:** The active gorge of AChE were closed at non-active state when there were no ACh and were opened at the active state when there were ACh which reacted with it; the substrate ACh had active role on the structure of the enzyme AChE, which facilitates itself to enter the active center of AChE through the active gorge, opening a new window for the AChE molecular function.

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**O07 Chiglitazar, a novel PPARalpha/gamma dual agonists with beneficial effects on insulin resistance and lipid metabolism in MSG rats**

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**KEY WORDS** insulin resistance; insulin sensitizer; MSG rats

**AIM:** Peroxisome proliferator-activated receptor (PPAR) alpha and PPAR gamma agonists lower lipid accumulation by different mechanisms. We investigated whether benefits could be achieved on insulin sensitivity and lipid metabolism by the dual PPARalpha/gamma agonist chiglitazar in MSG rats. **METHODS:** Chiglitazar was orally administered in 5, 10, 20 $mg \cdot kg^{-1} \cdot d^{-1}$ dosages in MSG rats for 40 d. The drug therapeutic effect was evaluated by glucose tolerance tests, insulin tolerance tests, and hyperinsulinemic-euglycemic clamps technique. The level of blood insulin, free fatty acid, cholesterol and triglycerides were also measured. With RT-PCR technique the level of gene expression was calculated, which were involved in the oxidation of fatty acid. **RESULTS:** Chiglitazar ameliorated insulin resistance and lowered plasma free fatty acid, cholesterol and triglycerides. Moreover chiglitazar increased PPAR-responsive genes expression, such as CPT-1a, ACOX, bifunctional enzyme and CYP4A10 in the liver of MSG rats. **CONCLUSION:** Chiglitazar substantially improves insulin sensitivity and potently improves overall lipid homeostasis. Lipid lowering in MSG rats correlated with the magnitude of hepatic gene expression changes. These data suggest that PPARalpha/gamma agonists, such as chiglitazar with these properties may provide favorable means for treatment of type 2 diabetes to restore insulin sensitivity and correct diabetic dyslipidemia.

**O08 Serotonin-induced proliferation of pulmonary artery smooth muscle cells is serotonin transporter and ERK pathway dependent**

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**KEY WORDS** 5-hydroxytryptamine; 5-hydroxytryptamine transporter; pulmonary artery smooth muscle cells; extracellular signal-regulated kinases

**AIM:** To investigate the effect of serotonin transporter (5-HTT) inhibitor fluoxetine and antisense oligodeoxynucleotide (ODN) to extracelluar signal-regulated kinases (ERKs) on pulmonary arterial smooth muscle cells (PASMCs) proliferation induced by 5-HT. **METHODS:** Liposomal transfection was used to introduce ODNs to ERK1/2 into cultured rat PASMCs and the transfection efficiency was measured by observing the uptake of the fluorescein isothiocyanate (FITC)-labeled antisense ODN in PASMCs. The effects of 5-HTT selectively inhibitor fluoxetine and ODNs on the proliferation of PASMCs were evaluated by cells number counting and cell cycle analysis, and measured by microculture tetrazolium (MTT) assay and flow cytometry (FCM), respectively. **RESULTS:** Fluoxetine concentration-dependently inhibited the proliferation of PASMCs induced by 5-HT *in vitro*. Meanwhile, liposomes mediated the transfection of ODNs into PASMCs with high efficiency, and antisense ODN to ERK1/2 inhibited 5-HT-induced proliferation of PASMCs. **CONCLUSION:** 5-HTT mediates the mitogenic effect of 5-HT on PASMCs and the intracellular signal transduction of 5-HT in PASMCs is dependent on ERKs signal pathway.

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**O09 Experimental study of the hexosamine biosynthesis pathway and insulin resistance**

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**KEY WORDS** hexosamine biosynthesis pathway (HBP); insulin resistance (IR); GDH method; GFAT inhibitors; insulin sensitizer

**AIM:** To set up the GDH method and the insulin resistance cell model for screening the glutamine:fructose-6-phosphate amidotransferase (GFAT) inhibitors. **METHODS:** Glutamine can be converted to glutamate by GFAT, then, affected with APAD to produce APADH by GDH. APADH showed a peak at the 360 nm wavelength. Each factor of the active system was regulated. After the insulin administration in HIRc cells, the GFAT activity and the insulin-induced glucose uptake were measured. The samples were screened with the GDH method and HIRc cell stimulated by insulin. **RESULTS:** The standard curve showed linear ($r$=0.983) when glutamate, the standard, was less than 62.5 nmol. The suitable value of pH was 8.5. The adequate concentrations of the reactants, glutamine, F-6-P, APAD, and GDH were 6 mmol/L, 0.8 mmol/L, 0.3 mmol/L, and 6 U, respectively. The high concentration of APAD maybe interfere the APADH measurement. Administration with the insulin, the GFAT activity showed a valley at 12 h, and 2 peaks at 2 h and 36 h, respectively. In this case, the insulin-induced glucose up-take decreased about 20 %. Azaserine was used as the positive drug for screening GFAT inhibitors. **CONCLUSION:** the GDH method is an enzyme method for GFAT assay in the cultured cells. Insulin induced insulin resistance in HIRc cells that can be used for the GFAT inhibitors screening.

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**O10 Chronic amiodarone remodels expression of ion channel transcripts in the mouse heart**

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**KEY WORDS** pharmacogenomics; amiodarone; cardiac ion channel; microarrays

**AIM:** The basis for the unique effectiveness of chronic amiodarone on cardiac arrhythmias is incompletely understood. The present study investigated the pharmacogenomics profile of amiodarone on genes encoding ion channel subunits. **METHODS AND RESULTS:** Adult male mice were treated for 6 weeks with vehicle or oral amiodarone at 30, 90, or 180 $mg \cdot kg^{-1} \cdot d^{-1}$. Plasma and myocardial levels of amiodarone and *n*-desethyl-amiodarone increased dose-dependently, reaching therapeutic ranges in human. Plasma T3 levels decreased whereas rT3 levels increased in amiodarone-treated animals. In ECG recordings, amiodarone dose-dependently prolonged RR, PR, QRS, and QTc intervals. Specific microarrays containing probes for the complete ion channel repertoire and real-time RT-PCR experiments demonstrated that amiodarone induced a dose-dependent remodeling in multiple ion channel subunits. Genes encoding for $Na^+$ (SCN4A, SCN5A, SCN1B), connexin (GJA1), $Ca^{2+}$ (CaCNA1C) and $K^+$ channels (KCNA5, KCNB1, KCND2) were down-regulated. In patch-clamp experiments, lower expression of $K^+$ and $Na^+$ channel genes was associated with decreased Ito,f ,IK,slow and INa currents. Inversely, other $K^+$ channel alpha- and beta-subunits such as KCNA4, KCNK1, KCNAB1 and KCNE3 were up-regulated. **CONCLUSION:** Chronic amiodarone induces a dose-dependent remodeling of ion channel expression that is consistent with its cardiac electrophysiologic effects (slowed conduction and prolonged repolarization). This profile cannot be attributed solely to the amiodorane-induced cardiac hypothyroidism syndrome. Thus, in addition to the direct effect of the drug on membrane proteins, part of the therapeutic action of chronic amiodarone is likely related to its effects on ion channel transcripts.
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**O11 Association between lipid-lowering drugs (statins and fibrates) and venous thromboembolism: a case-control study**

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**KEY WORDS** statins; fibrates; venous thromboembolism; case-control study

**AIM:** Previous studies of selected patients have suggested a reduction in the risk of venous thromboembolism (VTE) with the use of statins, and no effect of fibrates. The aim of this study was to evaluate the relationship between statin and fibrate use and the risk of VTE. **METHODS:** The present report used data from an ongoing hospital-based case-control study designed to investigate genetic and environmental risk factors of VTE. This study started in May 2000 in a single centre in Brest, France, and included patients consecutively hospitalized for a documented venous thromboembolic event. Controls were matched on age, sex and the main risk factors of VTE (cancer, surgery, pregnancy, *etc*. **RESULTS:** In June 2004, 857 cases and 736 controls were included. The mean age of patients was 67.7 year. Controls had more often previous vascular events (coronary heart disease, stroke or arteriopathy of the lower limbs) than cases but the difference was not significant. Statin use was associated with a significant decreased risk of VTE (OR = 0.58; 95% CI, 0.41-0.82), whereas fibrate use was associated with a significant increased risk of VTE (OR=1.60; 95% CI, 1.09-2.34). After adjustment on the main confounding factors including aspirin use and cardiovascular disease, these associations remained significant. Among pleiotropic effects of statins, some antithrombotic mechanisms could be proposed to explain their possible protective effect. Concerning the possible negative effect of fibrates, some authors found that the most prescribed fibrates, but not statins, caused hyperhomocysteinemia. In our study, analysis of homocysteinemia are ongoing. **CONCLUSION:** In this case-control study of hospitalized patients, statin use was associated with a significant decreased risk of VTE, whereas fibrate use was associated with a significant increased risk. Homocysteinemia may be involved in the difference between the effects of these two categories of lipid-lowering drugs on VTE. Because our study was observational, the protective effect of statins as regards the risk of VTE remains questionable and further prospective studies are needed.

**O12 Upregulation of voltage-activated potassium channels in hippocampus of $A\beta_{25-35}$-treated rats**

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**KEY WORDS** β-amyloid peptide; hippocampus; pyramidal neuron; potassium channels; patch-clamp techniques

**AIM:** Potassium channels dysfunction has been indicated in Alzheimer disease. In the present study, the mRNA and protein

expression alterations and the functional changes of voltage- activated potassium channels were studied in rat hippocampus after a single intracerebro- ventricular injection of β-amyloid peptide 25-35 ($A\beta_{25-35}$). **METHODS:** The expressions of mRNA and protein were assessed using RT-PCR and Western blot, respectively. The corresponding functional delayed rectifier $K^+$ current ($I_{K(DR)}$) and transient outward $K^+$ current ($I_{K(A)}$) were investigated in acutely dissociated rat hippocampal pyramidal neurons using the whole-cell patch-clamp technique. **RESULTS:** After the injection of Ab, the spatial memory of rats was significantly impaired in the Morris water maze. Expressions of five main Kv channel subunits (Kv1.5, Kv2.1, Kv1.4, Kv4.2 and Kv4.3) were studied. The mRNA levels of Kv2.1 and Kv1.4 were increased by 72 % ($P < 0.01$) and 67 % ($P < 0.01$) in hippocampus, respectively. No other significant mRNA expression changes were found in Aβ-treated rats. The protein expression of Kv2.1, and Kv1.4 was detected using Western blot. Kv2.1 and Kv1.4 in protein levels were increased by 48% ($P<0.01$) and 50 % ($P < 0.01$), respectively, in hippocampus of Aβ-treated rats. When the cells were depolarized from -50 to +40 mV, the current amplitude and current density of $I_{K(DR)}$ were increased by 68.9 % ($P<0.01$) and 66.1 % ($P<0.01$) in Aβ-treated rats, respectively. The current amplitude and density of $I_{K(A)}$ were increased by 43.1 % ($P<0.05$) and 66.6 % ($P<0.01$), respectively. The steady-state activation curve of $I_{K(DR)}$ was shifted towards more negative potential by -8 mV ($P<0.01$) after Aβ treatment. The kinetics of $I_{K(A)}$ showed that the time constant of recovery from inactivation was markedly increased ($P<0.01$), while no significant changes of the steady-state activation and inactivation properties for $I_{K(A)}$ were found after Aβ injection. **CONCLUSION**: Upregulation of voltage-activated K channels in molecular and functional levels in Aβ-induced cognitive impairment might play an important role in the pathogenesis of Alzheimer's disease.

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### O13 Long-term treatment with an aldosterone synthase inhibitor improves cardiac function and myocardial structure in rats with chronic heart failure

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**KEY WORDS** heart failure; aldosterone synthase inhibition

**AIM:** Aldosterone receptor antagonists reduce total and cardiovascular mortality in patients with chronic heart failure (CHF) under active angiotensin converting enzyme inhibition treatment, illustrating the deleterious involvement of aldosterone in the progression of CHF. The reduction of aldosterone synthesis through inhibition of aldosterone synthase is an alternative way to prevent the effects of aldosterone. However, whether chronic aldosterone synthase inhibition exerts beneficial effects in CHF remains unknown. We assessed, in rats with CHF, the long term effects of an aldosterone synthase inhibitor on cardiac hemodynamics, as well as left ventricular function and structure. **METHODS***:* The selective aldosterone synthase inhibitor FAD286 was administered in rats with CHF (4 $mg \cdot kg^{-1} \cdot d^{-1}$ as food additive for 90 d starting 8 d after coronary ligation). Left ventricular systolic and diastolic diameters and cardiac output were assessed by conventional echocardiography in anaesthetized rats. Arterial blood pressure and left ventricular end-diastolic pressure and relaxation constant Tau were investigated in anaesthetized rats using Millar microtip catheter. **RESULTS:** After 90 d, FAD286 decreased left ventricular systolic and diastolic diameters (-16.3 % and -11.5 % respectively *vs* control rats) and increased cardiac output (+22.5 % *vs* control rats) determined in anaesthetized rats using conventional echocardiography. At the end of treatment, FAD286 decreased left ventricular end-diastolic pressure (-65.5 % *vs* control rats) and left ventricular relaxation constant Tau (-38.6 % *vs* control rats) but did not modify arterial blood pressure investigated in anaesthetized rats using Millar microtip catheter. Simultaneously, FAD286 significantly reduced heart weight (-11.7 % *vs* control rats). **CONCLUSION***:* Long-term aldosterone synthase inhibition with FAD286 improves cardiac hemodynamics as well as left ventricular diastolic and systolic function and prevents left ventricular hypertrophy. These beneficial effects of FAD286 suggest that aldosterone synthase inhibition might be a new therapeutic option in the treatment of CHF.

### O14 Experimental study of icariin on vascular dementia in rats induced by 2-VO method

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**KEY WORDS** icariin; vascular dementia; Morris water maze; immunohistochemistry; apoptosis; electron microscope

**AIM:** To study the effects of icariin (ICA) on the learning and memory of ischemic vascular dementia (VD) model of rats,and explore the protective mechanisms. **METHODS:** ICA was administered to the VD model rats induced by a permanent bilateral occlusion of both common carotids arteries(2-VO method) and by cerebral ischemia-reperfusion (I10-R10-I10 method). Morris water maze was used to examine the abilities of spatial learning and memory of VD model rats. The activity of SOD, level of MDA in brain, and the concentration of AChE in brain and serum was detected, respectively. The expression of AChE and ChAT in hippocampus was detected by immunohistochemistry (IHC), then were quantitated and analyzed by BI2000 image analysis system. Brain tissues were made into paraffin section of 6 μm thickness and were stained with hematoxylin-eosin (HE) , then observed with optical microscope. The ultrastructrue of cerebral cortex and hippocampus tissues were observed by electron microscope. The protective of ICA on apoptosis of cerebral cortex and hippocampal neurons were observed by TUNEL

technique. **RESULTS:** In the place navigation test the mean escape latency was significantly shortened and the exploring distance was obviously decreased in ICA groups compared to model group, while in the spatial probe test the mean explore time in target area was increased. With the 2-VO method, the average activity of SOD was apparently higher, the average activity of MDA and the concentration of AChE was apparently lower in ICA groups than those in model group. No significant effect of ICA on the concentration of AChE in serum was observed. The immunohistochemistry and quantitative analysis found that the level of AChE in model group was not significantly different from that in sham group, but was significantly higher in ICA groups compared to model group. The level of ChAT in model group was significantly lower than that in sham group ,while the level was significantly higher in ICA groups compared to model group. With the I10-R10-I10 method, these results of optical microscope showed that hippocampal neurons was obviously deleted in model group compared to sham group, and that was obviously increased in ICA groups compared to model group. The changes of the ultrastructrue were found among the cerebral cortex neurons of model group that was characterized by condensed chromatin, clumped apoptotic nuclei and the broken nuclear membrane into apoptotic body. And there were obviously degeneration, necrosis and deletion, mitochondrial tumefaction, vascular degeneration and synaptic degeneration, but the neuronal injury in ICA groups were obviously reduced. **CONCLUSION:** ICA can improve the abilities of spatial learning and memory of VD model rats by 2-VO method. The active mechanisms may be involved in that (1) increase the activity of SOD, decrease the activity of MDA, and anti-lipid-peroxidation; (2) promote the function of injuried neurons and increase the synthesis and expression of AChE and ChAT; (3) enhance the function of mitochondria and relieve the apoptosis of neurons.

## O15 Fluorescence polarization assay for identification of lox-1 ligand through high throughput screening

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**KEY WORDS** lectin-like low-density lipoprotein receptor-1; fluorescence polarization; high throughput screening; atherosclerosis

**AIM:** Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) was identified as special receptor for oxidized low-density lipoprotein (oxLDL) in endothelial cells. LOX-1 critically mediated oxLDL stimulating the progression of atherosclerosis. Identification and discovery LOX-1 antagonist to therapy atherosclerosis will be an interested exploration. **METHODS:** Human LOX-1 was expressed as a His-fusion in bacteria and purified by metal ion affinity and gel filtration chromatography. Sequential ultracentrifugation at 4 °C from normolipidemic fasting volunteers obtain low density lipoprotein (LDL), and LDL was modified by $CuSO_4$ (5 μmol·$L^{-1}$) at 37 °C for 24 h. A fluorescence polarization assay was developed to identify ligands of LOX-1 by high throughput screening (HTS). The assay based on the interaction receptor-ligand, human LOX-1 was labeled by FITC and bound to its special ligand of oxLDL. A total of 3200 compounds were screened by FP-based competitive displacement assay, at excitation filter of 485 nm and emission filter of 530 nm. Z' factor was used to assess the assay. **RESULTS:** The developed FP-based HTS has been formatted in a 384-well microplate with a Z' factor of 0.75, and total 3200 compounds were screened and three compounds of hLOX-1 ligands with an $EC_{50}$ below 40 nmol/L were identified. **CONCLUSION:** The results indicated that developed fluorescence polarization assay was robust and well suited for high throughput screening efforts.

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## O16 Mitochondrial calcium-activated potassium channel: another potential target for neuroprotection?

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**KEY WORDS** mitochondrial $Ca^{2+}$-activated potassium channel; mitochondrial adenosine triphosphate-sensitive $K^+$ channel; neuroprotection

**AIM:** It has recently been reported that large-conductance $Ca^{2+}$-activated potassium channel is present in the inner mitochondrial membrane (mito$K_{Ca}$) of the neuron cell, which has been reported to have cardioprotective effect similar to that of mitochondrial ATP-sensitive $K^+$ channel (mito$K_{ATP}$). Hence the aim of this study was to clarify if mito$K_{Ca}$ is neuroprotective and compare this potential effect with that of mito$K_{ATP}$. **METHODS:** Male Sprague-Dawley rats were subjected to middle cerebral artery occlusion (MCAO) for 90 min followed by reperfusion. A total amount of 30 μL diazoxide (2 mmol/L), an opener of mito$K_{ATP}$, or NS1619 (0.1 mmol/L), an opener of mito$K_{Ca}$, was infused into the right lateral cerebral ventricle 15 min before ischemia. Neurological scoring was made 24 h after the MCAO and then infarct area was determined by using standard 2,3,5-triphenyltetrazolium chloride staining techniques. To further clarify the capacity of NS1619 to protect mitochondria from swelling induced by $Ca^{2+}$, which is supposed to be the leading pathological event during ischemia-reperfusion injury, we isolated brain-derived non-synaptosomal mitochondria and evaluated the effect of NS1619 and diazoxide on $Ca^{2+}$-induced swelling through measurement of spectrophotometric alterations in light scattering. **RESULTS:** Neurological score and the percent of infarct area were improved in the animals with pretreatment of 2 mmol/L diazoxide (2.0±0.6, $n$=10, $P$<0.05 *vs* sham; 16.7 %±5.1 %, $n$=10, $P$<0.01 *vs* sham) and 0.1 mmol/L NS1619 ($P$>0.05 *vs* diazoxide) when compared with

sham treatment (1.0±0.1, *n*=6; 27.7 %±3.5 %, *n*=6). In the isolated mitochondria model, permeability transition was readily induced at the level of 100 μmol/L $Ca^{2+}$ and was effectively inhibited by 200 μmol/L diazoxide (61.4 %±0.7 %) and 10 μmol/L NS1619 (65.2 %±0.4 %, *P*>0.05 *vs* diazoxide) when compared with the basal (42.1 %±0.5 %). **CONCLUSION:** Our results indicate that selective opening of the mito$K_{Ca}$ has neuroprotective effect against ischemia-reperfusion injury in the rat brain and this effect is comparable to that of mito$K_{ATP}$. As a result, we propose that mito$K_{Ca}$ should be another important neuroprotective target worth further studying.

## O17 Larger adaptive response of 5-$HT_{1A}$ autoreceptors to chronic fluoxetine in a mouse model of depression than in healthy mice

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**KEY WORDS** antidepressant; mutant mice; 5-$HT_{1A}$ autoreceptors; dorsal raphe

Vulnerability to major depressive disorders, in particular depression, is often associated with both hypoactivity of the central serotoninergic (5-HT) system and hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis. Extensive studies in normal healthy rodents showed that chronic treatment with SSRI antidepressants produced a marked functional desensitization of somatodendritic 5-$HT_{1A}$ autoreceptors, and this adaptive change has been claimed to play a key role in the therapeutic action of these drugs. However, the relevance of such observations in normal animals to the pathological situation can be questioned. The generation of transgenic mice (GR-i), with an ~ 60 % decrease in brain density of glucocorticoid receptors (GR), caused by transgene-driven expression of antisense GR mRNA under the control of human neurofilament gene promoter, offered the opportunity to further assess the effects of SSRI antidepressants in animals with a hyperactive HPA axis like that associated with depression in humans. Indeed brain GR deficiency causes antidepressant-responsive behavioral alterations and a marked deficit in glucocorticoid-mediated inhibitory feed back control of HPA axis in these transgenic mice.

GR-i and paired wild-type (WT) mice were treated for 21 d with fluoxetine (5 mg/kg, ip daily) or saline, and the functional characteristics of 5-$HT_{1A}$ autoreceptors in the dorsal raphe nucleus (DRN) were assessed using *in vitro* electrophysiological and autoradiographic techniques. After chronic saline, 5-$HT_{1A}$ autoreceptor functional characteristics were similar in GR-i mutants and paired WT mice, and corresponded to those found in untreated mice of both groups. Fluoxetine treatment produced both a decreased potency of the 5-$HT_{1A}$ receptor agonist, ipsapirone, to inhibit the firing of DRN 5-HT neurons, and a reduction in 5-$HT_{1A}$ receptor-mediated increase in [$^{35}$S]GTP-γ-S labeling of the DRN by 5-carboxamido-tryptamine (5-CT). Although such fluoxetine-induced changes were observed in both groups of mice, they were significantly larger in GR-i mutants (ipsapirone $EC_{50}$x12; 60 % reduction in 5-CT-induced [$^{35}$S]GTP-γ-S labeling, compared with respective values in saline-treated GR-i mice) than in WT mice (ipsapirone $EC_{50}$x5; 49 % reduction in 5-CT-induced [$^{35}$S]GTP-γ-S labeling, compared to saline-treated WT mice). These data indicated that tonic hyperactivity of HPA axis like that occurring in depressed patients was associated with enhanced 5-$HT_{1A}$ autoreceptor functional desensitization in response to chronic SSRI treatment. Such observations further emphasize the idea that investigations aimed at elucidating the actual neurobiological effects underlying therapeutic action of antidepressants have to be performed in validated animal models of depression rather than in normal healthy rodents.

## O18 The dissociation of heroin-seeking patterns induced by contextual, discriminative, or discrete conditioned cues in a model of relapse to heroin in rats

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**KEY WORDS** heroin; cues; relapse; association learning

**AIM:** To characterize the patterns of resumption of drug-seeking induced by drug-related cues and the extent to drug-seeking controlled by these cues in rats after withdrawal. **METHODS:** Nosepoke responding by male rats was reinforced with intravenous heroin (0.05 mg/kg per infusion, 4 h session daily) under a PR schedule of reinforcement for 14 d. A green light in active nosepoke served as discriminative cue (DS). Each earned heroin injection was also paired with 5 s red light and a house light, the compound lights and sound of infusion pump served as the discrete conditioned stimuli (CSs). **RESULTS:** Response rates of heroin seeking induced by contextual stimuli (chamber) were comparable to the average responding during the self-administration training, but response rates of heroin seeking induced by either DS or CSs were greater than those induced by contextual cues ($P < 0.05$). The responding magnitude induced by CSs was higher than that of DS after extinction of instrumental behavior. The drug seeking induced by CSs or DS can be recovered respectively after 3 days extinction of DS versus CSs in original context. **CONCLUSION:** The resumption of drug-seeking can be elicited separately by the drug-paired environmental cues, heroin-predictive DS, or discrete CSs in the same rat after abstinence, suggesting that there are different neural substrates for these cues.

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**O19 Involvement of the tachykinin $NK_3$ receptor in the regulation of MMP-2 human lung fibroblast production**

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**KEY WORDS** lung fibroblast; metalloproteinase; tachykinin; $NK_3$ antagonist

**AIM:** Overexpression of matrix metalloproteinases (MMPs) by lung fibroblasts has been blamed for much of the tissue destruction associated with airway inflammation. It has been previously demonstrated that a tachykinin $NK_3$ receptor antagonist, SR142801, reduced MMP-9 production associated with pulmonary inflammation in mice (Nénan *et al*, Eur J Pharmacol, 2001; Véron *et al*, Clin Exp Physiol Pharmacol, 2004, in press). The current study was designed to evaluate the *in vitro* effect of SR142801 on the MMP-2, TIMP-2, and collagen production by lung fibroblasts. **METHODS:** To examine the expression of the tachykinin $NK_3$ receptor in human fetal lung fibroblasts, HFL-1, flow cytometry experiments were performed. Cells were collected by trypsinization and incubated for 30 min at 4 ℃ with a primary antibody specific to the human $NK_3$ receptor (Abcam). Cells were then washed and incubated with FITC-conjugated secondary IgG for 30 min and analyzed by flow cytometry using FACScalibur (CellQuest software; BD Biosciences). To evaluate the effect of SR142801, HFL-1 were plated at a density of $1\times10^4$ cells into a 96-well plate until sub-confluence and were drawn to a quiescent state by incubation in serum-free medium for 24 h before experiments. Then HFL-1 was incubated for 1 h with SR142801 (0.001 to 10 μmol/L) and stimulated with IL-1(10 μg/L) during 24 h. Gelatinolytic activities of secreted MMPs were analyzed by zymography, the amount of enzyme was then quantified by measuring the intensity of the negative bands using a densitometric analyser. TIMP-2 concentration was quantified by ELISA. Soluble collagen was quantified by colorimetric Sircol assay (Biocolor). **RESULTS:** By flow cytometry analysis we observed the expression of tachykinin $NK_3$ receptor on the surface of HFL-1 cells. Stimulation of lung fibroblasts with IL-1 induced a significant increase in production of latent MMP-2 and collagen, which was significantly inhibited by the SR142801 from 0.1 μmol/L and 10 μmol/L respectively. **CONCLUSION:** These results suggest that $NK_3$ receptor antagonism can modulate the degradation/synthesis balance of extracellular matrix induced by pro-inflammatory cytokine. Then $NK_3$ receptor antagonist should display a relevant interest in lung diseases associated with abnormal remodelling.

**O20 Pharmacological profile of a new and potent gonadotropin releasing hormone antagonist LXT-101**

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**KEY WORDS** GnRH antagonist; serum testosterone; chemical castration

**AIM:** To investigate the pharmacological effect of LXT-101 on serum testosterone level in intact male rats. **METHODS:** Rats were injected subcutaneously with LXT-101 while control animals received only vehicle (5 % mannitol). Blood samples were collected at different times after administration of LXT-101. The serum testosterone was determined by specific immunochemiluminescence assay using kits produced by Beckman-Counter Co. The mRNA expressions of hormone receptor genes related to the HPT axis were investigated by real-time PCR technique. **RESULTS:** LXT-101 produced a dose- and time-dependent suppression of serum testosterone level after single subcutaneous injections and showed excellent characters of chemical castration when rats received intermittent treatment. The time of onset and the dose needed to maintain the effect of chemical castration decreased when the frequency of injection increased. Using ALZET® osmotic pump, the effective dose of LXT-101 was reduced to 1/6 to 1/24 of that needed for intermittent subcutaneous injection. The weight of testicular, prostate and seminal vesicles, rather than the body weight, decreased significantly after long-term repeated treatment of LXT-101. The gene expression of pituitary GnRH receptor was also down-regulated after multiple administrations but this effect was reversed after 24 h of withdrawal. **CONCLUSION:** LXT-101 is fit for depot formulation and it might possibly be developed as an ideal candidate for treating sex hormone-sensitive tumors and other disorders.

(Work was supported by the Chinese National Key Project of Technology, No 2002AA2Z3121)

**O21 Activation of the elastin-laminin receptor (S-Gal) induces preconditioning in isolated rat heart submitted to ischemia and reperfusion**

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**KEY WORDS** preconditioning; elastin peptides; isolated heart

**AIM:** Elastin-laminin receptor (S-Gal), was described to belong to G-protein-coupled receptors (GPCRs). Using an isolated non-working rat heart model, we investigated whether S-Gal stimulation was able to mimic ischemic preconditioning as observed with some other GPCRs. **METHODS:** Hearts, after 6-hydroxydopamine pretreatment and a 20-min stabilization period,

were perfused for 25 min then subjected to 40 min of global ischemia and 30 min of reperfusion (I/R, Ctrl); exposed to 0.66 μmol/L kappa elastin (ke) or 0.66 μmol/L $(VGVAPG)_3$, a sequence repeated several fold in tropoelastin (the soluble precursor form of elastin), for 10 min followed by a 10-min drug-free perfusion before I/R (ke-PC)); treated during 20 min with lactose ($1\times10^{-4}$ mol/L), an antagonist of elastin-laminin receptor with an infusion starting 5 min before ke (Lke-PC). The main endpoints were mean coronary flow, left ventricular end-diastolic pressure (LVEDP), rate-pressure product (RPP), CK release, and myocardial infarct size. **RESULTS:** Elastin peptides improved the recovery of coronary flow (90 %±2 % *vs* 66 %±4 % in Ctrl group, $P<0.05$ at 95 min), lowered postischemic LVEDP (16 ±4 mmHg *vs* 42±9 mmHg in Ctrl group, $P<0.05$ at 95 min), improved RPP recovery (83 %±6 % *vs* 31 %±9 % in Ctrl group, $P<0.05$ at 95 min), decreased CK release ( 246±33 $mIU\cdot min^{-1}\cdot g^{-1}$ *vs* 525±43 $mIU\cdot min^{-1}\cdot g^{-1}$ in Ctrl group, $P<0.05$ at t95 min) and reduced the myocardial infarct size. All these effects were reproduced by $(VGVAPG)_3$ and abolished by lactose. **CONCLUSIONS:** GPCRs including adenosine, opioid, bradykinin, acetylcholine, angiotensin, and catecholamine receptors, have been reported to mediate cardioprotection when these mediators were administered before I/R. In this study, we showed that preischemic stimulation of S-Gal by elastin-derived peptide induced a protective effect against myocardial dysfunction similar to pharmacological preconditioning.

## O22 Effects of DPCPX on the hippocampal BDNF protein expression and endoplasmic reticulum content

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**KEY WORDS** 8-dyclopentyl-1,3-dipropylxanthine; adenosine $A_1$ receptor; endoplasmic reticulum; brain-derived neurotrophic factor

**AIM:** To study the effect of blocking adenosine $A_1$ receptors on brain-derived neurotrophic factor (BDNF) protein expression and the endoplasmic reticulum (ER) content of the rat hippocampus. **METHODS:** Using immunohistochemical method (two-step polymer detection reagent, PV) and transmission electron microscope to observe the effect of 8-dyclopentyl-1,3-dipropylxanthine (DPCPX, ip, 15 d). **RESULTS:** DPCPX (0.5 $mg\cdot kg^{-1}$) significantly increased the amount of positive staining cells which indicated BDNF protein expression of granular cells of the rat hippocampus, and increased the surface density, numerical density, boundary density, mean optical density of positive cells, and reduced the mean gray of positive cells. DPCPX (0.1 $mg\cdot kg^{-1}$) could only significantly increase the surface density, numerical density and boundary density, but it did not affect the mean optical density and the mean gray of positive cells. We also observed that ER crowded obviously and ranged continuously like net in hippocampus neurons obtained from the animals treated with DPCPX (0.5 $mg\cdot kg^{-1}$) , and the density of ER calculated by point counting method increased significantly. **CONCLUSION:** DPCPX via blockade of adenosine $A_1$ receptors significantly increases BDNF protein expression in the granular cells of the rat hippocampus, and ER content of the rat hippocampus neurons. The increase in BDNF protein expression in the rat hippocampus neurons may be related to the increase in ER content induced by DPCPX.

## O23 Dimerization of the D1 dopamine receptors is related with agonist and inverse agonist-induced receptor internalization

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**KEY WORDS** D1 dopamine receptor; dimer; internalization

**AIM:** To examine the relationship between D1 dopamine receptor dimer formation and ligand-induced receptor internalization. **METHODS:** FLAG-tagged D1 dopamine receptor was transiently expressed in Sf9 cells. The cells were treated with SKF38393 or (+)butaclamol 1 μmol/L for different periods time or at different doses for 30 min respectively. Western blot assay was performed to assess dimer formation, and flow cytometer and radioligand binding assays were performed to detect receptor internalization. **RESULTS:** Treatment with agonist SKF38393 and inverse agonist (+)butaclamol led to increase in the proportion of dimers and receptor internalization in a dose-dependent manner. Antagonist SCH23390 blocked dimer formation and receptor internalization. Time course of SKF38393-induced dimer formation showed that dimer formation increased after 5 min treatment and reached maximal value at 10 min treatment, and then appeared decrease in dimer formation after 30 min treatment. A similar time course was also observed in SKF38393-induced receptor internalization. Interestingly, time courses of (+)butaclamol-induced dimer formation and receptor internalization were different from those of SKF38393-induced dimer formation and receptor internalization. However, the relationship between dimer formation and receptor internalization is similar to that of SKF38393-induced dimer formation and receptor internalization. A dramatic receptor internalization occurred after 1 min (+)butaclamol treatment and reached maximal value at 5 min treatment and then maintained such level of internalization during 120 min treatment. Similarly, Western blot result showed that the D1 receptor dimers increased with 1 min treatment and reached maximal value at 5 min treatment and then stabilized such level of dimers during sustained exposure. **CONCLUSION:** These results demonstrate a relationship between the ability of ligands to mediate the levels of dimer and to induce receptor internalization, suggesting that interconversion between monomeric and dimeric forms may be important for D1 dopamine receptor internalization.

**O24 Effects of *Ganoderma lucidum* polysaccharides on CIK cells proliferation and cytotoxicity**

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**AIM:** To study the effect of *Ganoderma lucidum* polysaccharides (*Gl*-PS) on proliferation, cytotoxicity and phenotype in cytokine-induced killer (CIK) cells as well as anti-tumor activity of CIK cells induced by *Gl*-PS and cytokines on mice bearing tumor *in vivo*. **METHODS:** Nonadherent splenocytes were incubated at $1\times10^9$/L in complete medium with IFN-γ (1000 U/mL) 24 h before IL-2 (300 U/mL) plus anti-CD3 (50 ng/mL) and IL-1 (100 U/mL) stimulation to generate CIK cells as routine protocol. Experimental groups were the same as routine protocol except additional stimulus of 400 mg/L or 100 mg/L *Gl*-PS and decrease in dose of anti-CD3 and IL-2 by 50 % and 75 %; addition of soluble starch or methylcellulose (100 mg/L or 400 mg/L) instead of *Gl*-PS in the same protocol as experimental groups was used as negative control. CIK cells proliferation, cytotoxicity and phenotype were determined by Trypan blue exclusion method, MTT assay, and flowcytometry. The mice bearing H-22 tumor or S180 tumor were treated with adoptive immunotherapy by CIK cells prepared in different protocol. **RESULTS:** Synergizing with cytokines, 400 mg/L or 100 mg/L *Gl*-PS could decrease the dose of IL-2 and anti-CD3 by 75 % and 50 % in inducing CIK cells, and had insignificant change on proliferation, cytotoxicity and phenotype of CIK cells compared with routine protocol in which CIK cells expand about 80-fold and the main effectors $CD3^+$ $NK1.1^+$ cells expanded by more than 15 % significantly. Cytotoxicity of CIK cells in routine protocol against P815 and YAC-1 was 80.73 % ± 6.83 %, 89.75 %± 4.46 % at E: T ratio of 20:1 (Cytotoxicity of CIK cells against P815 and YAC-1 in experimental group added *Gl*-PS 100 mg/L: 76.87 %±6.81 %, 84.65 %± 7.88 %; Cytotoxicity of CIK cells against P815 and YAC-1 in experimental group added *Gl*-PS 400 mg/L: 79.32 %± 4.68 %, 88.87 %± 5.48 %). However, proliferation and cytotoxicity of CIK cells in negative control was lowered markedly than that in experimental groups or routine protocol. CIK cells induced by *Gl*-PS and cytokines were effective on mice bearing H-22 tumor or S180 tumor in adoptive therapy. **CONCLUSION:** Synergizing cytokines, 400 mg/L or 100 mg/L *Gl*-PS could enhance CIK cells proliferation and anti-tumor activity. *Gl*-PS was shown to be a promising biological response modifier and immune potentiator.

**O25 Evaluation of cellular delivery of phosphorothioate oligonucleotides targeting Her-2 mRNA in breast cancer cell line**

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**KEY WORDS** oligodeoxyribonucleotides; radioactivity; confocal laser scanning microscope

**AIM:** To examine the amount of antisense phosphorothioate oligodeoxyribonucleotides designed for inhibiting Her-2 mRNA after tranfection *in vitro* with a commercially available cationic lipid. **METHODS:** Breast cancer cells were transfected with $^{32}P$ and FAM labeled phosphorothioate oligodeoxyribonucleotides complexed with lipfectamine™. The cells were isolated and the other radioactivity was collected by centrifugation and determined the isotope labeled antisense phosphorothioate oligodeoxyribonucleotides by liquid scintillation counting. Confocal laser scanning microscope was utilized for measuring fluorescence intensity in cells. **RESULTS:** After 4 h exposure, 50 % radioactivity was determined in cells for the potent sequence, while just only 10 % was detected in cells for control sequence. The image of confocal laser scan microscope showed that the transfection efficacy of potent sequence treated group were higher than that of control sequence. **CONCLUSION:** The results indicate that different sequence with different pharmacodynamics is transferred into cells differently. The potent sequence is more effective than control sequence.

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**O26 Effect of nitric oxide on CYP450 content and expression in chemic-immune hepatocarcinogenesis induced by DEN plus BCG in rats**

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**AIM:** To investigate the effect and the possible mechanism of *N*-diethylnitrosamine (DEN) plus *Mycobacterium* bovis bacillus Calmette-Guerin (BCG) on hepatic CYP450 total content and expression in chemic-immune hepatocarcinogenesis in rats. **METHODS:** The chemic-immune hepatocarcinogenesis model was established by a single administration of DEN (150 mg/kg, ip) and by a single dose of BCG (50 mg/kg, iv, before 2 weeks at collection of sample) in rats. Aminoguanidine (AG), a selective nitric oxide synthase (iNOS) inhibitor, was administered by intraperitoneal injection (50 mg/kg, every other day) before one week at collection of sample. After 1st, 2nd, and 3rd month end stimulated by DEN or/and BCG, the samples of serums and hepatic tissues were collected and frozen at -20 ℃ until determinated. Alanine aminotransferase(ALT) and nitrite levels in serurm, and CYP450 total content in hepatic homogenate were determined by the method of spectrophotography. The protein expression of iNOS, CYP1A2, CYP2E1, GSTpi, and PCNA (the marker of hepatocarcinogenesis) in hepatic tissue were determined by methods of immunohistochemistry. **RESULTS:** Com-

pared with DEN only stimulation, DEN plus BCG immune stimuli increased the ratio of liver weight and body weight (Lw/Bw). Both ALT activity and nitrite concentration in serums, and both protein expressions of iNOS and PCNA were significantly increased ($P$<0.05) under similar chemic-immune stimulated condition. On the other hand, CYP450 total content, the protein expression of CYP1A2 and CYP2E1 were decreased ($P$<0.05). Administration of AG reversed above reactions induced by DEN plus CG in rats. **CONCLUSION:** The results suggest that NO participates in the down-regulating mechanism of CYP450 in hepatocarcinogenesis induced by DEN plus BCG in rats.

### O27 Comparative effects of type I interferon (huIFN-α and ovIFN-τ) and type II interferon (huIFN-γ) on the tryptophan-to-kynurenine pathway in human uninfected or HIV-1-infected macrophages

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**KEY WORDS** kynurenine; tryptophan; HIV; interferon

**AIM:** Ovine type I IFN-τ displays the same antiretroviral properties than human IFN-α but is less toxic *in vitro* and *in vivo*. Clinical use of type I IFN is associated with severe neuropsychiatric side effects, in part linked to a stimulation of the kynurenine pathway. Activated macrophages and microglia are apparently the only cells capable of catabolizing tryptophan to quinolinic acid via the kynurenine pathway in the central nervous system. The rate limiting enzyme of this pathway is the indoleamine 2,3-dioxygenase (IDO). In this study, we compared the effects of human IFN-γ and IFN-α to those of ovine IFN-τ on the kynurenine pathway in macrophages infected or not with HIV. **METHODS:** Human primary macrophages treated with 100 IU/mL IFN were infected or not with HIV-1/Ba-L. Tryptophan and kynurenine concentrations in cell culture supernatants were simultaneously measured by high performance liquid chromato-graphy. In parallel, IDO mRNA expression was quantified by PCR and the (kynurenine/tryptophan) ratio allowed an estimation of IDO activity. HIV replication was assessed in cell culture supernatants. **RESULTS:** Tryptophan levels in cell culture supernatants were undetectable after 3 d of treatment with IFN-γ and remained low throughout the period of treatment, whether the macrophages were infected or not. In parallel, a correlated induction of the kynurenine pathway was observed, with high levels of IDO mRNA expression. In contrast, the effects of type I IFN (-α and -τ) were weak and transient in uninfected macrophages. The low activation of the kynurenine pathway was more pronounced with IFN-α than with IFN-τ. In infected and untreated macrophages, the kynurenine pathway was activated by high HIV replication, but the antiretroviral properties of type I IFN prevented this stimulation. **CONCLUSION:** At antiretroviral concentrations, IFN-τ had a weaker impact than IFN-α on the kynurenine pathway and might be considered as a future therapy in HIV or HIV/HCV infections.

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### O28 Therapeutic drug monitoring of amoxicillin and cloxacillin

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**KEY WORDS** beta-lactams; therapeutic drug monitoring; renal insufficiency

**AIM:** Beta-lactams (BL) are broad-spectrum antibiotics currently used in number of infectious diseases and some infections need high dose of antibiotics. BL studied here are eliminated rather quickly by the kidney. A renal insufficiency involves an increase in BL concentrations. Therapeutic drug monitoring could help in adapting the target concentration. **METHODS:** We developed a rapid (less than 20 min), sensitive, and specific HPLC method for simultaneous assay of 12 BL in plasma. Extraction was achieved by a simple protein precipitation with acetonitril. Chromatographic separation was achieved on a column ATLANTIS™ with a linear gradient. Assay was performed with high precision (CV<5 %) and recovery (>90 %) in range from 2 to 250 mg/L. For amoxicillin and cloxacillin, 81 levels between 2003 January and 2004 June were retrospectively analysed. Patients were classified in two groups according to creatinin level (1: creatinin level=100 μmol/L, 2: creatinin level >100 μmol/L) and their concentrations were compared. **RESULTS:** Among patients experiencing intravenous administration, 49 have been treated with amoxicillin and 32 with cloxacillin for various pathologies. Higher BL levels were measured in group 2 ($P$<0.05) and demonstrated an accumulation. Therefore, adaptation based only on renal function was not efficient despite a mean dose decrease of 4.5 and 1.8 g/d (for amoxicillin and cloxacillin respectively). **CONCLUSION:** For BL, therapeutic drug monitoring easily performed in routine practice, could help in adapting the target concentration in order to achieve long-term safety, especially in patients with renal impairment.

### O29 Influence of C3435T and G2677T/A MDR1 polymorphism on morphine side effects.

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**KEY WORDS** morphine; P-glycoprotein; pharmacogenetics; clinical study

**AIM:** Morphine efficacy and side effects present large interindividual pharmacodynamic variability. Single nucleotide polymorphisms (SNPs) of P-glycoprotein (MDR1), an efflux transporter considered as a major component of the blood-brain-barrier, may explain a part of this variability. We aimed to determine the potential relationships between MDR1 C3435T and G2677T/A SNPs and morphine efficacy and the need for ondansetron after morphine parenteral administration. **METHODS:** Patients following colorectal surgery, and receiving systematic post-operative parenteral morphine were included in this prospective study. Renal or hepatic failure, and opioid or corticoid treatment prior surgery, were exclusion criteria. Demographic, biological data, and concomittant drugs were collected for each patient during and after surgery. Post-operative analgesic and adverse effects of morphine were evaluated during 24 h after surgery. Genotyping for MDR1 C3435T and G2677T/A SNPs were performed using a Taqman allelic discrimination assay. **RESULTS:** Seventy-three patients were included in this study. Anaesthesia procedure was standardized for all patients. Sixteen patients were found homozygous CC, 41 heterozygous CT, and 16 homozygous TT for C3435T MDR1 SNP. Twenty-one patients were found homozygous GG, 37 heterozygous GT or GA, and 15 patients were found TT, TA or AA for MDR1 G2677T/A. Twenty-three patients required ondansetron for nausea during first post-operative 24 h. No relationship was found between C3435T and G2677T/A MDR1 SNPs and cumulative dose of parenteral morphine during 24 h in our population. MDR1 C3435T and G2677T/A polymorphisms were associated with a trend for a low need of ondansetron with a protective OR of 0.24 [95 % CI 0.05-1.18] for MDR1 C3435T SNP and 0.27 [95 % CI 0.07-1.02] for MDR1 G2677T/A SNP. **CONCLUSION:** Whereas MDR1 SNPs does not affect morphine analgesic effect, wild type homozygous for C3435T and 2677T/A MDR1 SNPs, associated with high P-glycoprotein expression levels and drug resistance, protects against morphine side effects.

## O30 Metabolism, distribution and excretion of recombinant human glucagons-like peptide-1 (7-36) in rats

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**KEY WORDS** rhGLP-1(7-36); distribution; metabolism; excretion

**AIM:** To study the metabolism, distribution, and excretion profiles of recombinant human glucagon-like peptide-1(7-36) [(rhGLP-1(7-36))] in Wistar rats. **METHODS:** The dose was set at 25 $\mu g \cdot kg^{-1}$. $^{125}$I-rhGLP-1(7-36) was prepared by iodogen method and determined by size exclusive HPLC (SHPLC) or trichloroacetic acid (TCA) precipitation analysis. **RESULTS:** SHPLC analysis after iv or sc administration of rhGLP-1(7-36) showed that the small molecule metabolites increased with the decreasing of the parent drug. TCA precipitable radioactivity in tissue showed that the highest radioactivity level was found in kidney, the second was pancreas, then were plasma, adrenal gland, lung, small intestine, thymus gland, bladder, lymph node, spleen, heart, liver, gonad, eyeball, muscle and brain in turn, and that of the fat was the lowest. 82.6 %±2.4 % of injected dose was excreted through urine within 72 h. **CONCLUSIONS:** Total radioactivity does not represent the concentration of parent drug. TCA precipitable radioactivity in target tissue was rather high. The main excretion route of rhGLP-1(7-36) was urinary system.

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## O31 Pharmacokinetics and bioequivalence of lorazepam tablets in Chinese healthy volunteers

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**KEYWORDS** Lorazepam; high performance liquid chromatography; pharmacokinetics; bioavailablity; bioequivalence

**AIM:** To study the pharmacokinetics and bioequivalence of lorazepam tablets in Chinese healthy volunteers. **METHODS:** Twenty Chinese healthy male volunteers were involved in the study. Each subject received a single dose of 3 mg Lorazepam tested formulation (T, Hubei Zhongtian Airbeck Pharmaceutical Limited Company) or Lorazepam reference formulation (R, Thailand Atlatic Pharmaceutical Limited Company) with a randomized crossover study. Blood sampling was conducted consequently within 48 h. The plasma Lorazepam concentration was determined by HPLC. The plasma Lorazepam concentration was calculated by 3p97 pharmacokinetical program and the pharmacokimetical parameters was caculated using non-compartment model. The relative bioavailability was calculated based on $AUC_{0-t}$. To evaluate the bioequivalence of the two formulations, analysis of variance of the pharmacokinetical parameters of AUC

and $C_{max}$ were done after logarithmic transformation, simultaneously two one-side test were done. **RESULTS:** After a single dose of 3 mg tested or reference formulation, the pharmacokinetic parameters of lorazepam were as follows: $C_{max}$ were 35.770±6.839 μg·L$^{-1}$ and 36.945±4.598 μg·L$^{-1}$; $t_{max}$ were 2.375±0.535 h and 2.225±0.472 h; $t_{1/2}$ were 13.716±2.072 h and 13.830±2.490 h; $AUC_{(0-48)}$ were 542.187±84.332 μg·h·L$^{-1}$ and 527.531±63.515 μg·h·L$^{-1}$; $AUC_{(0-inf)}$ were 605.217±93.524 μg·h·L$^{-1}$ and 599.37±1.56 μg·h·L$^{-1}$ for tested and reference formulation, respectively. The relative bioavailability of tested formulation to reference formulation was $F$=(103.5±15.9) %. **CONCLUSION:** The tested and reference formulations were bioequivalent.

**O32 Relationship between G-proteins associated transmembrane cascades and MAPK phosphorylation induced by recombinant interleukin-1α in fibroblast-like synoviocytes from rats with adjuvant arthritis**

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**KEY WORDS** adjuvant arthritis; fibroblast-like synoviocytes; G protein; mitogen-activated protein kinases

**AIM:** To elucidate the relationship between G protein-associated transmembrane cascade and mitogen-activated protein kinases (MAPKs) phosphorylation in recombinant rat IL-1α(rIL-1α)-induced fibroblast-like synoviocytes (FLS) from rats with adjuvant arthritis (AA). **METHODS:** The expressions of MAPKs phosphorylation, the stimulatory subunit of G alpha protein ($G_s$) and the inhibitory subunits of G alpha protein ($G_i$) were detected by Western blot in rIL-1α-induced FLS. Cyclic adenosine monophosphate (cAMP) accumulation was measured by enzyme-linked immuno-absorbant assay (ELISA) and prostaglandin $E_2$ ($PGE_2$) was measured by radioimmunoassay. Activities of protein kinase A (PKA) and PKC were detected by colorimetric assay. **REDULTS:** Pertussis toxin (PT) (1 mg/L) and cholera toxin (CT) (10 mg/L) decreased the phosphorylation of extracellular regulating kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 induced by rIL-1α in FLS. The membrane-associated expression of $G_{i1}$, $G_{i2}$, $G_{i3}$, and $G_s$, which were stimulated by rIL-1α, were decreased by U 0126 (25 or 50 μmol/L) and SB 203580(2.5 or 25 μmol/L). SB 203580 and U 0126 inhibited both cAMP accumulation and $PGE_2$ induction induced by rIL-1α. SB 203580 and U0126 not only decreased the activities of PKC and PKA but also decreased the PKC/PKA in FLS stimulated by rIL-1α. **CONCLUSION:** This study suggests that activation of MAPKs is regulated by at least two of G protein-coupled cascades, Gi and Gs signal transduction pathway, in rIL-1α-induced FLS from rats with AA. Furthermore, inhibitors of MAPKs cascades, SB203580 and U0126, show a negative feedback to G-protein associated transmembrane signal.

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## POSTER PRESENTATION
## Cardiovascular Pharmacology

**PC01 Inhibitory effects of KXS01 on angiogenesis *in vitro***

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**KEY WORDS** KXS01; angiogenesis; rat aortic ring; endothelial cell

**AIM:** To evaluate the inhibitory effects of new compound KXS01 on angiogenesis. **METHODS:** Aortae from Wistar rats were cut into rings, embedded in a fibrin clot and cultured for 12 d in serum-free medium and the microvessels were counted. Human umbilical vein endothelial cells(HUVEC) were cultured with or without $VEGF_{165}$ for 72 h and cell proliferation was studied by MTT assay. Migration of HUVECs induced by $VEGF_{165}$ was studied by wound-healing assay. For the *in vitro* angiogenesis assay, HUVECs were cultured on Matrigel for 18 h with or without 10 %FCS. The typical capillary networks formed on Matrigel by HUVECs as a result of cell migration and differentiation were determined by photography. **RESULTS:** KXS01 $1\times10^{-5}$ mol/L and $1\times10^{-6}$ mol/L significantly inhibited microvessel sprouting from cultured rat aorta. KXS01 could significantly inhibit $VEGF_{165}$-induced HUVEC proliferation in a dose-dependent manner ($IC_{50}$= $8.16\times10^{-7}$ mol/L) but had no cytotoxic effect on HUVEC in VEGF-free culture. KXS01 $1\times10^{-5}$ mol/L and $1\times10^{-6}$ mol/L significantly inhibited HUVEC migration induced by $VEGF_{165}$. KXS01 at concentrations of $1\times10^{-5}$ mol/L and $1\times10^{-6}$ mol/L significantly prevented the formation of capillary-like tubules by HUVEC in Matrigel containing 10 % FCS but had no effect without FCS. **CONCLUSION:** KXS01 can inhibit angiogenesis *in vitro* by preventing proliferation and migration of endothelial cells.

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**PC02 Beta3-adrenoceptor agonist-induced relaxation of human placental arteries is reduced in pregnancy-induced hypertension**

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**AIM:** Preeclampsia is one of the leading causes of neonatal and maternal morbidity and mortality. The purpose of this study is to investigate the functionality of β2- and ß3-adrenoreceptors (β-ARs) in human placental arteries and to assess the influence of pregnancy-induced hypertension on β-ARs responsiveness. **METHODS:** We performed *in vitro* functional and biochemical studies as well as RT-PCR experiments. **RESULTS:** SR59119 and salbutamol (β3- and β2-AR agonists respectively) induced a concentration-dependent relaxation of placental artery rings obtained from women with uncomplicated or hypertensive pregnancies and contracted by U46619. In normotensive arterial rings SR59119 was more efficient than salbutamol (maximal relaxation at $1\times10^{-5}$ mol/L; $E_{max}$: 44 %±4 % *vs* 21 %±4 % for SR59119 and salbutamol respectively, $P<0.05$) whereas -log$EC_{20}$ values were not significantly different. SR59119A-induced relaxation was unaffected by the blockade of $\beta_{1,2}$-ARs by propranolol $1\times10^{-7}$ mol/L whereas it was antagonized by blockade of $\beta_{1,2,3}$-ARs by propranolol $1\times10^{-5}$ mol/L ($E_{max}$: 30 %±5 % *vs* 20 %±5 %, $P<0.01$). SR59119A and salbutamol both stimulated cAMP production (4.9±0.4 and 4.5±0.5 pmol/mg protein, respectively compared to 2.3±0.4 for basal value, $P<0.05$). Salbutamol- but not SR59119A-induced cAMP production was inhibited by propranolol $1\times10^{-7}$ mol/L. SR59119A-induced relaxation ($E_{max}$ 30 %±5 % *vs* 44 %±4 % respectively, $P<0.05$) and cAMP production (2.7±0.5 *vs* 4.5±0.5 pmol/mg protein respectively, $P<0.05$) were decreased in arteries obtained from hypertensive compared to normotensive women. $\beta_2$- and $\beta_3$-AR mRNAs were both and equally expressed in normotensive and preeclamptic arteries. **CONCLUSION:** We suggest that (1) $\beta_2$- and $\beta_3$-ARs are present and functional in human placental arteries., (2) pregnancy-induced hypertension is associated with an impairment of $\beta_3$-AR responsiveness.

## PC03 Gi-dependent negative inotropic effect of adrenomedullin: relevance to septic shock.

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**AIM:** We incidentally have observed that a truncated adrenomedullin peptide (ADM 22-52) could greatly improve contractility of rat ventricular myocytes isolated from rats with sepsis-induced shock, suggesting a cardio-depressant activity for this native vasoactive peptide in this context. Severe sepsis often leads to shock, which is fatal in many instances. Our limited understanding of the involved mediatorsand their complex interactions makes it difficult to restore perfusion to key vascular beds, which is impaired by massive vasodilatation, ventricular failure and disseminated coagulation, and to prevent multiple organ failure and death. With regard to the ventricular failure associated with septic shock, our observation could thus be of significance, and was examined further. Our goals were to confirm a cardio-depressant activity of adrenomedullin in conditions of septic shock, and to gain insight into the mechanisms involved. **METHODS:** All experiments were conducted using ventricular myocytes freshly isolated from control rats, and from rats suffering shock 4 h after ip injection of 10 mg/kg lipopolysaccharides (LPS). **RESULTS:** In control myocytes, adrenomedullin (ADM 1-52) had time-dependent effects, which were blocked by ADM (22-52). Initially (<1/2 h incubation), the peptide acted as a positive inotropic substance in an adenylate cyclase-dependent manner. After prolonged incubation (> 1 h) the peptide acted as a negative inotropic substance via the production of prostacyclin. This negative inotropic effect was abolished when PKA or $G_i$ were inhibited. Adrenomedullin production was significantly up-regulated in ventricular myocytes isolated from shocked rats. Shortening of these myocytes in response to electrical field stimulation was decreased due to a decreased $I_{Ca}$ and the associated decreased sarcoplasmic reticulum loading. ADM (22-52) could restore $I_{Ca}$ (and contraction) back to control levels as well as prostacyclin synthase and $G_i$ inhibition. **CONCLUSION:** Adrenomedullin production is increased during septic shock, perhaps initially as a compensatory mechanism to increase ventricular performance via activation of adenylate cyclase and production of cAMP. However, when associated with the induction of COX II, ADM (1-52) becomes a cardiodepressor, perhaps due to a PKA-dependent switch from $G_s$ to $G_i$ stimulation. Because it is also a potent vasodilatator, adrenomedullin most probably plays an important role in the cardiovascular failure associated with septic shock.

## PC04 Inhibition of mitochondrial permeability transition pore contributes to the neuroprotection induced by activation of mitochondrial ATP-sensitive potassium channel

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**KEY WORDS** brain ischemia/reperfusion; mito$K_{ATP}$; neuroprotection; MPTP

**AIM:** To investigate whether the neuroprotection via activating mitochondrial ATP-sensitive potassium channel (mito$K_{ATP}$) is mediated by the inhibition of mitochondrial permeability transition pore (MPTP). **METHODS:** Adult male Sprague-Dawley rats were undergoing 90 min of middle cerebral artery occlusion (MCAO) by introducing a nylon monofilament through the external carotid artery to middle cerebral artery, followed by reperfusion with withdrawing the nylon monofilament for 22 h. All drugs were introduced into lateral cerebral ventricle. Neurological deficits of the animals were evaluated 22 h after reperfusion, using a 6-point neurological function scoring system. The infarct size of the brain slices stained with 2,3,5-triphenyltetrazolim was determined by area analysis. **RESULTS:** Application of 2 mmol/L diazoxide 20 min before MCAO significantly reduced the total percent infarction compared with the

sham group (sham *vs* diazoxide, 26.7 %±3.5 % *vs* 16.7 %±2.1 %, $P<0.01$), and increased neurological function score (1.0 %±0.1 % *vs* 2.2 %±0.2 %, $P<0.01$). The subsequent application of 2 mmol/L atractyloside 10 min before reperfusion significantly attenuated the neuroprotective effect of 2 mmol/L diazoxide given 20 min before MCAO (the total percent infarction: 25.7 %±2.3 %, $P<0.01$; neurological function score: 1.1 %±0.1 %, $P<0.01$). **CONCLUSION:** The opening of mito$K_{ATP}$ protects brain against cerebral ischemia and reperfusion, which is probably mediated by the inhibition of MPTP.

**PC05 Effects of glycosaminoglycan from scallop skirt on foam cell**

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**KEY WORDS** glycosaminoglycan from scallop skirt; foam cell; VEGF; calcium

**AIM:** To study effects of glycosaminoglycan from scallop skirt (SS-GAG) on NO production, antioxidative enzyme activity, and formation of macrophage-derived and smooth muscle cell-derived foam cell; to study the effects of SS-GAG on VEGF expression, intracellular $Ca^{2+}$ level, and cytokines secretion of macrophage-derived foam cell. **METHODS:** Foam-like cells were generated by incubating the U937 cells or porcine artery smooth muscle cells with oxidized low density lipoprotein (ox-LDL). U937 cells were incubated with medium RPMI-1640 containing 80 mg/L ox-LDL for 48 h and porcine artery smooth cells were cultured with medium 199 containing 15 mg/L ox-LDL for 72 h. The NO release, alterations of GSH-px and SOD in medium and intracellular cholesterol contents were investigated in the two kinds of foam cell. In U937 cells, the concentrations of VEGF, TNFα, IL-6 and IL-8 in medium were determined by ELISA. The intracellular $Ca^{2+}$ level was measured by flow cytometry. **RESUITS:** Compared with control groups, after treatment with different concentrations of SS-GAG, (1)the intracellular cholesterol contents, NO, and GSH-px release, the concentrations of VEGF, TNFα, IL-6, and IL-8 and the intracellular $Ca^{2+}$ level significantly decreased; (2)NO and GSH-px release significantly increased. **CONCLUSION:** The antiatherogenic effects of SS-GAG are probably due to its ability to inhibit the foam cell formation, increase NO and GSH-px release and decrease the concentrations of VEGF, TNFα, IL-6, IL-8 and the intracellular $Ca^{2+}$ level.

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**PC06 Angiotensin II induces cerebral arteriolar constriction and dilatation of cerebral arterioles in normotensive rats.**

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**KEY WORDS** angiotensin II; $AT_1$ receptor blocker; $AT_2$ receptor blocker; cerebral arterioles

**AIM:** Angiotensin I converting enzyme inhibitors (ACEIs) improve the autoregulatory dilator response to systemic hypotension (Chillon and Baumbach, 2001) suggesting that angiotensin II constricts cerebral arterioles. The goal of this work is to evaluate constrictor and dilator effects of angiotensin II on cerebral arterioles. **METHODS:** Experiments were performed in adult, male Wistar rats (400-450 g body weight) anesthetized with sodium pentobarbital (induction 60 $mg \cdot kg^{-1}$, ip, maintenance 20 $mg \cdot kg^{-1} \cdot h^{-1}$, iv). We measured changes in pial arteriolar internal diameter (μm) following topical administration of drugs with a computerized video system using the cranial window technique (Chillon and Baumbach, 2001). **RESULTS:** Angiotensin II produced concentration-related decreases in diameter with a maximum of -6.2±0.5 μm at $1\times10^{-6}$ mol/L ($n$=7). Curve fitting using GraphPad Prism v3.03 showed a best fit with a 2 site non linear model [$EC_{50}$: $7.5\times10^{-11}$ ($3.1\times10^{-11}$-$1.8\times10^{-10}$) and $5.4\times10^{-8}$ mol/L ($2.0\times10^{-8}$–$1.7\times10^{-7}$)]. The $AT_1$ blocker telmisartan $1\times10^{-5}$ mol/L reversed the effects of angiotensin II with an increase in internal diameter at a maximum of 4.7±0.6 ($1\times10^{-6}$ mol/L, $n$=7). The $AT_2$ blocker PD123319 ($1\times10^{-5}$ mol/L) abolished the vasodilatation induced by angiotensin II in presence of telmisartan ($1\times10^{-5}$ mol/L): -5.1±0.5 μm for angiotensin II alone ($1\times10^{-6}$ mol/L) *vs* 2.2±0.8 μm after telmisartan and –0.1±0.1 μm after telmisartan and PD123319 ($n$=6). Finally, The $AT_2$ blocker PD123319 ($1\times10^{-6}$ mol/L) enhanced the vasoconstriction induced by angiotensin II: -4.9±0.5μm for angiotensin II alone ($1\times10^{-6}$ mol/L) *vs* -8.7±0.8 μm after PD123319 ($n$=7). **CONCLUSION:** These results suggest that 1) angiotensin II applied to the outside of the pial arteriole modifies vasomotion, 2) the overall effect of angiotensin II on the cerebral arteriole is vasoconstrictor following stimulation of $AT_1$ receptor, but a vasodilator response is also evoked following stimulation of $AT_2$ receptor.

(Chillon and Baumbach, H*ypertension 2001*, 37:1388-1393 )

**PC07 Protective effects of isoliquiritigenin against cerebral ischemia-reperfusion injury in mice**

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**KEY WORDS** isoliquiritigenin; cerebral ischemia-reperfusion; energy metabolism; hemorheology; reversed-phase high performance liquid chromatography

**AIM:** To investigate the protective effects of isoliquiritigenin (ISL) against cerebral ischemia-reperfusion injury in mice. **METHODS:** The cerebral ischemia-reperfusion models in mice were made by repeated occlusion of bilateral common carotid arteries and reperfusion. To observe the hypoxia tolerance ability by means of recording the respiratory duration after cutting heads in repeated cerebral ischemia-reperfusion mice. The whole blood viscosity, hematocrit and blood coagulation time were measured. The brain energy state was analyzed by reversed-phase high performance liquid chromatography. **RESULTS:** ISL 20 and 40 mg·kg$^{-1}$ ig significantly prolonged the respiratory duration after head-cutting ($P$<0.01), decreased the whole blood viscosity and hematocrit, prolonged the blood coagulation time ($P$<0.01). ISL (10-40 mg·kg$^{-1}$, ig) improved the brain energy metabolism in a dose-dependent manner. In repeated cerebral ischemia-reperfusion mice treated with ISL (10-40 mg·kg$^{-1}$ ig ), EC and TAN were enhanced by 28 %, 43 %, 45 %, and 41 %,76 %, 90 % compared with model group, and ATP levels were restored to 55 %,75 %, 83 % of sham value respectively. **CONCLUSION:** ISL has protective effects against cerebral ischemia-reperfusion injury in mice£¬which may be related to the improvement of the abnormal hemorheological changes and brain energy metabolism.

**PC08 Effects of pravastatin on pulmonary arteries and aorta reactivity in monocrotalin-induced pulmonary hypertension**

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**KEY WORDS** pulmonary hypertension; endothelium; statin

**AIM:** Vascular injury caused by monocrotalin (MC) can affect endothelial regulation and induces pulmonary hypertension and heart failure. We showed previously that pravastatin prevented the development of MC-induced pulmonary hypertension by improving pulmonary arteries (PA) endothelium dependent vasodilation. The aims of this study were to compare the protective effects of pravastatin (PS) on PA or aorta and assess the role of cholesterol in this effects. **METHODS:** PS (10 mg·kg$^{-1}$·d$^{-1}$) or vehicle were given orally for 28 d to rats injected or not with MC (60 mg/kg intraperitonealy) giving four groups MC, MC+PS, PS and controls. Endothelium-dependent and independent vasodilation of PA and thoracic aorta were studied by constructing cumulative concentration-response curves to acetylcholine (ACh) and sodium nitroprusside (SNP) respectively. Total cholesterol and high density lipoproteins (HDL) were measured by enzymatic assays. **RESULTS:** Four weeks after injection, PA dilation induced either by ACh ($E_{max}$=46 %±3 % *vs* 83 %±5 % in controls, $P$<0.05), or SNP ($E_{max}$=92 %±2 % *vs* 99 %±0 % in controls, $P$<0.05) was significantly reduced in MC rats. In MC rats, ACh-induced aorta dilation was reduced although not significantly ($E_{max}$=43 %±9 % *vs* 55 %±8 % in controls. Treatment with PS was shown to prevent impairement of Ach-induced dilation of PA ($E_{max}$=65 %±5 % *vs* 46 %±3 %, $P$<0.05) in MC rats. On the contrary, such treatment was without effect on the SNP-induced PA dilation ($E_{max}$=92 %±1 % *vs* 92 %±2 %) and ACh-induced aorta dilation ($E_{max}$=41 %±6 % *vs* 43 %±9 %). No difference was found for the SNP-induced aorta dilation in the four groups. Serum cholesterol levels (total or HDL) were similar in rats treated and non-treated with PS. **CONCLUSION:** These results suggest that MC impairs endothelium-dependent and independent PA, but not aorta dilation. Pravastatin-induced protection does not appear to be mediated through cholesterol-lowering.

(Study funded by University of Burgundy)

**PC09 Involvement of ET-1 in diabetic cardiomyopathy, vascular abnormality and nepropathy which are regressed by a novel endothelin receptor antagonist Dajisentan.**

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**KEY WORDS** diabetes; heart; blood vessels; kidney; endothelin antagonists

**AIM:** An impaired endothelium contributes to diabetic cardiomyopathy (CMP), vascular pathy (VSP) and nephropathy (NPP) in diabetes. It is hypothesized that these disorders which are the consequence to damaged endothelium could be recovered by Dajisentan, a novel dual endothelin receptor antagonist, developed by us as an investigated new drug. **METHODS:** Rat diabetes model was developed by ip streptozotocin and the assessment of cardiomyopathy, vascular and renal abnormality were conducted in the absence and presence of long term treatment with Dajisentan *po*. **RESULTS:** An enhanced ET-1, iNOS activity and a reduction in NO were involved in the formation of the CMP, VSP, and NPP in diabetic rats. After 28 d treatment of *po* Dajisentan and aminoguanidine, an antagonist to AGEs and iNOS, the recovery of the CMP, VSP and NPP was significant and mediated by a reduction of ET-1 levels, iNOS activity and oxidative stress, and an increase in NO. The attenuation of urinary albumin and histological lesion in the kidney were remarkable in the treated group against the diabetic model. **CONCLUSION:** The onset of the CMP, VSP, and NPP is attributed to the compromized endothelium in diabetic rats. An endothelin antagonist Dajisentan which is very effective to relieve the pathogenesis from hyperglycemia offers a new approach to treat diabetes via a dual blockade on the ETA and ETB receptors.

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**PC10 Influence of etomidate and sevoflurane on the angiotensin II- induced aorta vasoconstriction of WKY rats: role of intracellular calcium mobilization.**

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**KEY WORDS** angiotensin II; etomidate; sevoflurane; vasoconstriction

**AIM:** To investigate the influence of two anaesthetic agents, etomidate (ETO) and sevoflurane (SEVO) i) on relaxation of angiotensine II (AngII)- pretreated aorta, from Wistar Kyoto (WKY) rat, and ii) on vascular smooth muscle cells calcium mobilization in relation with the modification of intracellular calcium stores implicated in these effects. **METHODS:** ETO ($1\times10^{-7}$-$1\times10^{-3}$ mol/L) and SEVO (0.5 %-4 %) have been tested on the maximal isometric tension induced by Ang II ($1\times10^{-6}$ mol/L) of endothelium free aortic rings and on calcium release in Fura-2 loaded cultured aortic vascular smooth muscle cells isolated from 6-weeks-old WKY rats, using fluorescent imaging microscopy. Caffeine ($4\times10^{-4}$ mol/L) has been used to deplete the intracellular calcium stores. Results are expressed in % of the control to AngII-induced response, and analysed by ANOVA. **RESULTS:**ETO produced a concentration-dependent inhibition of the tension induced by AngII ($EC_{50} = 9.1\times10^{-5}$ mol/L, $P<0.01$). In the presence of caffeine, the kinetics of inhibition was modified without modification of the $EC_{50}$ value ($8.9\times10^{-5}$ mol/L). SEVO alone did not decrease the tension induced by AngII. In the presence of caffeine, the AngII- induced tension is significantly decreased between 1.5 % to 4 % SEVO ($P<0.05$). On vascular smooth muscle cells, ETO ($1\times10^{-6}$ and $1\times10^{-4}$ mol/L) decreased the calcium release from internal stores ($P<0.05$ and $P<0.01$) in presence of extracellular calcium and decreased significantly the calcium influx at $1\times10^{-5}$ mol/L ($P<0.01$). SEVO induced a concentration-dependent decrease of Ang II-induced calcium mobilization in presence of extracellular calcium ($P<0.05$). This effect is associated with a decrease of intracellular calcium release ($P<0.05$) and of calcium influx ($P<0.05$). **CONCLUSION:** This work exhibits the inhibitory effects of sevoflurane and etomidate on the angiotensin II- induced tension of aortic rings and on calcium mobilization of vascular smooth muscle cells of WKY rats. Our results suggest different transduction pathways for these two anaesthetic agents. Furthermore, ETO appears implicated in a modification of the intracellular calcium mobilization, that could explain the hypotension induced by this compound in case of hypovolemia.

## PC11 Dose-effect relationships of intravenous enoxaparin in patients undergoing percutaneous coronary intervention: a population and bayesian approach based study

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**KEY WORDS** anticoagulants; pharmacokinetics; catheterization; angioplasty; heparin

**AIM:** Recent studies have suggested that intravenous enoxaparin can be used as an alternative therapy in patients percutaneous coronary intervention (PCI); yet the optimal regimen is to be defined. **METHODS:** Anti-Xa activities were measured in 556 patients who received a single 0.5 mg/kg dose of enoxaparin intravenously immediately before PCI. A population pharmacokinetic model was developed (NONMEM) and using a bayesian approach, individual anticoagulation profiles were then estimated. Effects of higher doses (0.75 mg/kg and 1 mg/kg) and/or additional bolus after the initial administration were similarly studied. **RESULTS:** Enoxaparin anti-Xa-time profiles were best described by a one-compartment-model with first-order kinetics. Mean population parameters were $K_{in}$ 17.9 $h^{-1}$, $V_d$ 4.1 L, and $K_{out}$ 0.31 $h^{-1}$. With a single bolus of 0.5 mg/kg, 546 patients (98 %) reached an effective anticoagulation level (>0.5 anti-Xa IU/mL) and 19 patients (3.5 %) reached levels above 1.5 IU/mL. While greater doses (0.75 mg/kg and 1 mg/kg) showed a prolonged duration of effective anticoagulation (4.2 and 5.4 h, respectively) compared to the 0.5 mg/kg bolus (2.6 h) and an important increase of over-anticoagulated patients (35 % and 82 %, respectively). For delayed and/or prolonged procedures, patients could be administered a second bolus of half of the initial injection, between 90 min to 2 h later, in order to maintain similar anticoagulation profile levels. **CONCLUSION:** A single 0.5 mg/kg intravenous dose of enoxaparin is effective and should be safer compared with greater doses for anticoagulation in patients undergoing elective PCI. An additional second bolus could be proposed in patients if needed.

## PC12 Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density and improve heart function in a rat cellular cardiomyoplasty model

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**KEY WORDS** cell therapy; stem cells; angiogenesis

**AIM:** Cellular cardiomyoplasty is promising for improving postinfarcted cardiac function. Over the past decade, a variety of cell types have been proposed including mononuclear bone marrow cells. The latter contains different lineages including mesenchymal stem cells (MSCs). The aim of this study was to analyse the differentiation pathways of engrafted syngenic mesenchymal progenitor cells (MPCs) obtained in culture from bone marrow MSCs and their effects on the left ventricular function in a rat model of myocardial infarction. **METHODS:** Bone marrow was obtained from Lewis inbred rats and then cultured. MPCs were isolated by bone marrow cell adherence. *In vitro* differentiation was assessed by immunohistochemical analysis using anti-alpha SM actin, anti-vimentin, anti-beta actin, anti-CD31, anti-myosin heavy chain (MHC), and anti-desmin. A ligation model

of left coronary artery was used. Seven days after ligation, MPCs labeled with 4,6-diamidino-2-phenylindole (DAPI) were injected into the infarcted myocardium ($n$=8). For control, culture medium was injected ($n$=8). Transthoracic echocardiography was performed 6 d after myocardial infarction (baseline measurements) and 30 d after cell implantation. For assessment of the cell grafting and vascular density, rats were sacrified 30 d after implantation. Histological and immunohistochemical analysis were performed using the same antibodies as above. **RESULTS:** *In vitro*, all the cells express vimentin showed their mesenchymal origin. Moreover, they expressed alpha SM actin and beta actin filaments which were respectively specific to smooth muscle and non-muscle cells, but they did not express skeletal MHC and desmin. DAPI-labeled cells were observed in the luminal face of endothelium vessels expressing the endothelial marker CD31 and not alpha SM actin or desmin. Many loci positively stained for alpha SM actin were observed which were discrete positively stained for desmin. Furthermore, vessel density was augmented in the MPC group in comparison with the control group (8.4±0.9/0.2 mm² *vs* 5.4±0.9/0.2 mm²; $P$=0.001). After 30 d, echocardiography showed an improvement on left ventricle ejection fraction (42 %±2.7 % *vs* 28 %±1.5 %; $P$=0.002) and fractional shortening (16.7 %±1.3 % *vs* 10.4 %±0.7 %; $P$=0.003) in the MPCs compared to the control group. **CONCLUSION:** The implantation of syngenic MPCs into a rat model of myocardial infarction was demonstrated. Some engrafted cells appeared to differentiate into endothelial cells. MPC engraftment seemed to contribute to the improvement of the cardiac function.

### PC13 Cell based assay for hypoglycemic drugs screening

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**KEY WORDS** hypoglycemic drug; cell-based assay; glucose consumption

**OBJECTIVE:** To establish a cell based assay for hypoglycemic drugs. **METHODS:** The five cell lines, BALB/c3T3, HepG2, NIH3T3, Bel7402, and L929 were incubated with insulin (0-125 n mol/L) for 48 h. Their sensitivities to insulin were studied by detecting glucose consumption. The dose-response and time-response relationship between the sensitive cell line (BALB/c 3T3) and insulin were observed. The cell proliferation induced by insulin was measured by MTT. **RESULTS:** BALB/c 3T3 was preferably sensitive to insulin than the other four cell lines. Insulin elevated glucose uptake in BALB/c 3T3 in a concentration- and time- manner. Insulin 30 nmol/L could accelerate glucose consumption of BALB/c 3T3 by 30 % and increase the proliferation of BALB/c3T3 in a time-dependent manner. Two thousand samples were screened by detecting glucose consumption of BALB/c 3T3 and the hit rate was 0.5 %. **CONCLUSION:** The cell based assay by detecting glucose consumption in BALB/c 3T3 was suitable for high through put screening and was feasible to identify hypoglycemic drugs.

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### PC14 Experimental study on regulation of blood glucose by 1400w (*N*-3-aminomethyl-benzyl-acetamidine)

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**KEY WORDS** hyperglycemia; iNOS; AG; 1400w

**AIM:** To study the prevention and cure of *N*-3-aminomethyl-benzyl-acetamidine (1400w), a high selective iNOS inhibitor, on hyperglycemic mellitus induced by STZ, which inhibits NOS activity 100 times stronger than equal dose of AG in rat. **METHODS:** Hyperglycemic mice model was established by 130 mg/kg STZ. Blood glucose and serum NO concentration was measured by blood glucose meter and NO kit separately. Effects of 1400w on blood glucose of normal mice, on the time course hyperglycemic response of STZ and on hyperglycemic mellitus induced by STZ were observed. Moreover, reverse effects of isosorbide (NO donor) to AG and 1400w on the time course of hyperglycemic response of STZ and on serum NO concentration induced by STZ were observed. In prevention experiment, mice were pretreated with 1400w (10 mg/kg), AG (50 mg/kg) or saline, 1 h before STZ, and blood samples were analyzed before 0 h and after 24, 48, and 72 h. In cure experiment, mice were pretreated with STZ 72 h before, then treated with similar dose of 1400w, AG or saline, and blood samples were analyzed before 0 h and after 0.5, 1, 2, and 4 h. **RESULTS:** The 1400w could neither prevent the time course of hyperglycemic response of STZ in mice nor cure hyperglycemic mellitus induced by STZ. **CONCLUSION:** 1400w has no effect on prevention and cure experimental hyperglycemia. Effects of AG on the time course of hyperglycemic response of STZ in mice is independent onNO pathway.

### PC15 Effects of 2-(1-hydroxypentyl)-benzoate on platelet aggregation and thrombus formation in rats

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**KEY WORDS** 2-(1-hydroxypentyl)-benzoate; platelet aggregation; thrombus formation; cerebral ischemia.

**AIM:** Potassium 2-(1-hydroxypentyl)-benzoate (*dl*-PHPB), derivated from 3-*n*-butylphthalide (NBP), is a newly synthesized compound which is under development as a therapeutic

drug for cerebral ischemia. In the present study, we used *ex vivo* platelet aggregation method and *in vivo* arteriovenous shunt model to evaluate the antiplatelet and antithrombotic effects of *dl*-PHPB. **METHODS:** In the test of platelet aggregation, *dl*-PHPB was given orally or intravenously, then platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared. Platelet aggregation induced by ADP, arachidonic acid (AA), and collagen (COL) was determined by Born's method. The antithrombotic activity of *dl*-PHPB was observed in the rat A-V shunt model after *dl*-PHPB was given orally. The extracorporeal circulation between the left jugular vein and right common carotid artery was maintained for 15 min, and the thrombus forming was determined. **RESULTS:** *dl*-PHPB significantly inhibited platelet aggregation induced by ADP, AA, and COL in a dose-dependent manner when it was given orally (12.9-129.5 mg/kg). The inhibitory potency was similar to NBP and aspirin (ASP). At the highest dose, *dl*-PHPB inhibited ADP, AA, or COL-induced aggregation by 26 % ($P<0.01$ *vs* control), 20 % ($P<0.01$) and 12 % ($P<0.01$), respectively. The inhibition on platelet aggregation was also observed after *dl*-PHPB (1.29-12.9 mg/kg) was intravenously administered. At the highest dose, *dl*-PHPB inhibited ADP, AA and COL- induced platelet aggregation by 30 % ($P<0.01$ *vs* control), 35 % ($P<0.01$) and 19 % ($P<0.01$) , respectively. The time-course of above effects showed the maximal inhibition on platelet aggregation appeared at 1 h after oral administration and 30 min after intravenous injection. *dl*-PHPB caused dose-dependent inhibition of thrombus formation in the rat A-V shunt thrombosis model. At doses of 12.9, 38.8, and 129.5 mg/kg, *dl*-PHPB decreased thrombus weight significantly from a 48.2 mg (control) to 39.9, 36.8, and 31.8 mg, respectively ($P<0.05$ *vs* control). **CONCLUSION:** *dl*-PHPB is an orally and intravenously antiplatelet and antithrombotic agent and therefore it might be useful for treatment of ischemia stroke.

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### PC16 Streptozotocin induced diabetes in lyon hypertensive rats

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**KEY WORDS** rats; hypertension; genetics; diabetes

**AIM:** Lyon hypertensive (LH) rats, compared to their normotensive controls (LL) exhibit an increased blood pressure (BP) associated with a marked proteinuria and a metabolic syndrom including elevated plasma lipids and insulin/glucose ratio. The aim of the present work was to determine wether a type 2 diabetes could be induced in LH rats so as to obtain a model suitable for study of the relationships between diabetes and hypertension. **METHODS:** Various doses of streptozotocin (STZ) were administered ip in sevaral groups of 2 d-old male LH and LL rats. Starting at the age of 4 weeks plasma glucose was measured once a month using an enzymatic method. BP was determined by plethysmography. At 16 weeks of age, 24 h urines were collected to measure the excretion of proteins. Finally, an oral glucose tolerance test was performed (2 g/kg given by gavage). **RESULTS:** Neonatally given STZ did not change BP of LH and LL rats. Plasma glucose in fasted rats increased shortly before returning to baseline in 16 week-old rats. The urinary excretion of proteins was not changed in LL rats while it was dose-dependently elevated in LH animals (141±13; 182±43; 193±13; 223±19 mg/d in rats given 0, 50, 75, and 100 mg/kg of STZ respectively). **CONCLUSION:** LH rats are more sensitive than LL controls to the effects of neonatally given STZ. The use of STZ75 mg/kg in LH rats allows to obtain a model which associates spontaneous hypertension to a type 2 diabetes.

### PC17 Effects of temperature and intracellular sodium, ATP and pH on $Na^+$-$Ca^{2+}$ exchange currents of intact guinea-pig myocytes

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**KEY WORDS** $Na^+$-$Ca^{2+}$ exchange currents; temperature; ATP; ions

**AIM:** The $Na^+$-$Ca^{2+}$ exchange is a major pathway for removal of cytosolic $Ca^{2+}$ in cardiac myocytes. There have been many researches reporting about the effects of temperature, intracellular sodium, ATP, and pH on $Na^+$-$Ca^{2+}$ exchange currents. But most of these researches were made with giant-patch voltage-clamp technique or with $Ca^{2+}$ flux studies in sarcolemmal vesicles. During myocardial ischemia-reperfusion, these factors would change, the effects of temperature, intracellular sodium, ATP and pH on $Na^+$-$Ca^{2+}$ exchange currents of intact guinea-pig myocytes were investigated in this study. **METHODS:** The whole-cell patch-clamp technique was used for recording $I_{Na/Ca}$ in isolated guinea-pig ventricular myocytes. **RESULTS:** At room temperature, when pipette solution contained 5 mmol/L ATP, 25 mmol/L $Na^+$ and $pH_i$ (7.4), there were nearly no outward current at +50 mV and slightly inward or outward current at -80 mV. Pipette solution with 25 mmol/L $Na^+$, without ATP and $pH_i$ (6.8), the result was similar. At 34-37 ℃, pipette solution with 5 mmol/L ATP, 25 mmol/L $Na^+$ and $pH_i$ (7.4), the outward current was 5.3 pA/pF at +50 mV and the inward current was -2.4 pA/pF at -80 mV. Pipette solution with 25 mmol/L $Na^+$, without ATP and $pH_i$ (6.8), the outward current was 3.6 pA/pF at +50 mV and the inward current was -1.1 pA/pF at -80 mV, both currents decreased obviously. Pipette solution with 100 mmol/L $Na^+$, without ATP and $pH_i$ (6.8), the outward current was 4 pA/pF at +50 mV and the current was outward at -80 mV. **CONCLUSION:** At room temperature, intracellular ATP and pH had no influence on $Na^+$-$Ca^{2+}$ exchange currents. Intracellular acidification and ATP depletion could decrease $Na^+$-$Ca^{2+}$ exchange currents at body temperature, and higher intracellular sodium could active reverse exchange persistently to induce calcium overload, leading to

apoptosis and necrosis of myocytes.

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**PC18 5-Hydroxytryptamine transporter was involved in monocrotaline-induced pulmonary hypertension in the rat***

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**KEY WORDS** 5-hydroxytryptamine; 5-HT transporter; pulmonary hypertension

**AIM:** To compare the expression of 5-hydroxytryptamine transporter (5-HTT) gene in PAs from normal and chronic "inflammatory" PHT rats. **METHODS:** MCT-treated rats were used as a model for chronic PHT. Arterial pressure and pulmonary arterial pressure were measured with a pressure transducer connected to a polygraph and recorded with a thermal recorder. The right ventricle (RV), septum and the left ventricle (S+LV) were dissected and the ratio of RV weight to S+LV weight was calculated as index of the right ventricular hypertrophy. RT-PCR to identify mRNA expression of 5-HT transporter in rat PAs was performed. 5-HT transporter was assayed by RT-PCR. **RESULTS:** Chronic PHT model in rats induced by MCT was successfully established at the end of 3 weeks and confirmed by a significant increase of mean pulmonary arterial pressure (1.42±0.23 kPa *vs* controls 2.19±0.37 kPa, $P<0.01$) and right ventricular hypertrophy index (0.46±0.07 *vs* controls 0.32±0.03, $P<0.01$). The maximum contraction to 5-HT was enhanced in MCT rats *vs* controls, with an $E_{max}$ value increased from 68.2 %±16.9 % (response to 50 mmol/L KCl) to 105.9 %±15.2 % ($P<0.01$) and the $pEC_{50}$ value were 3.53±0.28 for controls and 3.42±0.31 for MCT rats. The thickness of pulmonary vascular medial walls was increased in MCT rats (17.3±2.6 μm *vs* controls 3.9±0.5 μm, $P<0.01$). The ratio of the PCR products of 5-HTT gene to those of β-actin gene was much higher in MCT rats than in control rats (2.0±0.4 *vs* control 1.2±0.4, $P<0.01$). 5-HTT mRNA expression of pulmonary arteries correlated with the thickness of pulmonary vascular medial walls in rats ($r = 0.741$, $P<0.01$). **CONCLUSIONS:** MCT-induced pulmonary vascular remodeling was accompanied with an increase in contractile response of pulmonary artery rings to 5-HT. 5-HTT mRNA expression in the PAs was increased and the level of 5-HTT mRNA expression was correlated with the thickness of pulmonary vascular medial walls. Taken these evidences from hypoxia- and MCT-induced pulmonary hypertensive animal models together, we considered that 5-HTT played a key role in the pathophysiological processes of pulmonary hypertension and this may provide a potential therapeutic target for this disease.

**PC19 Therapeutic effect of *Oenanthe javanica* flavone on streptozotocin-induced diabetic mice**

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**KEY WORDS** *Oenanthe javanica* flavone; blood glucose; streptozotocin; islets of Langerhan; insulin

**AIM:** To observe the therapeutic and preventive effects of *Oenanthe javanica* flavone (*Oj*F) on streptozotocin induced diabetic mice. **METHODS:** Mice were given injected ip with streptozotocin (STZ) 150 mg/kg to induce diabetic model. The diabetic mice were given *Oj*F 50 mg/kg, 100 mg/kg,ig. The blood samples were taken at 1, 5 h after treatment for observing the effects of *Oj*F. After the latter time point (5 h) the blood were taken by exsaguinating, then the serum was centrifugated and retained to determine insulin (Ins). The mice were prevented with *Oj*F for 3 d, then injected ip with STZ 105 mg/kg. After intoxication, the mice were treated with *Oj*F for another 3 d. Blood glucose (Glu) was determined by using One Touch Basic Glu meter. Pathological examination and serum insulin were determined by HE stain and radio-immunoassay, respectively. **RESULTS:** The treatment with *Oj*F (50 and 100 mg/kg) could decrease the Glu significantly ($P<0.05$; $P<0.01$). The pretreatment plus treatment with OjF (50 and 100 mg/kg) showed the antagonism against the STZ-induced hyperglycemia ($P<0.01$, $P<0.001$). Under the light microscopy, the size and cell number of islets were reduced in control group, while those in *Oj*F -treating groups manifested approximate normal pathology. **CONCLUSION:** *Oj*F has the effects of hypoglycemication and islet-protection in STZ diabetic mice. The hypoglycemic action may be produced by promoting release of Ins from B cells in islet.

**PC20 Effect of iptakalim hydrochloride on hemodynamics**

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**KEY WORDS** iptakalim hydrochloride; hemodynamics; hypotension

**AIM:** To study the effect of iptakalim hydrochloride (Ipt) on hemodynamics. **METHODS:** Effect of Ipt on hemodynamics were studied in anesthetized nomotensive dogs, conscious nomotensive rats (NTR), and stroke prone spontaneously hypertensive rats (SHRsp), respectively. **RESULTS:** In pentobarbital anesthetized nomotensive dogs, Ipt at doses of 0.125, 0.25, 0.5,1.0, and 2.0 mg/kg iv could dose-dependently decrease blood pressure (BP), with the decrease of systolic BP equivalent to that of diastolic BP. Ipt of 1.0 to 2.0 mg/kg iv caused significant reductions of heart rate (HR), left ventricular systolic pressure (LVSP), $+dp/dt_{max}$, $-dp/dt_{max}$, the physiologic velocity of contractile element shorting (*V*pm), the aorta blood flow (ABF), and coronary blood flow (CBF), which remained to be unchanged at

doses of 0.125 to 0.5 mg/kg. Under the same conditions, BP was reduced accompanied by the enhancement of HR, the mild decrease of LVSP, $+dp/dt_{max}$, *V*pm, $-dp/dt_{max}$, and the mild increase of ABF, CBF, by Pin of 0.25 mg/kg iv. In conscious SHRsp rats, HR, ventricular contractile wave, heather index and cardiac output were decreased with total electromechanical systole time, left ventricular ejection time and left ventricular diastolic time prolonged by Ipt of 0.5 mg/kg iv, whereas those parameters remained to be unchanged in NTR by the same dose of Ipt. **CONCLUSION:** The antihypertensive characteristics of Ipt revealed a sure, steady and long lasting effect and hemodynamic properties of Ipt were some what different from those of Pin. Moreover, the cardiovascular effects of Ipt were related to the level of blood pressure (nomotensive and hypertensive) and the state (conscious and anesthetized) of the experimental animals.

### PC21 Baroreflex dysfunction promotes the induction of atherosclerosis in rats

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**KEY WORDS** arterial baroreflex; atherosclerosis; rat; sinoaortic denervation

**AIM:** To testify the hypothesis that arterial baroreflex dysfunction promotes the induction of atherosclerosis. **METHODS AND RESULTS:** Experiment 1: The baroreflex sensitivity (BRS) was measured in 30 SD rats in conscious state with a computerized blood pressure monitoring system. Four weeks later, the rats were fed with a high-cholesterol diet for 8-week duration to induce atherosclerosis. The hearts and aortae were removed for pathological examination and the scores of coronary and aortic atherosclerosis were determined. A negative correlation was found between BRS and the scores of coronary ($r$=-0.464, $P$<0.01) or aortic atherosclerosis ($r$=-0.524, $P$<0.01) in SD rats. Experiment 2: Sinoaortic denervation (SAD) and sham operation were performed in 20 SD rats followed by 8-week high-cholesterol diet. The atherosclerotic scores in SAD rats were significantly higher than those in sham-operated rats. Experiment 3: The expression of CRP, ICAM-1, and VCAM-1 in coronary artery and aorta was examined by immunohistochemistry and Western blotting methods in SAD and sham-operated rats. It was found that the expression of CRP, ICAM-1 and VCAM-1 increased in SAD rats significantly. **CONCLUSION:** It can be concluded that baroreflex dysfunction promotes the development of atherosclerosis in SAD rats, and inflammation is involved in this process.

### PC22 Relationship between protecitve effect of probucol on endothelial cells and asymmetrical dimethylarginine levels

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**KEY WORDS** probucol; oxidative-low density lipoprotein; asymmetric dimethylarginine; dimethylarginine dimethylaminohydrolase

**AIM:** To investigate the relationship between protective effect of probucol on endothelial cells and endogenous nitric oxide synthase inhibitor levels. **METHODS:** Endothelial cells were treated with oxidative-low density lipoprotein (ox-LDL) (100 mg/L) or lysophosphatidyl choline (LPC) (5 mg/L) for 48 h, and the release of lactate dehydrogenase (LDH), levels of nitric oxide (NO), malondialdehyde (MDA), and asymmetric dimethylarginine (ADMA) in conditioned medium were determined. The protein expression of protein arginine methyltransferases-I (PRMT-I) and the activity of dimethylarginine dimethylaminohydrolase (DDAH) in endothelial cells were measured. **RESULTS:** Incubation of ox-LDL (100 mg/L) or LPC (5 mg/L) for 48 h markedly increased the release of LDH, levels of ADMA and MDA, and the protein expression of PRMT-I, concomitantly with a significant decrease in the activity of DDAH and the level of NO. Pretreatment with probucol (1.0, 2.5, or 5.0 μmol/L) markedly attenuated the increased levels of LDH, ADMA and MDA, inhibited the increase in expression of PRMT-I, and reduced levels of NO and activity of DDAH by ox-LDL or LPC. **CONCLUSION:** Probucol protects endothelial cell against damages induced by ox-LDL or LPC, and the beneficial effects of probucol may be related to the reduction of ADMA concentration via decreasing PRMT-I expression and increasing DDAH activity.

## Cancer Chemotherapy

### PT01 Effect of age on 6-mercaptopurine metabolic profile during the maintenance phase in children with acute lymphoblastic leukaemia

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**KEY WORDS** 6-mercaptopurine; pharmacogenetics; acute lymphoblastic leukaemia

**INTRODUCTION:** 6-Mercaptopurine (6-MP) is a thiopurine analogue administered for the treatment of acute lymphoblastic leukaemia (ALL). It is an inactive pro-drug that undergoes extensive metabolism resulting in the formation of active metabolites 6-thioguanine nucleotides (6-TGN) and inactive 6-mercaptopurine methylated metabolites (6-MMP) under the genetic control of the enzyme thiopurine methyltransferase (TPMT). 6-MP metabolic profile (6-MMP/6-TGN) was proposed as a tool to optimize therapeutic response while monitoring the risk for drug induced toxicity. **METHODS:** Fifty-two children aged 6.7±4.

1 years at the time of ALL diagnosis, treated according to the protocol of EORTC 58951, were included. TPMT genotype was determined before the initiation of treatment. During maintenance therapy, the individual daily dose of 6-MP (50 mg/m$^2$ per day) was adapted to maintain white blood cell count within the therapeutic range of 2000-3000 cells/mm$^3$ and 6-MMP/6-TGN ratio was measured by HPLC every month. **RESULTS:** Fourty-seven children were homozygous wild type for TMPT activity while 5 children were heterozygous. Due to TPMT pharmacogenetics, impact of long-term 6-MP metabolism on 6-MP metabolic profile during maintenance therapy was only analyzed in homozygous patients. As prognosis is dependant on age, patients were divided into two groups: younger than 6 years ($n$=30); 6 years or older ($n$=17). A repeated measures analysis of variance showed that when the whole group of children was considered, 6-MMP/6-TGN ratio did not vary according to age ($P$=0.14) while a significant difference in the evolution of 6-MMP/6-TGN ratio was observed between the two age groups ($P$=0.02) with repeated 6-MP administrations. **CONCLUSION:** In the patients of homozygous wild type for TPMT activity, and using a repeated measure model, changes in the 6-MMP/6-TGN ratio was significantly different between patients younger and older than 6 years. This could suggest a possible saturation of the 6-TGN pathway with repeated 6-MP administrations in the younger patients. The impact on 6-MP efficacy and toxicity remains to be evaluated.

**PT02 Cell density mediated pericellular hypoxia and $CoCl_2$ induced hypoxia leads to AQP1 induction under isotonic conditions *in vitro***

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**KEYWORDS** aquaporin1; hypoxia; p38 MAPK; PKC; calcium

Recently, many reports have linked aquaporin 1 (AQP1) protein overexpression with tumorigenesis, tumor growth and progression, but the underlying mechanisms on regulating of AQP1 protein expression are still not clear. We hypothesized that hypoxia might play an important role in AQP1 induction during tumorigenesis as well as at the late stages of tumor development. To prove this hypothesis, we studied the AQP1 protein expression and its regulation by using laser confocal fluorescence microscope (LCFM) and flow cytometric technologies in a prostate cancer cell line - PC-3M. We found that AQP1 was induced under density-induced pericellular hypoxia and $CoCl_2$-induced hypoxia, and that such induction specifically happened at the transcription level. Further investigations revealed that p38 MAPK was an important signal transducer in AQP1 induction; PKC and intracellular calcium were also responsible through activating p38 MAPK pathway. In addition, we demonstrated that the mechanism controlling AQP1 induction in dense cultures was, however, dependent on lowered $O_2$ tension. Moreover, in dense culture, secretory proteins could regulate AQP1 induction indirectly as conditioned media from dense culture were observed to upregulate AQP1 expression in sparse culture. In conclusion, the results suggested that AQP1 could be induced by hypoxia at transcription level through various mechanisms, and that this regulation is dependent on p38 MAPK, PKC and calcium, as well as oxygen tension in the media.

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**PT03 Biodistribution profiles of recombinant anti-breast cancer immunotherapeutic infusion protein HSP-MUC1 vaccine after subcutaneous in tumor-bearing mice**

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**KEY WORDS** HSP-MUC1; distribution; tumor-bearing mice

**AIM:** To study biodistributional profiles of recombinant anti-breast cancer immunotherapeutic fusion protein HSP-MUC1 vaccine after subcutaneous in tumor-bearing mice. **METHODS:** HSP-MUC1 of variant tissue was detected by $^{125}$I-HSP-MUC1 combined with trichloroacetic acid (TCA) precipitation or size-exclusive high performance liquid chromatography (SHPLC). **RESULTS**: $^{125}$I-HSP-MUC1 distributed widely. The highest concentration was found in regional lymph node, the second was in injection site and the lowest is in encephalon. The order of the AUC to TCA precipitation part from low to high is encephalon, fat, eyeball, pancreas, intestinal contents, heart, spleen, small intestine, liver, ovaries, muscle, mesenteric lymph node, bladder, urine, thymus, stool, tumor, blood serum, lung, kidney, adrenal gland, glandula angularis, injection site, and local lymph node. **CONCLUSION:** The concentration of regional immune organ and injection site in all tissues was the higher than that of other tissues. This result accorded with the hypothesis of mechanism for therapeutic vaccine HSP-MUC1.

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**PT04 Lovastatin potentiates antitumor activity and improves hemorheology in lewis lung carcinoma in C57 mice**

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**KEY WORDS** lovastatin; hemorheology; lewis lung carcinoma

**AIM:** Lovastatin is commonly used in the clinic to treat hypercholesterolemia. The aim of the present studies was to investigate whether lovastatin had an antitumor activity on lewis lung carcinoma and evaluate whether lovastatin had a direct effect on hemorheology in tumor model. **METHODS:** The C57 mice which with lewis lung carcinoma were treated with lovastatin at concentrations of 10, 20, and 40 mg/kg ig for 21 d. We examined the antitumor properties of lovastatin on the lewis lung carcinoma, a highly metastatic murine tumor model. **RESULTS:** Treatment significantly reduced tumor formation and metastatic dissemination to the lungs from established oxter tumors ($P$<0.01). Lovastatin-treated mice also exhibited decreased viscosity in blood ($P$<0.05) and enriched the electric charge on rbc ($P$<0.05). **CONCLUSION:** Lovastatin is effective in slowing the growth of tumor formation and metastasis, at the same time, lovastatin ameliorates the hemorheology in tumor model. These *in vivo* results support further investigation of lovastatin as an antitumor agent in animal models

### PT05 Anti-tumor activity of a lignanic compound from *Schisandrapropinqua* (Wall) Baill, var sinensis Oliv

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**KEY WORDS** anti-tumor; cytotoxicity; flow-cytometry; PTK

**AIM:** To discover anti-tumor activity of a lignan from *Schisandrapropinqua* (Wall) Baill, varsinensis Oliv. **METHODS:** The cytotoxic activity of compound IE2503 was investigated on several cancer cell lines including solid tumor (HepG2), blood tumor (HL-60), drug resistant tumor (R-HepG2) and one normal cell line NIH3T3. To further prove the apoptosis and investigate the specific cell cycle distribution of HepG2 and HL-60 induced by IE2503, its effect on the cell cycle progression of HepG2 and HL-60 were determined by flow cytometry. And the method of ELISA coupled with HRP system was used to detect the activity of protein tyrosine kinase in cytoplasm. **RESULTS**: Compound IE2503 showed relatively selective cytotoxicity on cancerous cells based on the higher $IC_{50}$ values of them on normal cells than that on tumor cells, and it brought out similar $G_2$/M cell cycle arrest in HepG2 and HL-60 cells. As a specific kinase inhibitor, compound IE2503 decreased the activity of protein tyrosine kinase. **CONCLUSION:** Compound IE2503 is potential anti-tumor agents on both parental and drug resistant tumors with low toxicity. It exerts cytotoxic activity via apoptosis and $G_2$/M arrest. Furthermore, inhibition of PTK is one of the probably mechanisms of anti-tumor activity.

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### PT06 Comparison of the antitumor activity on spore polysaccharides (*Gl*-SP) and broken spore polysaccharides (*Gl*-BSP) isolated from *Ganoderma lucidum*

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**AIM:** To compare the antitumor activity of spore polysaccharides (*Gl*-SP) and broken spore polysaccharides (*Gl*-BSP) from spores of *Ganoderma lucidum* (Leyss ex fr) Karst. **METHODS:** BALB/c mice were implanted with Sarcoma 180 and administered intragastrically with *Gl*-SP or *Gl*-BPS (50, 100, 200 mg/kg) respectively for 14 d. At the end of experiment, the tumor were removed and weighted. At the same time, spleens of tumor-bearing mice were prepared to observe the effect of *Gl*-SP and *Gl*-BSP on the proliferation of lymphocytes induced by mitogen, the cytotoxic activity of NK cells with MTT assay and the serum level of TNF-α were detected by biological assay. The effect of *Gl*-SP, *Gl*-BSP and *Gl*-SP-, *Gl*-BSP-treated serum on proliferation of human lung carcinoma cell line (PG) and the effects of these two kinds of polysaccharides on mixed lymphocyte culture reaction (MLR), lymphocyte proliferation induced by mitogen and the cytotoxic activity of spleen NK cells were also detected with MTT assay *in vitro*. **RESULTS:** *Gl*-SP and *Gl*-BSP (50, 100, and 200 mg/kg) inhibited the growth of implanted Sarcoma 180 in BALB/c mice *in vivo*. The inhibitory rates were 7.8 %, 18.1 %, 37.4 % ( $P$<0.05) and 30.7 %, 40.1 % ($P$<0.05), 59.9 % ($P$<0.01), respectively. *Gl*-SP and *Gl*-BSP (100 and 200 mg/kg) could promote the spleen lymphocytes proliferation induced by ConA or LPS, augment the NK cytotoxic activity, and increase the serum level of TNF-α in tumor-bearing mice. The level of TNF-α in *Gl*-BSP-treated serum was higher than that of in Gl-SP-treated serum at the same concentration. In further study, *Gl*-SP and *Gl*-BSP (0.1, 1, 10, 100, and 200 mg/L) directly adding to the cultured medium could not inhibit the PG cell proliferation *in vitro*, however *Gl*-SP (100, and 200 mg/kg)-treated serum or *Gl*-BSP (50, 100, and 200 mg/kg)-treated serum could significantly inhibit PG cell proliferation, the inhibitory rates were 5.6 % ($P$<0.05), 11.2 % ($P$<0.01) and 38.7 % ($P$<0.001), 49.3 % ($P$<0.001), 51.2 % ($P$<0.001), respectively. At the concentrations of 0.2-12.8 mg/L, *Gl*-SP and *Gl*-BSP were shown to promote MLR and to antagonize the inhibitory effect of mitomycin on MLR *in vitro*. *Gl*-BSP had much higher activity than *Gl*-SP. *Gl*-SP and *Gl*-BSP also could increase lymphocyte proliferation induced by Con A or LPS, and enhance the NK cytotoxic activity at the same concentrations, but there was no significant difference between these two kind of polysaccharides. **CONCLUSION:** *Gl*-SP and *Gl*-BSP can inhibit the tumor growth and improve the immune function in tumor-bearing mice *in vivo*, *Gl*-BSP shows much higher bioactivities than *Gl*-SP. The antitumor activity of *Gl*-SP

and *Gl*-BSP may be related to its immunomodulating effects.

## Neuropharmacology (PN01-PN19)

### PN01 Memory enhancing properties of acutumine, an alkaloid isolated from menispermum dauricum

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Certain alkaloids structurally related to acutumine have been shown to possess extensive pharmacological activities in both the nervous and cardiovascular systems, although no report has been published concerning such activities with acutumine. The present study was undertaken in order to determine if acutumine possessed memory enhancing properties. Using a water-maze task in mouse, acutumine (40 to 80 mg/kg, *po*) has been found to partially reverse scopolamine-induced spatial performance deficit. Moreover, in the social recognition test in the rat, acutumine (3-30 mg/kg, ip) improved in a dose-dependent manner the memory retention. The minimal effective dose was 10 mg/kg and a higher effect was observed at 30 mg/kg. Moreover, at a dose of 30 mg/kg ip, acutumine was systematically active on memory retention in the case of retroactive inhibition of social memory. Furthermore, acutumine (10-100 mg/kg, ip) also induced an improvement of memory retention in the object recognition test in the rat. The positive effects of acutumine on memory retention in social recognition, and object recognition in Wistar rats and the anti-amnesic properties of acutumine in the mouse water-maze, are in favour of a promising therapeutic potential of acutumine in age-related cognitive disorders.

### PN02 Research for SIPI0856 in receptor-binding of the 5-HT

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**KEY WORDS** SIPI0856; radio ligand-receptor binding assay; 5-$HT_1$ and 5-$HT_2$ receptors

**AIM:** Using the radio ligand-receptor binding assay to study the combined effect of SIPI0856 with 5-$HT_1$ and 5-$HT_2$ receptors, then, to study the bio-effect of SIPI0856 using isolated organ. **METHODS:** Using [$^3$H]-5-HT as the specific ligand of 5-$HT_1$ receptor and [$^3$H]-spiperone as the specific ligand of 5-$HT_2$ receptor to draw the saturation curves of each. On the basis of the membrane protein concentration provided by the saturation test, we studied the competitive binding ability of SIPI0856. Using the method of isolated organ to study whether SIPI0856 have contracted effect on aorta of rabbit. **RESULTS:** The maximum binding density ($B_{max}$) for 5-$HT_1$ was 28.8 fmol/mg protein, the dissociation constant ($K_d$) was 7.66 nmol/L. In the ligand receptor competition test, the $IC_{50}$ for SIPI0856 was 1.584 μmol·$L^{-1}$, and the Hill coefficient (nH) was 0.96. In the ligand receptor saturation test of 5-$HT_2$, $B_{max}$ was 121 fmol/mg protein and $K_d$ was 5.91 nmol/L for 5-$HT_2$. In the ligand receptor competition test, the $IC_{50}$ for SIPI0856 was 1.0 μmol·$L^{-1}$, and the nH was 0.87. The method of isolated organ has shown that SIPI0856 have not the contracted effect for aorta ring of rabbit, but it can resist partly the contracted effect produced by 5-HT. **CONCLUSION:** SIPI0856 combined with single binding site of 5-$HT_1$ receptor and its binding with 5-$HT_2$ receptor is irregular, there are maybe negative interactions and several binding sites. SIPI0856 can partly antagonize the contraction of aorta ring produced by 5-HT.

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### PN03 The effects of orientvine stem [*Sinomenium acutum* (Thunb) et Wils] and sinomenine on withdrawal syndrome induced by morphine-dependence in animal models

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**KEY WORDS** orientvine stem; sinomenine; morphine-dependence; naloxone; withdrawal syndrome

**AIM:** To study the effects of orientvine stem [*Sinomenium acutum* (Thunb) et Wils] (QFT) and its active component, sinomenine on naloxone-precipitated withdrawal syndrome. **METHODS:** The isolated ileum of guenea pig was incubated with morphine (3 μmol·$L^{-1}$) at 37.5 ℃ for 4 h to form the morphine-dependent ileum and a contracture was elicited by the addition of naloxone (1 μmol·$L^{-1}$). The morphine-dependent ileum was treated with the extract of orientvine stem and sinomenin, respectively, and effects of drugs were observed. Mice and rats were given increasing doses of morphine to produce a physical dependence. A biased procedure induced the strong place preference. The effect of sinomenine (10, 30, and 60 mg·$kg^{-1}$, ip) and the extract of orientvine stem (20 g·$kg^{-1}$, ip) on the rewarding property of morphine was observed in a conditioned place preference(CPP) paradigm in mice. **RESULTS:** The extract of orientvine stem (1, 2, and 4 g·$L^{-1}$) and sinomenin (10, 50, and 250 μmol·$L^{-1}$) could significantly inhibit the naloxone-precipitated contracture in isolated ileum in a dose-dependent manner. After administration of the extract of orientvine stem (20 g·$kg^{-1}$) and sinomenin (60 mg·$kg^{-1}$) for 3 d, respectively, the jumping reactions, wrest body, forepaw tremor, "wet dog" shakes and body weight loss of morphine- dependent mice were decreased. The extract of orientvine stem (10 g ·$kg^{-1}$) and sinomenin (20 and 60 mg·$kg^{-1}$) could alleviate whithdrawal symptom of rats. Sin and the extract of orientvine stem significantly decreased the time of staying in morphine-pair compartment. **CONCLUSION:** Orientvine stem and sinomenin can effectively inhibit withdrawal syndrome induced by morphine-dependence in animal.

**PN04 *R*-(+)-ABP a novel derivative of 3-*n*-butyl-phthalide possesses anti-convulsant and neuroprotective properties in rodents**

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ABP is a novel phthalide derivative of 3-*n*-butyl-phthalide (NBP) synthesized at the Beijing Institute of Materia Medica. NBP was isolated from several plants including *Apium graveolens* Linn. The juice squeezed from fresh celery leaves has long been used in Southeastern China for the treatment of epilepsy, and NBP has been reported to possess anti-convulsant properties (Drugs Future 2000; 25: 16-23). The present study was performed in order to assess the potential anti-convulsant and neuroprotective properties of ABP, a novel phthalide derivative of NBP, using electroshock seizures test in NMRI mice and transient global forebrain ischemia in rats. *R*-(+)-ABP (50 mg/kg ip and 100 mg/kg *po*), in a dose-dependent manner, antagonized seizures induced by a maximal corneal electroshock in NMRI mice. Moreover, *R*-(+)-ABP was found to be more active than *S*(-)-ABP and NBP in this test. Transient (10 min) global forebrain ischemia was induced using the four-vessels occlusion model in Wistar rats. Seven days after ischemia, neuroprotective effects of pre-treatment (30 min before and 30 min after ischemia) with *R*-(+)-ABP (20 mg/kg ip) and *S*-(-)-ABP (20 mg/kg ip) were assessed histologically, in brain sections through the dorsal hippocampus, by counting viable CA1 neurons. Results demonstrated that *R*-(+)-ABP and S-(-)-ABP had neuroprotective effects in pre-ischemic treatment condition. *R*-(+)-ABP seems to be more active than *S*-(-)-ABP. These results indicate that *R*-(+)-ABP possesses anti-anticonvulsant and neuroprotective properties that could be related to the effects of *R*-(+)-ABP on calcium and sodium channels. Further studies are needed to better define the therapeutic potential of ABP as a neuroprotective drug.

**PN05 Treatment with dehydroepiandrosterone sulfate increases NR1 and NR2 subunits of NMDA receptors in rat hippocampus.**

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Neurosteroids are present in the central nervous system (CNS) and can be allosteric modulators of neurotransmitter receptors. One of them, the dehydroepiandrosterone sulfate (DHEAS) has been shown to modulate the NMDA receptor, a subtype of glutamate receptor. These NMDA receptors are known to be involved in long-term potentiation and in learning and memory (Tsien 2000). Moreover, DHEAS has been reported to have significant memory-enhancing effects *in vivo* in mice and this effect could be the result of the increase of NMDA receptors. **AIM:** To study the effect of DHEAS treatment on NMDA receptors in rat brain and to precise which type of NMDA receptor subunits are able to be modified. **METHODS:** Male Wistar rats (200-220 g) were treated with DHEAS 30 mg/kg, twice a day during 5, 10, 20, and 30 d, then killed by decapitation 2 h after the last injection. The brain were rapidly removed from the skull, quickly frozen in liquid isopentane at -40 °C, and kept at -80 °C until sectioned for autoradiographic and immunocytochemical study. For autoradiographic study, [$^3$H] dizocilpine ([$^3$H]-MK 801) (7 nmol·L$^{-1}$) was used as specific ligand of NMDA receptors. Binding was measured as relative optical density (OD) units. The NR1 and NR2 subunits of NMDA receptor was identified by immunocytochemichal study using peroxydase technique, and can be quantified by the SCION program developed at the NIH (USA). **RESULTS:** Under DHEAS treatment (5 d) the autoradiographic study shows an increase of the apparent number of [$^3$H]MK801 binding sites in hippocampal areas (field CA1, CA3, dentate gyrus lateral blade and medial blade) and in cortex layer IV by 22 %, 18 %, 13 %, 19 %, and 14 %, respectively (OD: 54.01±1.58; 33.37±2.1, 42.15±1.2; 43.13±1.7; 24.88±1.8) as compared to the control group ($P< 0.01$). After DHEAS treatment (5, 10, 20, and 30 d), we observed an increase of 15 % to 20 % of NR1 subunits of NMDA receptors in hippocampus area as compared to control group, using immunocytochemical technique. A slight but not significant increase was also observed for NR2 subunits. **CONCLUSION:** These findings, together with the fact that during ageing, a physiological decrease of NMDA receptors is observed in human, making it of great interest to prevent the decrease in NMDA receptors by treatment with DHEAS.

**PN06 Protective effects of *Ginkgo biloba* leaf extract on model rats of brain dysfunction induced by aluminum salt**

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**KEY WORDS** *Ginkgo biloba;* aluminum; learning and memory; acetylcholinesterase

**AIM:** To examine the protective effects of *Ginkgo biloba* leaf extract (*GbE)* on the learning and memory in brain dysfunction model induced by aluminum salt in rats, and to investigate potential mechanisms. **METHODS:** Wistar rats were given daily aluminum chloride 500 mg·kg$^{-1}$·d$^{-1}$ ig, for one month, followed by continuous exposure via the drinking water containing 1600 ppm aluminum chloride for up to 5 months. The ability of spatial learning and memory was tested by Morris water maze. The acetylcholinesterase (AChE) activity in serum was detected by chemical colorimetry**,** the expression of AChE in hippocampus was examined by immunohistochemistry (IHC), and quantitated and analyzed by BI2000 image analysis system. **RESULTS:**

Aluminum administration significantly increased escape latency and searching distance, an indication of brain dysfunction. *Gb*E treatment (50-200 mg·kg$^{-1}$·d$^{-1}$, ig) significantly protected against brain dysfunction induced by aluminum, as evidenced by decreased escape latency and searching distance compared with the Al alone group. *Gb*E treatment reduced the expressions of AChE in hippocampus of rats treated with aluminum in a dose-dependent manner ($P$<0.01 respectively). At the highest dose of *Gb*E (200 mg·kg$^{-1}$·d$^{-1}$), the immuno-stain for AChE was returned to normal level. However. The AChE activity in serum of the rats in treatment groups at the dose of 200 mg·kg$^{-1}$·d$^{-1}$ increased compared with the rats in model group ($P$<0.05). **CONCLUSION:** *Gb*E is effective in improving the ability of spatial learning and memory of aluminum-intoxicated rats. This protection appears to be relative to a decreased expression of AChE in hippocampus.

**PN07 Neuroprotective role of pseudoginsenoside-F11 on activated microglia induced by lipopolysaccharide**

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**KEY WORDS** mitogen-activated protein kinases; tumor necrosis factor; nitric oxide; central nervous system

**AIM:** In the present study, the neuroprotective effect and its possible molecular mechanisms of pseudoginsenoside-$F_{11}$ ($PF_{11}$), a saponin existed in American ginseng, on activated N9 microglia induced by lipopolysaccharide (LPS) were studied. **RESULTS:** The results showed that $PF_{11}$ inhibited the activation of p38, p42/44 mitogen-activated protein kinases (MAPKs), and the degradation of IκB alpha (IκBα) induced by LPS. However, it had no effect on iNOS/NO synthesis induced by the activation of microglia with LPS. Further investigation showed that PF11 significantly suppressed tumor necrosis factor (TNF)-α production, but it did not influence the production of nitric oxide (NO) induced by the activation of microglia with LPS. **CONCLUSION:** The data suggested that $PF_{11}$ might have a potential protective effect on CNS by suppressing TNF-α production, but it perhaps has no effect on the central nervous system disease where NO is part of pathophysiology.

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**PN08 Morphine decreases extracellular levels of glutamate in the anterior cingulate cortex: an *in vivo* microdialysis study in freely moving rats**

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**KEY WORDS** morphine; glutamate; anterior cingulate cortex; microdialysis

**AIM:** The anterior cingulate cortex (ACC), an important region of prefrontal cortex for cognitive functions, has been implicated in drug abuse and addiction. In the present study, we intended to investigate the effect of morphine on the extracellular levels of glutamate in the ACC in freely moving rats. **METHODS:** *In vivo* microdialysis coupled to high performance liquid chromatography and electrochemical detection had been used for the determination of the extracellular levels of glutamate. **RESULTS:** The results showed that either acute (5 and 10 mg/kg, ip) or chronic (twice daily for 5 d in incremental doses 5, 10, 20, 40 and 50 mg/kg, ip) morphine treatment decreased the extracellular levels of glutamate in the ACC. The lower basal levels of glutamate were found after chronic morphine treatment. When naloxone (2 mg/kg, ip) was given 3 h later after the last administration of chronic morphine treatment, the reverse of the above phenomena was observed, that is, extracellular glutamate levels increased dramatically. **CONCLUSION:** The present study provides the first evidence that morphine decreases extracellular levels of glutamate in the ACC, suggesting that glutamate in ACC is involved in the central actions of morphine.

**PN09 Tetrandrine potentiated hypnotic effect of pentobarbital through serotonergic system**

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**KEY WORDS** tetrandrine; sleep; pentobarbital; 5-hydroxytryptophan; serotonergic system

**AIM:** To investigate the hypnotic activity and mechanism of tetrandrine (TET, a major component of *Stephania tetrandrae*) in mice. **METHODS:** Each mouse was observed for the onset and duration of sleep, with the criterion for sleep being loss of righting reflex. **RESULTS:** TET potentiated pentobarbital (45 mg/kg, ip)-induced hypnosis significantly by reducing sleep latency and increasing sleeping time in a dose-dependent manner and this effect was potetiated by 5-hydroxytryptophan (5-HTP). On the subhypnotic dosage of pentobarbital (28 mg/kg, ip treated mice, TET (60 and 30 mg/kg, *po*) significantly increased the rate of sleep onset and also showed synergic effect with 5-HTP. Pretreatment of *p*-chlorophenylalanine (PCPA, 300 mg/kg, sc), an inhibitor of tryptophan hydroxylase, significantly decreased pentobarbital-induced sleeping time and TET abolished this effect. **CONCLUSION:** Synergism of TET with pentobarbital on hypnotic effect is dependent on serotonergic syetem.

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sity and Research Fund of Shanghai Green Valley Holding Co, Ltd)

**PN10 Screening for MAO-A and MAO-B inhibitors by high-throughput screening methods**

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**KEY WORDS** monoamine oxidase; inhibitors; fluorescence assay; high throughput screening

**AIM:** The mitochondrial monoamine oxidase (MAO) existing as two isoforms of MAO-A and MAO-B is a flavin-containing enzyme responsible for the oxidation of biogenic amines. MAO has been proved not only physiologically to regulate the metabolism of amine-containing neurotransmitters, but also be highly involved in some central nervous system disorders such as neurodegenerative diseases, anxiety, depression, and psychiatric disorders as well. The current MAO inhibitors alleviate symptoms or slow the progress of these diseases to some degree, however, most of them have concomitantly distinctive adverse effects. The present study is conducted to establish a highly efficient model with a conveniently feasible one-step fluorescent assay to screen for more MAO inhibitors from our Chinese medicine library. **METHODS:** Mitochondrial purifications were performed with the examination of specificity and sensitivity of enzyme to its specific inhibitors to determine the optimal step. The fluorescent assay was evaluated by assessing its specificity and the precise kinetic properties of MAO-A and MAO-B. In view of characterization of high efficiency, reliability, and low consumable costs of high-throughput screening (HTS), the reaction system including concentration of enzyme and substrates, incubation volume and time were optimized to optimum conditions. The established HTS model was applied to screen for MAO-A or MAO-B inhibitors from 55 040 samples. **RESULTS:** Under the established reaction system conditions, 110 samples were discovered inhibitive effects on MAO-A, while 104 samples on MAO-B. **CONCLUSION:** The present HTS model is very efficient and suitable to screen for MAO inhibitors in drug discovery, providing a promising approach to the prevention and treatment of the pertinent diseases.

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**PN11 Study of effect of oxygen/glucose-deprived culture on the brain-pancreas relative protein in PC12 cells and the mechanism**

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**KEY WORDS** oxygen/glucose-deprived; brain-pancreas relative protein; p38; cell cycle; calcium

**AIM:** To study the effect of oxygen/glucose-deprived (OGD) culture on the expression of a novel protein, brain-pancreas relative protein (BPRP), and the possible regulating mechanism *in vitro*. BPRP was a key protein found in our previous study of cerebral ischemia. **METHODS:** PC12 cells was selected and exposed to the Eagle's solution containing 1 mmol/L $Na_2S_2O_4$ for 0, 1, 2, 4, 8, 12, and 24 h, then the cell viability, cellular content of MDA, activity of SOD, and lactate dehydrogenase (LDH) were investigated respectively. Cellular BPRP, VEGF, and P38 were examined by Western blotting or laser scanning confocal microscopy (LSCM) and flow cytometer (FCM). DNA ladder and FCM were used to assay apoptosis. Cell cycle was analyzed by FCM. Calcium fluorescence indicator Fluo-3/AM was used to measure free intracellular $Ca^{2+}$. **RESULTS:** OGD could significantly decrease cell viability, activity of SOD, but increase content of MDA, activity of LDH and apoptosis. Whereas the content of BPRP was decreased significantly after 1 h by Western blotting, FCM, and LSCM analysis. VEGF and P38 were up-regulated after OGD treatment for 1 h as well. S phase shortened and $G_2$-M phase lengthened during cell cycle. Intracellular $Ca^{2+}$ increased significantly. **CONCLUSION:** ODG impaired PC12 cells and significantly reduced intracellular BPRP, the possible mechanisms including P38 kinase pathway, elevation of intracellular $Ca^{2+}$, and change of cell cycle.

(Project supported by the National Natural Science Foundation of China, No 30270528).

**PN12 The protective effect of tetramethylpyrazine (TMP) against PC12 cells damages**

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**KEY WORDS** tetramethylpyrazine; PC12 cells; mitochondia

**AIM:** To discover the protective effects of tetramethylpyrazine (TMP) against PC12 cells damages and explore its protective mechanisms. **METHODS:** We established three *in vitro* models to investigate the protective effects of TMP against the injuries. In both of glutamate and natrium azide-induced PC12 injuries, the action of TMP on the cell viability was measured by MTT assay. The LDH efflux was measured by the assay kit, production of NO tested by Griess' method, and the intracellular free calcium concentration ($[Ca^{2+}]_i$) in PC12cells tested with Fura-2

double-wavelength fluoremetry method. In the *in vitro* natrium azide-induced rat brain mitochondria injury model, we detected the effect of TMP on the function of mitochondria by measuring the changes of the resarin fluorescence density. **RESULTS:** Compared with the model group, the cell viability of TMP-treated groups ($1\times10^{-5}$, $1\times10^{-6}$, and $1\times10^{-7}$ mol·L$^{-1}$) was increased dose-dependently. The production of NO and the effluxation of LDH in PC12 cell were decreased after treatment with TMP in a dose-dependent manner in both injury models. The elevation of the intracellular free calcium concentration ($[Ca^{2+}]_i$) after exposure to glutamate was attenuated in TMP-treated groups. The outcome of resarin fluorescence density reflected that TMP ($1\times10^{-5}$, $1\times10^{-6}$, and $1\times10^{-7}$ mol·L$^{-1}$) had a significantly protective effects on the isolated mitochondria function stimulated by natrium azide. **CONCLUSION:** TMP has a protective effect on both of PC12 cells injury models, which may be accounted for the attenuation of excitotoxication and preservation of the mitochondrial function.

(Project supported by National Natural Science Foundation of China, No 30171148 and 30472015, and National High Technology Research and Development Program of China, No 2004AA2Z3782)

### PN13 A model of subarachnoid hemorrhage in rats

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**KEY WORDS** animal models; subarachnoid hemorrhage; rats

**AIM:** To build a simple and repeatable animal model of subarachnoid hemorrhage (SAH). **METHODS:** SAH was introduced by passing a nylon thread up through the right internal carotid artery and piercing a hone in the right anterior cerebral artery. At 12 and 24 h, the rats were evaluated with rotarod test and the behavior scale (5-point scale). **RESULTS:** The rats were trained through rotarod test and then randomly divided into three groups, including vehicle group treated with vehicle after SAH, nimodipine treated group (ip 0.25 mg/kg 5 min, 6 h, and 12 h after SAH ) and sham group. At the point of the perforation there was usually a capping clot. There was always blood in the basal cisterns with some spread over the hemisphere. The time of rotarod test at 12 h was as follows [mean±SEM: vehicle ($n$=5), 40±22 s; nimodipine ($n$=5), 121±49 s; sham ($n$=8), 260±89 s; $P$=0.001 overall; $P$=0.002, vehicle *vs* sham; $P$=0.043, nimodipine *vs* sham]. The time of rotarod test at 24 h was as follows (mean±SEM): vehicle ($n$=5), 27±18 s; nimodipine ($n$=5), 73±34 s; sham ($n$=8), 184±45 s ($P$=0.03 overall; $P$=0.025, vehicle *vs* sham). The values for the 5-point scale at 12 h were as follows (mean±SEM): vehicle ($n$=5), 2.4±0.5 s; nimodipine ($n$=5), 1.6±0.5 s; sham ($n$=8), 0±0 s ($P$=0.000 overall; $P$=0.023, vehicle *vs* sham; $P$=0.007, nimodipine *vs* sham). The values for the 5-point scale at 24 h were as follows (mean±SEM): vehicle ($n$=5), 3.2±0.8 s; nimodipine ($n$=5), 1.8±0.4 s; sham ($n$=8), 0±0 s ($P$=0.000 overall; $P$=0.003, vehicle *vs* group; $P$=0.044, vehicle *vs* nimodipine). **CONCLUSION:** We present a simple and reliable model of SAH in the rats which allows to evaluate novel compounds and new drugs for treatment of SAH.

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### PN14 SO-3, a new O-superfamily conopeptide derived from *Conus striatus*, selectively inhibits N-type calcium currents in cultured hippocampal neurons in rats

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**KEY WORDS** SO-3; conotoxin; N-type calcium channels

**AIM:** To define the ion channel target of SO-3, a new O-superfamily conopeptide derived from *Conus striatus*. **METHODS:** Pharmacologically isolated whole-cell currents were recorded by patch-clamp technique in cultured hippocampal neurons. **RESULTS:** SO-3 had no effect on voltage-sensitive sodium currents, delayed rectifier potassium currents and transient outward potassium currents even at the dose of 100 μmol·L$^{-1}$. However, similar as the distinct N-type calcium channels blocker ω-conotoxin MVIIA, SO-3 could concentration-dependently inhibit the high voltage-activated (HVA) calcium currents and obtain its maximum inhibitory effect at the concentration of 3 μmol·L$^{-1}$. The $EC_{50}$ values for SO-3 and MVIIA were 0.16 and 0.20 μmol·L$^{-1}$, respectively. Further examination showed that SO-3 and MVIIA inhibited the overlapping components of the HVA currents, whereas no overlapping component was inhibited by SO-3 and nimodipine (L-type blocker), or by SO-3 and ω-agatoxin IVA (P/Q-type blocker). **CONCLUSION:** SO-3 selectively blocked N-type voltage-sensitive calcium channels in neurons. As N-type calcium channels are critical for pain transduction, SO-3 may have therapeutic potential as a novel analgesic agent.

(Project supported by the National High-Tech Research and Development Program of China, No 2001AA624150)

### PN15 Role of delayed rectifier K channel currents in β-amyloid (1-40)-induced caspase-3 activation and apoptosis in primary cultured cortical neurons

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**KEY WORDS** β-amyloid$_{(1-40)}$; delayed rectifier $K^+$ currents; caspase-3

**AIM:** To investigate the effect of chronic exposure to β-amyloid$_{(1-40)}$ ($A\beta_{1-40}$) on delayed rectifier $K^+$ currents ($I_K$) in primary cultured rat

cortical neurons and the effect of $I_K$ on caspase-3 activation and neurons apoptosis induced by $A\beta_{1-40}$. **METHODS:** $K^+$ currents were recorded using whole-cell patch clamp techniques; cells viability rate and apoptosis were studied using MTT and Hoechst 33342 staining; caspase colorimetric assay was applied to study caspase-3 activation by $A\beta_{1-40.}$ **RESULTS:** Neurons were exposed to $A\beta_{1-40}$ 2 μmol·L$^{-1}$, 5 μmol·L$^{-1}$, and 10 μmol·L$^{-1}$ for 12 h, delayed rectifier $K^+$ current density was increased in a concentration-dependent manner from the control (44.5±2.0 pA/pF) to 56.7±3.8 pA/pF, 63.3±3.6 pA/pF, and 70.3±3.8 pA/pF, respectively. It was increased by 27.48 %, 42.32 %, and 58.07 %. The increase of $I_k$ induced by $A\beta_{1-40}$ 5 μmol·L$^{-1}$ arrived to the peak after 12 h. The current was sensitive to TEA 5 mmol·L$^{-1}$. Transient outward $K^+$ currents ($I_A$) did not change obviously after exposure to $A\beta_{1-40.}$ Neurons viability decreased to 64.4 %± 8.8 % after exposure to $A\beta_{1-40}$ for 48 h. Meanwhile cell apoptosis increased to 19.6 %±0.6 %. In addition, $A\beta_{1-40}$ 5 μmol·L$^{-1}$ can increase the activity of caspase-3, it reached the peak after 24 h. TEA 5 mmol·L$^{-1}$ can attenuate the $A\beta_{1-40}$-induced caspase-3 activation as well as neuronal apoptosis. **CONCLUSION:** Delayed rectifier $K^+$ currents take part in the $A\beta_{1-40}$-induced cortical neurons apoptosis in the early phase. It can also increase the activation of caspase-3.

(Project supported by the State Key Basic Research and Development Program of China, 973 Project, No G1998051106)

**PN16 Possible neuroprotective role of arachidonic acid-sensitive potassium channels in rat acute cerebral ischemia**

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**KEY WORDS** potassium channels; TREK; cerebral ischemia; neuroprotection

**AIM:** To explore the actual role of arachidonic acid-sensitive potassium channels including TREK-1, TREK-2 and TRAAK in acute cerebral ischemia. **METHODS:** In acute rat MCAO model, RT-PCR and Western blot analysis were used to investigate the expression changes of TREK. Immunohistochemistry experiments were also used to study their protein expressions *in vivo*. **RESULTS:** TREK-1, TREK-2 and TRAAK mRNA expression levels increased significantly 2 h and 24 h after MCAO in both cortex and hippocampus. At the same time, all the three channel proteins showed significantly enhanced expressions 24 h after MCAO in cortex and hippocampus, but only TREK-1 showed increased expression 2 h after MCAO in cortex and both TREK-1 and TREK-2 in hippocampus. While in cortex and hippocampus neurons, the three channel proteins all had higher expression levels 24 h after MCAO. **CONCLUSION:** TREK-1, TREK-2 and TRAAK may play important roles in the neuroprotection in acute cerebral ischemia.

(Project supported by Major State Basic Research Development Program of China, No 91998051106)

**PN17 NT-1, an active constituents extracted from Tiaoxin Recipe, enhances long-term potentiation of CA1 area in rat hippocampal slices**

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**KEY WORDS** long-term potentiation (LTP); Tiaoxin recipe; Alzheimer disease; hippocampus

**AIM:** To investigate the effect of NT-1, an active constituent extracted from Tiaoxin Recipe (TXR), a traditional Chinese medicinal prescription, on long-term potentiation (LTP) in rat hippocampal slices. **METHODS:** Field excitatory postsynaptic potentials (fEPSPs) were recorded in CA1 region in rat hippocampal slices and the LTP was induced by high frequency stimulus. **RESULTS:** NT-1 significantly enhanced the induction of LTP without influence on the baseline of fEPSP slope. 0.2 μmol/L $A\beta_{25-35}$ and 2 μmol/L corticosterone (CORT) did not change the baseline of fEPSP slope but inhibited the induction of LTP. Furthermore, NT-1 significantly reversed the LTP inhibition by $A\beta_{25-35}$ or CORT. LTP induced in slices treated with NT-1 in addition to $A\beta_{25-35}$ or CORT appeared to be more potent than that in slices treated with TXR in addition to CORT, suggesting the specific action of NT-1 on restoration of LTP inhibited by $A\beta_{25-35}$ or CORT. **RESULTS:** NT-1 was more effective and specific to ameliorate synaptic plasticity inhibited by Aβ or CORT than TXR, indicating that the cognitive improving effect of NT-1 is closely related to the improvement of synaptic plasticity amelioration.

(Project supported by Key Project of National Natural Science Foundation of China, No 39830450.)

**PN18 *D*-serine enhances impaired long-term potentiation in CA1 subfield of hippocampal slice from aged senescence-accelerated mouse prone/8**

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**KEY WORDS** senescence-accelerated mice; *D*-serine; long-term potentiation

The molecular and cellular mechanisms underlying the cognitive deficient of senescence-accelerated mouse prone/8 (SAMP8) have been attributed to many pathobiological changes in neurons. Recently, increasing evidence have shown that astrocyte, by mean of *D*-serine, was involved in the process of

synaptic transmission. Here we reported the decrease of long-term potentiation (LTP) in SAMP8 along with aging, and the enhancement of *D*-serine on LTP in aged SAMP8. The fEPSP slope in CA1 area of hippocampal slices prepared from 2-, 6-, and 12-month-old SAMP8 significantly decreased with aging. Meanwhile, the LTP in the slices of 6- and 12-month-old mice markedly decreased below that of the age-matched normal strain SAMR1. Supplement with exogenous *D*-serine, a coagonist of *N*-methyl-*D*-aspartate (NMDA) receptor and a main product of the astrocyte, not only directly ameliorated the deficient LTP but also rescued the abolished LTP by *D*-amino acid oxidase (DAAO) in slices from 12-month-old SAMP8. This amelioration of *D*-serine was inhibited by AP-V and 5,7-dichlorokynurenic acid (DCKA). These results imply that absence of *D*-serine or dysfunction of the astrocyte contributes to the decrease of NMDA receptor-dependent LTP and cognition in aged SAMP8.

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**PN19 Antidepressant and anti-stress effects of curcumin in mice**

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**KEY WORDS** curcumin; forced swimming; tail suspension; antidepressant; hyperthermia; anti-stress; mice

Curcumin (diferuloylmethane), a yellow colouring agent contained in the rhizome of Curcuma Longa (turmeric), has a wide array of pharmacological and biological activities, such as antioxidant, anti-inflammatory, immunomodulating and anticarcinogenic effects. In this study, curcumin was examined for the antidepressant and anti-stress effects in forced swimming, tail suspension and stress-induced hyperthermia tests. Curcumin (2.5, 5, 10 mg/kg, *po*) significantly reduced the duration of immobility in forced swimming test and tail suspension test in mice. The effects of curcumin at the dose of 10 mg/kg were more potent than that of reference antidepressant fluoxetine (20 mg/kg, *po*). Neither curcumin nor fluoxetine, at the doses tested, produced significant effects on locomotor activity. In stress-induced hyperthermia in single housed mice, rectal temperature was measured at two consecutive time-points ($T_1$ and $T_2$) in 10-min intervals. $\Delta T$ ($T_1$-$T_2$) was significantly decreased at the doses of 5 to 20 mg/kg. Similar findings were obtained in stress-induced hyperthermia in group housed mice. These results suggest that curcumin exhibits antidepressant and anti-stress effects.

## Immunopharmacology and Immunotoxicology

**PI01 A novel immunopotentiator: MDP-C**

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**KEY WORDS** muramyl-dipeptide; immunopotentiator; SARS-CoV; dendritic cell

**AIM:** To identity a novel non-specific immunopotentiator and its primary action mechanisms, and to investigate the adjuvancy of a novel immunopotentiator, MDP-C. **METHODS:** MTT assay was used to detect the immunostimulatory activity of MDP derivatives on macrophages. The expression levels of surface molecules on dendritic cells (DCs), including CD11c, MHC class II, intercellular adhesion molecule-1 (ICAM-1), was determined by flow cytometry(FCM) and the levels of cytokines on DC, including interleukin-2 (IL-2), IL-12 and interferon-γ (IFN-γ), were quantified by ELISA kit. Additionally, NF-κB activation was assessed by EMSA and ELISA method using BD Mercury™ transfactor (NF-κB) kit, respectively. The adjuvancy of MDP-C with T-cell recognizing epitopes specific for severe acute respiratory syndrome-coronavirus (SARS-CoV) was investigated by enzyme-linked immunospot (ELISPOT) assay. **RESULTS:** Our data showed that MDP-C strongly induced cytolytic activity of macrophages in P388 leukemia cells. MDP-C was also found to be an effective stimulator of IL-2, IL-12 and IFN-γ production by DCs. Additionally, MDP-C elevated the expression level of surface molecules, such as CD11c, MHC class II, ICAM-1, and NF-κB protein in murine BMDCs. Importantly, MDP-C remarkably synergised immune responsiveness to T-cell recognizing epitope specific for SARS-CoV. **CONCLUSION:** MDP-C was a novel non-specific immuno-potentiator with apyrogenicity, non-allergicity and low toxicity, and great potential for immunotherapy to SARS.

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**PI02 Effect of inhaled fluticasone on airway reactivity and 8-Iso-PGF2α in bronchoalveolar lavage fluid of cats**

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**KEY WORDS** cat; inhaled fluticasone; airway reactivity; 8-iso-$PGF_{2\alpha}$

**AIM:** The objective of the this study was to assess the effect of inhaled fluticasone administrated daily during one or two weeks on bronchial reactivity (BR) and airway inflammation of experimental cats with mild, but persistent lower airway inflammation.

**METHODS:** Five European shorthair cats were investigated before (Pre) and after a one-week (Flu-1W) and or two-week (Flu-2W) period of treatment with inhaled fluticasone (250 μg/d). BR was determined using barometric whole body plethysmography and inhalation of stepwise increasing carbachol concentrations, allowing the calculation of the concentration inducing a 300 % increase of Penh. Cats also underwent bronchoscopy and bronchoalveolar lavage (BAL) under anaesthesia, allowing to establish bronchoscopy scores and to collect BAL fluid for cytological analysis and determination of 8-iso-$PGF_{2\alpha}$ (EIA, Cayman), an index of oxidative stress. **CONCLUSION:** These data showed that inhaled fluticasone allowed a significant reduction of BR and pulmonary oxidative processes although BAL cytology remained unchanged. An increase of the treatment period further decreased 8-iso-$PGF_{2\alpha}$ and BR and also led to a reduction of the bronchoscopy score. **RESULTS:** Tab 1 see 1568.

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**PI03 Effect of selective phosphodiesterase 4 inhibitor, rolipram, on cytokine and gelatinase B (MMP-9) release in the whole blood from adult patients with cystic fibrosis.**

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**KEY WORDS** cystic fibrosis; metalloproteinase; inflammation; PDE4 inhibitor.

**AIM:** Inflammation plays a critical role in lung disease progression in cystic fibrosis being able to be associated with the development of tissue remodeling. These processes are mainly due to pro-inflammatory cytokines release and to an imbalance between proteases and antiproteases involving matrix metalloproteinases (MMP). Phosphodiesterase 4 (PDE4) inhibitors, by elevating intracellular cAMP, are known to be potent inhibitors of cytokines release and MMP production. The aim of this study was to evaluate the effect of the selective PDE4 inhibitor, rolipram, on MMP-9 and pro-inflammatory cytokine release, IL-8 and TNF-β, in the presence of lipopolysaccharide (LPS), formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol 12-myristate 7-acetate (PMA) in the blood from adult patients with cystic fibrosis. **METHODS**: Adult patients with cystic fibrosis were recruited (last exacerbation of cystic fibrosis >3 months) at the CHRU of Rennes (France) accordingly to the local ethical committee. Whole blood was distributed in 96-well plate and pretreated with rolipram 0.1-10 μmol/L for 1 h. Then PMA 0.002–0.02 μmol/L, LPS 0.001-100 mg/L and fMLP 1-10 μmol/L were incubated overnight. Plasma supernatants were removed and analyzed by zymography for evaluation of MMP-9 secretion and by ELISA for cytokines quantification. **RESULTS:** PMA-, fMLP- and LPS-induced MMP-9, IL-8, and TNF-α levels increased in a concentration-dependent manner. Pre-incubation with rolipram resulted in a dose-dependent inhibition of LPS-induced TNF-α release with an $IC_{50}$ value of 1.56±0.23 μmol/L whereas it induced only 10 % of inhibition of MMP-9 secretion at 10 μmol/L, and it was ineffective against IL-8 production. **CONCLUSION:** LPS-induced TNF-α release is markedly inhibited by rolipram which was over 2 fold more potent in blood from patients with cystic fibrosis than in blood from patients with COPD ($IC_{50}$= 2.8±0.9 μmol/L, Ouaged *et al,* PPT, 2004, in press), suggesting that selective phosphodiesterase 4 inhibitors should be a therapeutic potential in the many pathological conditions associated with over-production of pro-inflammatory cytokines and MMPs such as cystic fibrosis.

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**PI04 Immunomodulatory effect of ginsenoside-Rg3: enhancement of cell proliferation and cytokines production in murine splenocytes**

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**KEY WORDS** ginsenoside-Rg3; cytokines; immunomodulation; splenocytes

**AIM:** Murine splenocytes were used to investigate the immunomodulatory effects of ginsenoside-Rg3 and explore its mechanisms of action. **METHODS:** Murine splenocytes were incubated with ginsenoside-Rg3 in presence of ConA for 72 h. The effect of ginsenoside-Rg3 on murine splenocytes proliferation was studied using [$^3$H]thymidine incorporation assay. Effects of ginsenoside-Rg3 on the production of Th1 cytokines interleukin-2 (IL-2), interferon-γ (IFN-γ), and Th2 cytokine interleukin-4 (IL-4) from murine splenocytes were detected by ELISA method. Effects of ginsenoside-Rg3 on mRNA level of Th1 cytokine IFN-γ and Th2 cytokine IL-4 were evaluated by reverse transcription polymerase chain reaction (RT-PCR) analysis. Effect of ginsenoside-Rg3 on NF-κB DNA binding activity in murine splenocytes was investigated by electrophoretic mobility shift assays (EMSA). **RESULTS:** The results demonstrated that ginsenoside-Rg3 not only enhanced the proliferation of murine splenocytes, but also increased the production and expression of Th1 and Th2 cytokines at the concentrations of 1-100 μmol/L in the presence of ConA. EMSA showed that 10 μmol/L ginsenoside-Rg3 augmented NF-κB DNA binding activity in murine splenocytes. **CONCLUSION:** Ginsenoside-Rg3 had immunomodulatory effects by increasing the production and gene expression of Th1 and Th2 cytokines in murine splenocytes,

which was, at least partly, due to enhancement of NF-κB DNA binding activity.

### PI05 Bifunctional effect of E2 on macrophage

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**KEYWORDS** 17β-estradiol; macrophage; bifunctional effect

**AIM:** Our previous study showed that the effect of 17β-estradiol ($E_2$) on macrophage does not strengthen when concentration increased. So the effect of $E_2$ on cytokines, intracellular free $Ca^{2+}$ ($[Ca^{2+}]_i$) and morphological change of macrophages at different concentrations were studied. **METHODS:** TNF-α was measured by MTT via L929 cell. Nitrate and nitrite level(NO) was measured by the method of Griess. $[Ca^{2+}]_i$ was examined by laser scanning confocal microscopy(LSCM). Fluorescent microscopy and environmental scanning electron microscopy(ESEM) were used to detect morphological changes of macrophages. **RESULTS:** TNF-α release of macrophages was enhanced by $E_2$ and this effect did not strengthen when the concentration increased to 1 μmol/L. And in the same way $E_2$ acted on NO release of macrophage. Apoptosis characters such as extensive nuclear condensation, DNA fragmentation and apoptosis bodies were induced by $E_2$ at 1-100 μmol/L which were detected by fluorescent microscopy and ESEM. Continual elevation of $[Ca^{2+}]_i$ were induced by $E_2$ from 1-100 nmol/L. When the concentration of $E_2$ increased more than 1 μmol/L, $[Ca^{2+}]_i$ elevated quickly and following a rapid fall which show a different character compared with that at concentration od nmol/L level. **CONCLUSION:** Bifunctional effect of $E_2$ on macrophage at different concentration was found and this may be related with the apoptosis of macrophage induced by high concentration of $E_2$ which may be a result of alter on intracellular free $Ca^{2+}$ of macrophage.

### PI06 NGF is released by IL-1β and induces hyperresponsiveness of the human isolated bronchus

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**KEY WORDS** NGF; IL-1β; airway hyperresponsiveness

**AIM:** NGF, a neurotrophic factor essential for the development and survival of neurons, is also an important mediator of inflammation involved in airway hyperresponsiveness. It is released by airway cells stimulated by IL-1β. Since IL-1β induces airway hyperresponsiveness to the tachykinin NK-1 receptor agonist [$Sar^9$,$Met(O_2)^{11}$]-substance P in human isolated bronchi, the aim of this study was to determine whether IL-1β was able to induce NGF release from isolated bronchi in conditions inducing airway hyperresponsiveness, and whether NGF may participate in this airway hyperresponsiveness. **METHODS:** Bronchial ring segments from patients undergoing surgery for lung carcinoma were placed in 1 mL Krebs-Henseleit solution at room temperature (21°C) for 15 h in the presence or absence of interleukin-1β (10 ng/mL). After incubation, the paired bronchi were taken for contractile studies, and the supernatants kept aliquoted at -80 °C until NGF measurement. In another set of experiments, anti-human NGF blocking antibodies or non relevant IgG antibodies were incubated simultaneously to IL-1β for 15 h at 21 °C, and bronchi taken for contractile studies. NGF was determined by ELISA method. **RESULTS:** IL-1β (10 ng/mL, 21 °C, 15 h) increased the release of NGF from human isolated bronchi *in vitro* ($P<0.05$), and, in organ bath studies, the response of human bronchi to [$Sar^9$,$Met(O_2)^{11}$]-substance P (0.1 μmol/L) ($P<0.05$). A significant correlation was found between both responses. Airway hyperresponsiveness induced by IL-1β was abolished by a blocking anti-human NGF antibody. Finally, NGF (1 ng/mL, 37 °C, 0.5 h) by itself induced a significant increase in [$Sar^9$,$Met(O_2)^{11}$]-substance P responsiveness ($P<0.05$). By contrast, it did not change the maximal contraction to acetylcholine. **CONCLUSION:** We have here clearly demonstrated that NGF may participate into the airway hyperresponsiveness induced by IL-1β, and that neuro-immune cross talk may be active in the airways.

### PI07 Effects of berberine hydrochloride on CYP450 total content and expression in BCG-induced immune hepatic injury in mice and its possible mechanism

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**AIM:**To investigate effects of berberine hydrochloride on hepatic cytochrome P450 in BCG-induced immunological hepatic injury in BALB/c mice and its possible mechanism. **METHODS:** Immunological liver injury was induced by intravenous injection of Mycobacterium bovis bacillus Calmette-Guerin (BCG, 125 mg/kg) in BALB/c mice. After one week stimulated by BCG, berberine hydrochloride (10, 25, 50, 75, and 100 mg/kg, respectively, qid 7 d) was administrated by intragastric administration, and aminoguanidine was given by intraperitoneal injection (50 mg/kg, every other day). On the 14 th d after injection of BCG, serum alanine aminotransferase (ALT) levels and cytochrome P450 (CYP450) total content in the hepatic homogenate were measured by the method of spectrophotometry. The hepatic tissue injury was estimated by histopathological H-E staining. The expression of inducible nitric oxide synthase (iNOS) in hepatic tissue was determined by immunohistochemical method. **RESULTS:** After two weeks BCG injection, serum ALT level was increased; granuloma was observed, over-expression of iNOS

protein was detected on the granulomas. The CYP450 total content in hepatic homogenate (2.86±0.586 nmol/g liver, P<0.05) was reduced to 36.3 percent of the control group (7.87 ± 0.63 nmol/g liver). Aminoguanidine, a selective iNOS inhibitor, reversed enhancement of serum ALT levels, partly increased the CYP450 total content, and significantly inhibited iNOS expression in hepatic tissue. Compared with BCG-liver injury model group, berberine inhibited liver haemorrhage and inflammation, and also reduced serum ALT levels and increased CYP450 total content by the dose-dependent manner in the range of 10 to 100 mg/kg. **CONCLUSION:** The present results indicates that berberine reversed the histological injury and the enhancement of CYP450 total content induced by BCG-immune liver damage, which mechanism was likely due to down-regulation of iNOS expression.

**PI08 Immunopharmacological effects and mechanism of dihydroqinghaosu on immunological liver injury mice**

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**KEY WORDS** dihydroqinghaosu; immunological liver injury; concanavalin A; alanine aminotransferase; aspartate aminotransferase; tumor necrosis factor-alpha; interferon-gamma

**AIM:** To investigate the immunopharmacological effects and the preliminary mechanism of Dihydroqinghaosu (DQHS) on the Con A-induced liver injury mice. **METHODS:** Hepatocytes of SD rats were prepared to examine the effect of DQHS on the ALT levels of hepatocytes content *in vitro*. The Con A-induced liver injury model of ICR mice was made. Dose-dependent and time-dependent difference of serum ALT activity was observed in Con A-induced liver injury mice. *In vivo,* effects of DQHS on the serum ALT, AST, TG levels after Con A injection were investigated. Serum ALT, AST, TG activity was determined by the standard photometric method with an automatic analyzer. The lymphocyte proliferation induced by mitogens was assayed by MTT method. The levels of NO were determined by Griess method. Time-dependent difference of the serum TNF-α and IFN-γ levels was observed after Con A injection. *In vivo,* effects of DQHS on the serum TNF-α and IFN-γ levels after Con A injection were investigated. TNF-α and IFN-γ levels were assayed with enzyme linked immunosorbent assay (ELISA). **RESULTS:** *In vitro,* DQHS had no effect on the serum ALT levels of SD rats hepatocytes at the concentrations from 0.25 mmol/L to 25 mmol/L, which showed that DQHS had no direct damage or other effects on hepatocytes. Serum ALT levels of Con A-induced hepatitis mice were in dose-dependent and time-dependent manner. The suboptimal dose of Con A to induce liver injury was 15mg/kg, which was selected to carry on the following experiments. After pretreatment introgastrically for ten days, DQHS 10 and 40 mg/kg reduced significantly the serum ALT, AST, TG levels of Con A-induced hepatitis mice. Pretreatment with DQHS 40 mg/kg for 10 d had no effect on the serum ALT, AST, TG levels of ICR mice. At the concentrations from 10 mmol/L to 100 mmol/L, DQHS can inhibit the proliferation of BALB/c mice lymphocytes induced by Con A and LPS *in vitro*. At the doses from 10 mg/kg to 40 mg/kg, pretreatment with DQHS for 10 days can inhibit the proliferation of lymphocytes stimulated by Con A and LPS *in vivo* in hepatitis mice. At the concentrations from 10 mmol/L to 100 mmol/L, DQHS can inhibit the NO secretion of BALB/c mice lymphocytes induced by Con A, LPS *in vitro* . At the doses from 10 mg/kg to 40 mg/kg, pretreatment with DQHS for 10 d can inhibit the NO secretion of Con A-induced hepatitis mice lymphocytes stimulated by Con A and LPS *in vivo.* Serum TNF-α levels of immunological hepatitis mice were dramatically increased at 0.5 h after Con A challenge ($P$<0.01). These elevated levels were not sustained and began to fall at 2 h after Con A challenge. Pretreatment with DQHS 10 mg/kg for 10 d had the same tendency in the serum TNF-α levels but lower than that of Con A model group. Serum IFN-γ levels of immunological hepatitis mice were dramatically increased at 4 h after Con A challenge and reached peak at 10 h. Pretreatment with DQHS reduced significantly the serum TNF-α and IFN-γ levels of Con A-induced hepatitis mice. **CONCLUSIONS:** *In vitro*, DQHS had no effect on the serum ALT levels of the SD rats hepatocytes, which showed that DQHS had no direct damage or other effects on hepatocytes. Pretreatment with DQHS can significantly decrease the ALT, AST, and TG levels in serum of Con A-induced liver injury mice, which reflected that DQHS had protective effects on Con A-induced liver injury in mice. It can inhibit the immunological functions of lymphocytes, such as proliferation and NO secretion, and reduce the serum TNF-α and IFN-γ levels after Con A challenge. Both may contribute to the mechanism of its protective effects on Con A-induced hepatitis mice.

**PI09 The protective effects of rutaecarpine on gastric mucosa injury in rats**

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**KEY WORDS** rutaecarpine; calcitonin gene-related peptide; vanilloid receptors

**AIM:** To examine the protective effects of rutaecarpine on gastric mucosa injury, and explore whether the protective effects of rutaecarpine are related to stimulation of endogenous CGRP release via activating vanilloid receptors in rats. **METHODS:** In rats models of the aspirin-induced ulceration and stress-induced ulceration, gastric mucosal ulcer index, pH value of gastric juice, and plasma concentrations of CGRP were determined. **RESULTS:** Aspirin significantly increased gastric mucosal ulcer index and the backdiffusion of $H^+$ through the mucosa. Rutaecarpine at the dose of 100 or 300 mg/kg (iv), and 300 or 600 mg/kg (ig) reduced ulcer index and backdiffusion of $H^+$, which were abolished by

pretreatment with capsaicin (50 mg/kg) or capsazepine (3 mg/kg), a competitive vanilloid receptor antagonist. Rutaecarpine significantly increased the plasma concentration of CGRP, which was also abolished by capsazepine. Rutaecarpine also reduced gastric mucosal damages induced by stress, which was abolished by capsazepine (5 mg/kg). **CONCLUSION:** These results suggest that rutaecarpine protects gastric mucosa against injury induced by aspirin or stress, and that the gastroprotection of rutaecarpine is related to stimulation of endogenous CGRP release via activating vanilloid receptors.

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**PI10 Total glucosides of paeony suppressed adjuvant arthritis in rats by intervening G-protein associated transmembrane signal transduction**

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**KEY WORDS** total glucosides of paeony; adjuvant arthritis; G-protein

**AIM:** To study the roles played by total glucosides of paeony (TGP), an effective compound of Chinese traditional herbal medicine (CTM), in the pathogenesis of arthritic diseases. **METHODS:** Adjuvants arthritis (AA) in rats was established and hind paw volumes of rats were measured by volume meter. Ultrastructure of synovioctes was observed under transmission electron microscope. Activities of IL-1 (interleukin-1) were determined by 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) assay. Tumor necrosis factor alpha (TNF-$\alpha$) and prostaglandin $E_2$ ($PGE_2$) were measured by radioimmunoassay. Phosphorylation of c-Jun N-terminal kinase (JNK), extracellular regulating kinase (ERK) and p38 kinase, expression of the stimulatory subunit of G alpha protein ($G_s$) and the inhibitory subunits of G alpha protein ($G_i$) were detected by Western blot analysis. Activities of G-protein associated protein kinase A (PKA) and PKC were detected with the colori metric PKA or PKC assay kit. **RESULTS:** TGP (50 and 100 mg/kg, ig × 7 d) inhibited inflammatory reactions, bone destructions, secretion and metabolism of synoviocytes, MAPKs phosphorylation, synoviocytes proliferation and G-protein associated signal transformation in synoviocytes from knees of AA rats. IL-1ra (30 and 120 mg/kg, ic × 7 d), used as positive control, had analogous effects as TGP and it had no effects on restoring the loss of body weight. **CONCLUSION:** TGP possesses anti-inflammatory actions and has a therapeutic effect on AA in rats due to G-protein associated transmembrane signal transduction.

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**PI11 Basis study on the model of hepatitis B—Vitro cell culture**

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**KEY WORDS** hepatitis B; hepatocytes culture *in vitro;* experimental models

**AIM:** To explore and set up many kinds of experimental model of hepatitis B in order to provide varies methods for application study on drugs to prevent and to cure hepatitis B. **METHODS:** According to the disorder and characters of hepatitis B, we used the models of duck primary hepatocytes which were infected duck hepatitis B virus (DHBV), the human hepatocellular carcinoma (cell 2215, Hep $G_2$) which was transferred with hepatitis B virus and rats primary hepatocytes cultured with $CCl_4$ *in vitro* respectively. The survival time and the levels of DHBV-DNA and ALT were observed in those models. **RESULTS:** The survival times of infected-duck, cell 2215 and rats primary hepatocytes were shorter than control, and the level of DHBV-DNA and ALT increased significantly. **CONCLUSION:** It is stable, and easy repeated to explore and set up many kinds of experimental model of hepatitis B in present study for application study on drugs to prevent and to cure hepatitis B.

**PI12 Differential regulation of δ-opioid receptor trafficking after internalization by TIPP and DPDPE**

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**KEY WORDS** Delta opioid receptor; internalization; recycling; desensitization

**AIM:** To explore the mechanisms underlying the difference between TIPP and DPDPE in desensitization of the δ-opioid receptors. **METHODS:** GH3 cells stably expressing HA-tagged δ-opioid receptors were treated with TIPP, DPDPE (1 μmol/L) or morphine (10 μmol/L) for different periods of time in the presence or absence of 50 μmol/L monensin or 10 nmol/L OA. Internalization of δ-opioid receptor was assessed using confocal laser microscopy and flow cytometry. **RESULTS:** In untreated GH3DOR cells (control), δ-opioid receptors were visualized primarily in the plasma membrane. After incubation of cells with DPDPE for 5-, 15-, and 30-min, a significant receptor internalization was observed. TIPP treatment also resulted in the internalization of the δ-opioid receptors, but to a lesser extent than DPDPE did. Consistent with previous studies, morphine failed

to trigger receptor internalization. Surprisingly, upon prolonged exposure to TIPP over 60 min, most of the internalized receptors were recycled back to cell surface, as indexed by a reappearance of the staining at the plasma membrane. In contrast, a substantial internalization of δ-opioid receptors was still observed after prolonged exposure to DPDPE over 60 min. The reappearance of the staining at the plasma membrane induced by TIPP was abolished in the presence of the recycling blocker monensin. TIPP-mediated the recycling of the δ-opioid receptors was also blocked in the presence of protein phosphatases inhibitor, OA. This indicated that the phosphatase activity was required for the receptor recycling to plasma membrane, suggesting a relationship between the dephosphorylation of the δ-opioid receptor and its recycling to cell surface. The differential down-regulation and desensitization of the δ-opioid receptors following chronic TIPP and DPDPE exposure further confirmed their distinct regulation of the trafficking of the δ-opioid receptors. Pretreatment of cells with DPDPE for 24 h produced a marked reduction of opioid binding sites and loss of inhibition of cAMP accumulation by agonists, while a significant down-regulation and desensitization of the δ-opioid receptors was not detected in the cells pretreated with TIPP for 24 h. **CONCLUSION:** There is Differential sorting of the δ-opioid receptors after internalization by TIPP and DPDPE.

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### PI13 Protective effect of Linomide on murine systemic lupus erythematosus (SLE)

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**KEY WORDS** linomide; chronic graft-versus-host disease; glomerulonephritis; cytokine; macrophage; $CD4^+/CD8^+$ cells; estradiol; immunomodulation

Linomide, a quinoline-3-carboxamide, has a pleiotropic immune modulating capacity and inhibits development as well as progression of disease in animal models of autoimmunity. Its benefical effects on experimental autoimmune disease models have been linked to regulation of Th1/Th2 balance and alter macrophage functions. We studied the effect of linomide on chronic graft-versus-host disease model mice. The oral administration of 32 mg/kg linomide from 3 d after the last cell injection, significantly delayed the onset of proteinuria and inhibited the production of pathogenic auto-antibodies. The elevation of serum blood urea nitrogen (BUN), creatinine, cholesterol and triglyceride levels resulting from the development of lupus nephritis was also inhibited. Treatment with linomide stimulated T cell proliferation reaction *in vivo*. At the same time, expressions of IFN-γ and IL-4 from stimulated spleen cells were also elevated. Macrophages from linomide-treated nephritis mice showed decreased TNF-α and IL-1β excrete in response to LPS. As SLE mice showed an increased $CD4^+/CD8^+$ ratio of spleen cells, treatment of the mice resulted in the balance of $CD4^+$ and $CD8^+$ subpopulations. Furthermore, we found that serum estradiol levels in nephritis mice were pronounced increased, treatment with linomide could reduce them. These results suggest that linomide may exert its actions in SLE and modulate the immune system while there are still many unknown mechanisms.

## Drug Metabolism and Dispositon

### PD01 Pharmacokinetic profiles of recombinant human parathyroid hormone (1-34) after subcutaneous injection in rats

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**KEY WORDS** recombinant human parathyroid hormone (1-34); pharmacokinetics; biodistribution

**AIM:** To study the pharmacokinetic profiles and biodistribution of recombinant human parathyroid hormone (1-34) [rhPTH(1-34)] after administration in rats. **METHODS:** $^{125}$I-rhPTH(1-34) was prepared by iodogen methods and purified by gel filtration. Immunoactivity of $^{125}$I-labeled peptide was assayed by enzyme immunoassay (EIA). Concentrations of $^{125}$I-rhPTH (1-34) in serum and tissues of rats were determined by the method of trichloroacetic acid (TCA) precipitation. Concentrations of radioactivity in serum, feces, urine and bile were measured by γ-counter. **RESULTS:** After sc injection of rhPTH(1-34) in rats at doses of 0.55, 1.10, and 2.19 MBq, $T_{max}$ were all 0.5 h, $C_{max}$ were 1.9, 5.8, and 4.6 kBq·mL$^{-1}$, respectively. Terminal $t_{1/2}$ after sc and iv administration were 8.2-10.7 h and 10.1 h, respectively. CLS was 1.2 - 2.6 L·h$^{-1}$·kg$^{-1}$ in all groups. Bioavailability after sc was 89.2 %. $^{125}$I-rhPTH(1-34) were widely distributed in all kinds of tissues after dosing, especially in the urinary system, which followed by the blood abundant organs. Distribution in the brain was the lowest. **CONCLUSION:** The concentration profiles of rhPTH(1-34) after administration were dependent on the loading dose. Clearance was not related neither to weight nor to sex. rhPTH(1-34) could hardly pass the blood-brain barrier and rhPTH(1-34) was cleared and excreted primarily by the kidney.

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### PD02 Pharmacokinetic profile of rhGLP-1(7-36) in Rhesus monkeys

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**KEY WORDS** rhGLP-1(7-36); pharmacokinetic; rhesus monkey

**AIM:** To study the pharmacokinetic profile of recombinant human glucagon-like peptide-1(7-36) [(rhGLP-1(7-36)] in Rhesus Monkeys. **METHODS:** The doses of sc groups were set at 1.05, 4.2, and 16.8 nmol·kg$^{-1}$, respectively and the dose of iv group was set at 4.2 nmol·kg$^{-1}$. RhGLP-1(7-36) concentrations in serum samples were determined by ELISA. The concentration-time curves were performed with the computer program Origin and the PK parameters were estimated by noncompartmental analysis. **RESULTS:** After administration of different doses (1.05, 4.2, and 16.8 nmol·kg$^{-1}$) of rhGLP-1(7-36), the mean $AUC_{(0-\infty)}$ were 1007±303, 5475± 2799 and 20689 ± 18101 pmol·L$^{-1}$·min$^{-1}$, respectively. The absolute bioavailability was calculated as 23.2 %. After multiple applications in the mid dose group, the serum rhGLP-1(7-36) levels were tended to elevate but with no significance between the same time point after the first and the last dosing. **CONCLUSIONS:** In the study range, the pharmacokinetic property exhibited linear. The absolute bioavailability was leaned to the low side, but there was no cumulation of rhGLP-1(7-36) in rhesus monkeys after administration time .

(Study supported in part by the National Natural Science Foundation of China, No 39930180, and National High-Tech R & D Program of China, No 2003AA2Z347B)

## PD03 Metabolic characteristics of methylsalicylate diglycoside *in vitro* and *in vivo*

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**KEY WORDS** methylsalicylate glycoside; analgesic; anti-inflammatory; anti-platelet; metabolism; β-glycosidase; carboxylesterase

**AIM:** Methylsalicylate diglycoside(MSDG) is a new aspirin-like natural product extracted from a folk drug: Gaultheria yunnanensis Rehd (GYR) in Yunnan province of China. GYR has been used in China as an anti-rheumatoid drug. It has also been used to treat flu, cough, asthma etc. To research and develop the folk medicine the pharmacological effects and pharmacokinetic character of its main constitute MSDG was studied. **METHODS:** Acetic acid-induced mice writhing model and croton oil-induced mice ear swelling model were used to study the analgesic and anti-inflammatory effects of MSDG respectively. The *in vivo* anti-platelet effect of MSDG after administration to rats was examined. The *in vivo* and in vitro metabolism character of MSDG was investigated to explain its pharmacological effects and its superiority over aspirin and aspirin-like drugs. **RESULTS:** MSDG was shown to have analgesic, anti-inflammatory and anti-platelet effects by animal experiment. *In vivo* metabolism study demonstrated that MSDG could metabolize to salicylic acid(SA) slowly and had less stimulating effect to gastrointestinal tract than aspirin. *In vitro* study showed that MSDG was metabolized by β-glycosidase of intestinal bacteria and serum carboxylesterase. **CONCLUSION:** MSDG is hopeful to be a new kind of analgesic, anti-inflammatory or anti-platelet drugs, maybe a substitute of aspirin.

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## PD04 Chiral pharmacokinetics and inversion of $N^G$-nitro-arginine in the rat

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**KEY WORDS** *L*-NNA; *D*-NNA; chiral inversion; chiral pharmacokinetics

**AIM:** To explore pharmacokinetics of $N^G$-nitro-*D*-arginine (*D*-NNA) and $N^G$-nitro-*L*-arginine (*L*-NNA) in conscious rats. **METHODS:** The plasma concentration of *D*-NNA and *L*-NNA were determined by chiral ligand exchange method with capillary electrochromatography (CEC). Pharmacokinetic parameters were estimated using non-compartment model and were fitted using a computer program DAS. Chiral inversion rate of *D*-NNA to *L*-NNA was calculated by the trapezoidal method. **RESULTS:** The metabolism of *D*-NNA and *L*-NNA exhibited significant isomeric selectivity. The CL and $t_{1/2}$ of *D*-NNA and *D*-NNA were 0.46±0.02 mL·h$^{-1}$·kg$^{-1}$ *vs* 0.17±0.03 mL· h$^{-1}$·kg$^{-1}$ ($P < 0.05$) and 1.44±0.28 h *vs* 3.48±0.41 h ($P < 0.05$), respectively. Unidirectional chiral conversion rate of *D*-NNA to *L*-NNA was 50.03 %±8.5 %. **CONCLUSION:** The stereoselective pharmacokinetics of $N^G$-nitro-arginine was maybe due to the unidirectional chiral inversion of *D*-NNA to *L*-NNA.

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## PD05 Effects of *Ganoderma lucidum* polysaccharides on diabetic nephropathy in mice

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**KEY WORDS** *Ganoderma lucidum;* polysaccharides; diabetic nephropathy; mice; streptozotocin

**AIM:** To study the effects of *Ganoderma lucidum* polysaccharides (*GL*-PS) on the renal damage in streptozotocin-induced diabetic mice. **METHODES:** Nine weeks old male C57 BL/6J mice were made diabetes with two or three consecutive intraperitoneal injection of streptozotocin, 72 h later, hyperglycemic mice with glucose levels higher than glucose 300 mg/dL were used. The diabetic mice were randomly divided into three groups and administrated intragastrically with vehicle or *Gl*-PS (125 mg/kg, 250 mg/kg), another group of normal mice were treated with the equivalent amount of vehicle. After the 8 weeks period of treatment, all mice were placed in individual metabolism cages to collect urine for 24 h, then the mice were sacrificed, and kidney tissues and blood samples were collected. Serum glucose, creatinine (Cr), blood urea nitrogen (BUN), triglyceride (TG), and total cholesterol (TC) were measured using commercial reagents. The urine volume and kidney weight were determined gravimetrically and urinary albumin concentration was measured by an enzyme-linked-immunosorbent assay using a mouse microalbuminuria kit. The degree of renal hypertrophy was expressed as the ratio of the two kidneys' weight to total body weight. Renal function was evaluated by measuring serum Cr and BUN level and 24 h urinary albumin excretion (UAE). **RESULTS:** Injection of streptozotocin significantly increased the blood glucose level in mice. At eight weeks after the streptozotocin injection, UAE levels of diabetic mice were significantly increased than the non-diabetic mice. The values were more than ten fold higher than that of non diabetic mice ($P$<0.001). Administration of *GL*-PS could dose-dependently reduce the glucose and UAE level, but only the dose of 250 mg/kg had statistic significance ($P$<0.05). A pronounced increase in Cr and BUN was observed in diabetic mice versus nondiabetic mice (76.5 $\pm$ 11.6 *vs* 5 $\pm$ 4.7 μmol/L and 43.6 $\pm$ 5.3 *vs* 31 $\pm$ 3.5 mg/dL respectively, $P$<0.01). However, *GL*-PS (125 mg/kg, 250 mg/kg) reversed these increase significantly ($P$<0.05). The diabetic mice had much higher TG and TC levels than normal mice ($P$<0.01), while administration of *GL*-PS (125 mg/kg, 250 mg/kg) reduced the TG level from 157 mg/dL to 124 mg/dL and 112 mg/dL ($P$<0.05, $P$<0.01), but it had no influence on TC level. Neither renal hypertrophy was decreased by *GL*-PS. **CONCLUSION:** *GL*-PS can improve the metabolic abnormalities of diabetic mice and prevent or delay the progression of the diabetic renal complication.

## Others

### PO01 Screening for potential inhibitors of influenza neuraminidase*

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**KEYWORDS** neuraminidase inhibitor; screening model; high throughput screening; virtual screening.

**AIM:** Applying the assay method of neuraminidase(NA) activity established for high throughput screening to find novel inhibitors of influenza virus NA. **METHODS:** Firstly, a virtual screening strategy was applied to our compound database to select drug-like compounds, and then a high throughput screening model of NA inhibitor was applied to test these compounds. **RESULTS:** Three compounds displayed higher inhibitory activities, the range of $IC_{50}$ was from 1 μmol/L to 6 μmol/L. **CONCLUSION:** The scaffolds of these compounds are very different from those of NA inhibitors approved for influenza treatment, their scaffolds are novel, and maybe they can give drug chemists some suggestion to design novel compounds with higher activities.

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### PO02 Neuroleptics are associated with an increased risk of venous thromboembolism: a case-control study.

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**KEY WORDS** neuroleptics; venous thromboembolism; case-control study

**AIM:** Preliminary reports suggest that use of antipsychotic drugs is associated with an increased risk of venous thromboembolism (VTE), but others did not confirm these results. The aim of this study was to evaluate the relationship between antipsychotic drugs and VTE. **METHODS:** The present report used data from an ongoing hospital-based case-control study designed to investigate genetic and environmental risk factors of VTE. This study started in May 2000 in a single centre in Brest, France, and included patients consecutively hospitalized for a documented venous thromboembolic event. Controls were matched on age, sex and the main risk factors of VTE (cancer, surgery, pregnancy). **RESULTS:** In June 2004, 857 cases and 736 controls were included. The mean age of patients was 67.7 year. No significant difference was found between cases and controls concerning the

main characteristics, except for smocking and body mass index. Among cases, 89 (10.4 %) were current users of neuroleptics compared to 35 (4.8 %) among controls. Current use of neuroleptics was associated with a significant increased risk of VTE (OR=2.32, CI 95 % 1.55-3.48). Excluding neuroleptics used for non psychiatric disorders, and after adjustment on the main confounding factors, this association remained significant (OR=3.48, CI 95 % 2.00-6.04). No difference was found between the different chemical categories of neuroleptics, but the number of patients in some groups had limited statistical power to demonstrate significant differences. Biological mechanisms of action have been proposed to explain this relation. Analyses are ongoing for anti-phospholipid antibodies and homocysteine. **CONCLUSION:** In this case-control study of hospitalized patients, neuroleptics use was associated with a significant increased risk of VTE. These results are concordant with previous reports. Nevertheless, further investigations are needed to explain wich mechanisms may be involved in such association and before use of neuroleptics can be definitely considered as risk factor for VTE.

**PO03 Evaluation and validation of drug targets**

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Drug target is one of the key factors for discovering and developing new drugs. To find and validate drug targets is a crucial technique required in drug discovery by the strategy of high throughput screening. Based on the knowledge of molecular biology, human genomics and proteomics, it has been predicted that 5000 to 10000 drug targets exist in human. So, it is important procedure to evaluate and validate the drug targets.

When we evaluate or validate the drug targets, we must know the definition and characteristics of drug targets. Under analysis of the known drug targets, we define the drug target as that the biomolecules which a drug could bind with and produce therapeutic effects. We also summarized drug target qualifications as the following: 1. Drug target is biomolecule(s), normally is protein, which could exist in isolated or complex modality. For example the GP receptors, channels and enzymes. 2. The biomolecules have special sites which match other molecules (commonly the small molecules) with special structures. These molecules could be the endogenous or extraneous substances such as chemical molecules (drugs). 3. The biomolecular structure could be changed when it binds to small molecules, but the change of structure normally are reversible. 4. Following the change of biomolecule's structure, there are various physiological responses occur and induce regulation on the cell, organ, tissue or the body status. 5. The physiological responses triggered by the changes of biomolecule structure play a major role in the complex regulation and exert therapeutic effect in pathological conditions. 6. The expression, activities and structures of the biomolecules could be changed in the duration of the pathological procedures. 7. The small molecules bind to the biomolecules are drugs.

The above qualifications of drug target describe the characteristics of biomolecules except the last one. So, if a biomolecule have the characteristics which match most of the above conditions, it is a candidate of drug target. The candidate may become a drug target if we could find drugs to match it. If a biomolecule only match some of the conditions, it may be a potential drug target. It is very important for potential drug targets to reveal their characteristics which match the above qualifications, i.e. validating the drug target.

Until now, numerous potential drug targets have been found by means of the human genomic and proteomic research. The understanding of drug target will promote the procedure of validating drug targets.

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**PO04 Analytical case reports: major discrepancy between digoxin immunoassay results in a context of acute overdose**

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**KEY WORDS** digoxin; overdose; level, underestimation

**AIM:** Digoxin, a heteroglycoside drug with cardiotonic effects, has a narrow therapeutic range, so therapeutic drug monitoring has been very helpful in improving patient management and preventing toxic effects. We report two cases of digoxin intoxication where the immunoassay techniques used for drug monitoring produced very different values with important clinical implications. **METHODS:** Serum levels of digoxin were determined with several analytical immunoassay techniques [Cobas Mira with enzyme multiplied immunoassay (EMIT) reagents, fluorescence polarization immunoassay (FPIA)/FLx with Digoxin II assays reagents, microparticle enzyme immunoassay (MEIA)/AxSYM] and verified by liquid chromatography/mass spectrometry (LC/MS). **RESULTS:** If results obtained with the EMIT and digoxin II reagents are comparable and coherent with the LC/MS results, the authors described discordant results with MEIA/AxYM; this test greatly underestimated the digoxin serum concentrations. On another hand, the authors demonstrated that results comparison between FPIA or MEIA using Abbott reagent obtained on 30 serum samples addressed to the laboratory for serum digoxin control, did not reveal any significant difference between both assays. **CONCLUSION:** In conclusion, although the underlying cause remains to be determined, these findings throw some doubt on the reliability of the MEIA technique for detecting digoxin overdose.

**PO05 Establishment of HEK293 cell line expressing GFP-AQP1 to determine water osmotic permeability**

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**KEY WORDS** osmotic water permeability; AQP1; stably transfected cell

**AIM:** To develop an osmotic water permeability assay. **METHODS:** We subcloned the rat AQP1 cDNA into pEGFP-C3 vector. HEK293 cells were transfected with pEGFP-C3/AQP1 or pEGFP-C3 and selected by G418. The expression of AQP1 was detected by RT-PCR and Western blot. Confocal laser fluorescence microscopy was used to record the change of fluorescent density corresponding to the volume change of the cells induced by hypotonic solution. Ratios of cell fluorescence to cell area were used to assess the cell volume change. The reliability and feasibility of the model was validated by the inhibitor $HgCl_2$. **RESULTS:** The stably transfected HEK293 cell line expressing AQP1 tagged with GFP can be recognized with confocal microscopy. The AQP1 expression in HEK293 cell stably transfected with pEGFP-C3/AQP1 was higher than that with empty vector. We found that the relative change rate of fluorescence intensity of the cells transfected with pEGFP/AQP1 was higher than that with pEGFP-C3. Treatment with $HgCl_2$ can remarkably weaken the relative change rate of fluorescence intensity in a dose-dependent manner. **CONCLUSION:** The relative change rate of fluorescence intensity of the HEK293 cells stably transfected with pEGFP-C3/AQP1 can be applied to evaluate the osmotic water permeability using confocal laser microscopy.

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**PO06 Effect of tea polyphenols on alcoholic liver disease**

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**KEY WORDS** Tea polyphenols; alcoholic liver disease; aldehyde

**AIM:** To investigate the scavenging effects of tea polyphenols on aldehyde *in vitro* and searching for the preliminary mechanism of tea polyphenols (TP) on alcoholic liver disease. **METHODS:** The effect of aldehyde absorption is tested at gaseous and liquid phases. High performance liquid chromatography (HPLC, HP1100Series) and UV-visible Detector (Wavelength: 235 nm) are used to analyze the components of the outcome of solution reaction. **RESULTS:** *In vitro* study showed that after 6h, the values would be about 0.076 $\mu g/L^3$ at gaseous phase and after 5 h, 87.5 % aldehyde is reacted at liquid phase. This approach could give adequate control of aldehyde evolution. The conclusion could be drawn by HPLC that the contents of outcome were dimer of TP and eletrophilic substituent product. **CONCLUSION:** The data suggest that TP could scavenge aldehyde both at gaseous and liquid phases, and we put forward that TP acts as antihepatotoxic drug, both at and subsequent to the time of manufacture of the product.

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**PO07 The (SNP) of multi-drug resistance 1 protein (MDR1, P-glycoprotein) in Chinese Han population**

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**AIM:** To investigate the single nucleotide polymorphism (SNP) of multi-drug resistance 1 protein (MDR1, P-glycoprotein) in the Chinese Han population. **METHODS:** DNA was extracted from 200 μL heparin-anticoagulated whole blood using QIAamp Blood Kit. A polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used for the detection of C3435T SNP. The PCR product of 248 bp was digested with restriction enzyme Dpn II. The digested products were separated on a 2.0 % agarose gel. **RESULTS:** The blood samples of one hundred Chinese Han population were evaluated for C3435T single nucleotide polymorphism (SNP) in exon 26 of the MDR1 gene. The samples included 25 males and 75 females with the age of $18 \pm 0.7$ years old. The frequencies of C allele and T allele are 66 % and 34 %, respectively. The genotype of C/C, C/T, and T/T had frequencies of 50 %, 41 %, and 9 %, respectively, which are different from the reported frequencies of 32 %, 42 %, and 26 % in Chinese population. **CONCLUSION:** In the present study, the C3435T SNP of MDR1 gene is observed in the Chinese Han population. This may constitute one of the genetic mechanisms in differences of drug response among different Chinese ethic groups. Further research is being developed in other Chinese ethnic population. Two more polymorphisms of the MDR1 gene (2677 and 1236 position ) are also being detected.

**PO08 Proteomics: a new approach for drug discovery**

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**KEY WORDS** proteomics; drug discovery; aquaporin 1

Proteomics is a bridge that crosses genome to drug discovery. Proteomic studies will provide possible targets for

therapeutic usage and moreover increase the efficiency of the downstream of drug discovery process. By using the 2-D electrophoresis combining with MS technology, which is most prevalent techniques of proteome, we have identified more than 20 proteins or peptides that were induced or inhibited by several compounds or in animal disease models. In addition, we have prepared polyclonal antibodies against 5 purified proteins and performed further functional studies. Establishment and application of high efficient and throughput cellular screening system is an important facet on a new target discovery and validation. Recently we have build up and applied the *Xenopus* oocytes transgenic system including their cell free format to observe compounds on water transportation of aquaporin1 (AQP1) gene and screen some drugs on apoptosis process. Structure-based drug design, docking and computer screening with protein and compound database are newly developed techniques on target discovery and targets and leading validations. Collaborated with chemists we have confirmed the binding sites of two carbonic anhydrase inhibitors in the AQP1 proteins 3D structure and conducted to synthesize more than 100 compounds that could regulate AQP1 protein. The pharmacological studies on these compounds were in operation.

### PO09 Effects of *Ganoderma* sterols (*GS*) on hepatic cytochrome P450 in BCG-induced immunological hepatic injury in BALB/c mice

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**AIM:** To investigate effects of *Ganoderma* sterols (*GS*) isolated from *Ganoderma lucidum* (Leyss ex fr) Karst on hepatic cytochrome P450 in BCG-induced immunological hepatic injury in BALB/c mice and its possible mechanism. **METHODS:** Immunological liver injury was induced by one intravenous injection of BCG (125 mg/kg) in BALB/c mice. One week later, successive intragastric administration of *GS* (20, 40, 80 mg/kg, per day) and intraperitoneal injection of aminoguanidine (50 mg/kg, every other day) was given. On the $d_{14}$ after injection of BCG, serum ALT and nitrite levels and CYP450 total content in homogenate sample were measured through spectrophotometry. The hepatic tissue injury was estimated by histopathological H-E staining. The expression of iNOS and CYP2E1 in hepatic tissue was determined by immunohistochemical method. **RESULTS:** Two weeks after BCG injection, serum ALT levels increased; granuloma was easily observed, over-expression of iNOS protein was detected on the granulomas, and decrease of CYP2E1 protein expression was also observed on mice hepatic tissue. The CYP450 total content in homogenate sample (4.666±0.699 nmol/g liver, $P<0.05$) reduced to 53.6 % of that of the control group (8.700±2.678 nmol/g liver). Aminoguanidine, a selective iNOS inhibitor, can lower the increased serum ALT levels, partly increase the CYP450 total content, and significantly inhibit iNOS expression. GS of three oral doses reduced serum ALT levels dose-dependently compared with liver injury model group, diminished liver haemorrhage and inflammation, increased the CYP450 total content and decreased iNOS expression. **CONCLUSION:** Morphology of liver tissue, levels of serum marker enzymes and hepatic CYP450 content indicated that *GS* regulate CYP450 expression under condition of immune liver injury by way similar to iNOS inhibitor.

**Tab 1. Effect of inhaled fluticasone on airway reactivity and 8-Iso-PGF2α in bronchoalveolar lavage fluid of cats. (see PI02, p 1559).**

| Treatment | % Carbachol (Penh300) | Bronchoscopy Score (0-6) | BAL % Macro | % Neutro | % Eosino | 8-Iso-$PGF_{2\alpha}$ (pg/mL) |
|---|---|---|---|---|---|---|
| PRE | 0.043 ± 0.019 | 4 (2-4) | 83±13 | 16±13 | 0.6±0.9 | 5.09±0.51 |
| Flu-1W | 0.053 ± 0.020[b] | 2 (1-3) | 86±3 | 13±3 | 0.2±0.5 | 2.81±0.59[b] |
| Flu-2W | 0.059 ± 0.020[b] | 1.5 (1-3)[b] | 79±8 | 15±3 | 0.6±1.3 | 1.06±0.56[b] |

[b] $P<0.05$ *vs* PRE.