

Therapeutic Effect of JCICM-4-07 on Visceral Pain and Diarrhea

Zhao-Xiang Bian¹, Xiao-jun Zhang¹, Shun Tan¹, Hong-xi Xu², Joseph J.Y. Sung³

¹School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

²Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong

³Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

In this study, we investigated the analgesic effect and anti-diarrhea effect of Traditional Chinese medicine formula JCICM-4-07 on rodent visceral pain and diarrhea models. The decoction of material herbs were either vacuum dried (water extraction) or processed by ethanol precipitation followed by vacuum dryness (ethanol precipitation). The analgesic effect of the above two preparations was evaluated by mice hot-plate test and writhing test. The anti-diarrhea effect was evaluated on Sennae leaf or castor oil induced mice diarrhea model. In addition, the therapeutic effect on visceral pain and stress-induced defecation on a rat model of irritable bowel syndrome induced by neonatal maternal separation were evaluated by observing the visceral muscular response induced by colorectal distension and number of fecal pellets during 1 hour water-avoidance stress. The results demonstrated that both kind of preparations showed analgesic effect and anti-diarrhea effect on mice models, whereas, ethanol precipitation-processed preparation showed analgesic and anti-diarrhea effect on a lower dosage compared with water extraction. The results revealed that JCICM-4-07 formula is effective on rodent visceral pain and diarrhea, and indicate that ethanol precipitation is an applicable processing method that not only can reduce dose volume but also guarantee effectiveness. The mechanism underlying the bioactivities of JCICM-4-07 is under further investigation.

The Role of Nerve Growth Factor in Visceral Hypersensitivity and Irritable Bowel Syndrome

Siu Wai Tsang, Zhaoxiang Bian

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder which can be characterized by cramping, abdominal pain, bloating, constipation and diarrhea, and thus causes a great deal of discomfort. Stress, either physical or psychological, seems closely correlated to the development of visceral hypersensitivity in IBS patients. In our study, animals which were subjected to neonatal maternal separation (NMS) showed to develop visceral hypersensitivity and with an increase in nerve growth factor (NGF) expression in the spinal cord and dorsal root ganglion (DRG) when comparing to the normal handling group. The expression levels of the NGF cognate receptor TrkA, Substance P and CGRP were also found up-regulated in those NMS rats. Most importantly, the group which was given exogenous administration of NGF demonstrated with visceral sensitivity as well, and the expression levels of TrkA, Substance P and CGRP in spinal cord and DRG were also induced correspondingly. However, the development of visceral hypersensitivity can be diminished when the NGF antagonist was applied. Both immunofluorescent images and Western blotting results showed that the expression levels of NGF, TrkA, Substance P and CGRP were reduced by the anti-NGF treatment. Therefore, NGF may play an important role in the development of visceral hyperalgesia, thus the pathogenesis of IBS.

Excitatory and Inhibitory Amino Acid Changes in Brain of Neonatal Maternal Separation Rats: An In Vivo Microdialysis Study

Shengda Qi^a, Xiaojun Zhang^a, Hongxi Xu^b, Joseph J. Y. Sung^c, Zhaoxiang Bian^{a,*}

^aSchool of Chinese Medicine, Hong Kong Baptist University, Hong Kong

^bHong Kong Jockey Club Institute of Chinese Medicine, Hong Kong

^cFaculty of Medicine, The Chinese University of Hong Kong, Hong Kong

Changes in amino acids levels in the brain of neonatal maternal separation (NMS) rats were studied. In the present experiments a combination of capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) and microdialysis was used to determine arginine, glutamate, aspartate, asparagine, glutamine, serine, γ -amino butyric acid, citrulline and glycine in the thalamus of rats. These amino acids were derivatized with 4-fluoro-7-nitrobenzo-2,1,3-oxadiazol (NBD-F), separated by capillary zone electrophoresis (CZE) including 30mM borate, 90 mM boric acid and 5% (v/v) methanol. Result was shown that increase in glutamate, aspartate, serine and citrulline of NMS rats were observed and other amino acids were not versus no handling or separation (NH). This indicated that the release of glutamate, aspartate, serine and citrulline may mediate early life stress in the thalamus.

Role of MicroRNA-214 in Ginsenoside-Rg1-induced Angiogenesis

Lai-Sheung Chan, Patrick Ying-Kit Yue, Nai-Ki Mak, Ricky Ngok-Shun Wong*

Department of Biology, Hong Kong Baptist University, Hong Kong

MicroRNAs (miRNAs) are small non-coding RNAs that act as post-transcriptional gene modulators. Ginsenoside-Rg1, one of the active components of ginseng, has been confirmed as an angiogenesis inducer. Using miRNA microarray analysis, a total of 17 (including miR-214) and 5 miRNAs were found to be down- or up-regulated by Rg1 in human umbilical vein endothelial cells (HUVECs), respectively. Since endothelial nitric oxide synthase (eNOS) is a target of miR-214 and is related to angiogenesis, its expression was further validated by qRT-PCR. We also investigated the role of miR-214 in eNOS expression and in tubulogenesis and motility of HUVEC by transfection of specific miRNA inhibitor or precursor. Our results suggested that Rg1 can down-regulate miR-214 expression, leading to an increase in eNOS expression, and in vitro cell migration and tube formation which can possibly promote angiogenesis. These results signify a new understanding towards how a simple natural compound can affect physiological changes through modulation of miRNA expression.

Activation of Canonical Wnt Signaling Pathway in Aortic Vascular Smooth Muscle Cells is Associated with Oxidative Stress Induction in Diabetic Macro-vasculopathy

Victor K. L. Hung*, Daniella P. K. Wong, Carmen K. M. Law, Christopher H.K. Cheng and Kevin K. M. Yue

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

School of Medical Sciences, The Chinese University of Hong Kong, Hong Kong

Hyperglycemia-induced oxidative stress is critical to the development of diabetic complications. Recently, the involvement of canonical Wnt signaling in oxidative stress induction has been demonstrated in diabetic nephropathy and retinopathy. Beta-catenin, the key transcription factor in the canonical Wnt pathway, was found to be differentially regulated among complications, implicating that the regulation may be tissue- or cell type-specific. In this study, it aims to determine the role of canonical Wnt signaling and oxidative stress in diabetic macro-vasculopathy. Here, the correlation between beta-catenin and oxidative stress in the macro-vasculature was investigated using streptozotocin (STZ)-induced diabetic animals and high-glucose challenge of vascular smooth muscle cells (VSMCs). To determine the time frame of beta-catenin alteration in the pathogenesis of diabetic macro-vasculopathy, a time-course study was performed in STZ-induced diabetic rats. The results showed that both expression and nuclear translocation of beta-catenin were significantly induced in the VSMC layer of the aortae as early as two weeks after STZ-injection, suggesting that activation of canonical Wnt pathway is an early event in response to hyperglycemia. Similarly, after 2-days of high-glucose challenge, primary VSMCs showed induction in ROS production and an increased in beta-catenin protein level in the nuclear fraction. In conclusion, our findings indicate that activation of the canonical Wnt signaling pathway is associated with oxidative stress induction in diabetic macro-vasculopathy.

GR/PI3K/Akt/eNOS Signaling Plays an Important Role in Cardioprotection of Ginseng Extract RSE in Rats

Hua Zhou; Shao Zhen Hou; Pei Luo; Bao Zeng; Yuen Fan Wong; Liang Liu

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Background: Previous studies demonstrated the cardioprotective effects of ginseng. However, the underlying mechanism remains unclear. In the present study, we investigated the effect of a standardized ginseng extract (RSE) in protecting rat heart against ischemia-reperfusion (I/R) injury and the role of nitric oxide (NO) generation via glucocorticoid receptor (GR) signaling in this effect.

Methods: Rats were subjected to 30 min ischemia followed by 90 min reperfusion by occluding and reopening of LAD. RSE standardized with HPLC fingerprint was orally given to rats 60 min before ischemia. Some rats received GR inhibitor RU468, NO synthase (NOS) inhibitor L-NAME, PI3K inhibitor LY294002, Akt inhibitor IV or transcriptional inhibitor ActD. eNOS^(-/-) and iNOS^(-/-) KO mice were employed to further identify the role of NOS.

Results: Compared with I/R control (10.2±2.1%), RSE treatment significantly decreased infarct size (TTC staining) of heart in a dose-dependent manner (0.3±0.3% and 5.8±1.2% for 80 and 40mg/kg of RSE, respectively). 80mg/kg RSE induced a significant increase in serum NO level (10.7±0.7µM vs. 6.5±0.9µM) while a remarkable decrease in CK activity (1518±65U/L vs. 916±55U/L) and LDH level (178±31U/L vs. 464±43U/L). Western blotting analysis showed that I/R treatment significantly suppressed activation of GR, PI3K, Akt, and eNOS in heart tissues, but RSE significantly reversed the suppressed activation of these proteins. In addition, the protection induced by 80mg/kg RSE was abolished by the blockage of GR (11.4±3.8%), PI3K (10.0±2.4%), Akt (8.8±3.9%), and eNOS (14.2±1.6%). However, ActD (0.6±0.3%) didn't reverse the protection. In gene knockout mice, knockout of eNOS completely blocked the effect of RSE. In contrast, knockout of iNOS didn't do this.

Conclusion: RSE affords rat hearts an acute cardioprotection, which is related to NO production and requires GR activation through non-genomic pathway in which PI3K, Akt, and eNOS are sequentially activated. Furthermore, eNOS instead of iNOS is essential for the cardioprotective effect of RSE.

Ginsenosides Increase Coronary Perfusion Flow in Rat Heart through Activation of PI3K/Akt-eNOS Signaling and Cardiac Energy-Associated Protein Expression

Xiao Qin Yi, Jing Rong Wang, Ting Li, Vincent Kam Wai Wong, Pei Luo, Shao Zhen Hou, Bao Zeng, Ivan Yuen Fan Wong, Zhi Hong Jiang, Liang Liu *, Hua Zhou *

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Background: Asian ginseng is the most popular herb used for treatment of ischemic heart diseases in Asia; ginsenosides are considered to be the major active ingredients. However, whether ginsenosides can enhance the coronary artery flow of ischemic heart and, if so, by what mechanisms they do this, remain unknown.

Methods: Isolated rat hearts with ischemia/reperfusion injury in Langendorff system were employed for examining the effect of total ginsenosides (TGS) on coronary perfusion flow (CPF). In addition, human aortic endothelial cells (HAECs) were used for mechanistic studies. Levels of various vasodilative molecules, intracellular calcium concentration [Ca²⁺]_i, NO, cell survival and apoptosis signaling pathways in heart tissues and HAECs were determined. Protein profiling of the rat heart tissues were established with 2D-gel/MALDI-TOF-MS/MS.

Results: TGS dose-dependently increased CPF and improved systolic and diastolic function of the ischemic heart, while the inhibitors of NOS, sGC, HO, COX, and potassium channel abolished the effect of TGS. Positive control verapamil was effective only in increasing CPF. TGS elevated levels of NO and 6-keto-prostaglandin F_{1α} in coronary effluents and supernatants of HAECs cultures, and [Ca²⁺]_i in HAECs. And, TGS could up-regulate expression of PI3K, p-Akt, p-eNOS, and p-p38 MAP kinase. 2D-gel image analysis detected over 1000 protein spots from the ischemic heart tissues, in which 11 protein spots were up-regulated by TGS, and of these, 6 identified proteins are related to cardiac energy metabolism.

Conclusions: TGS significantly increased CPF of the ischemic rat hearts through activation of PI3K/Akt-eNOS signaling and regulation of cardiac energy.

Synergistic Effects of the Combination of Baicalin with Ciprofloxacin and Gentamicin against Methicillin-resistant *Staphylococcus Aureus*

¹Ben Chung-Lap Chan, ²Margaret IP, ¹Clara Bik-San Lau, ^{1,2}Sau-Lai Lui, ¹Kwok-Pui Fung^{1,3} & ¹Ping-Chung Leung

¹Institute of Chinese Medicine, ²Department of Microbiology and ³School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong

Objective: Baicalin, the main active constituent derived from *Scutellaria baicalensis* Georgi, has previously been shown to significantly restore the effectiveness of β -lactam antibiotics against Methicillin-resistant *Staphylococcus aureus* (MRSA). The aim of the present study was to further investigate the resistance mechanisms of baicalin against MRSA.

Methods: The *in vitro* antibacterial activities against three *Staphylococcus aureus* (SA) strains with known specific drug resistance mechanisms were studied: (1) SA-1199B harbouring resistance to fluoroquinolones such as ciprofloxacin through over-expression of the NorA efflux pump, (2) SA-MsrA harbouring resistance to 14- and 15-membered macrolides include erythromycin through multicopies plasmid of pUL5054 and (3) SA APH2"-AAC6', harbouring resistance to gentamicin through modifying aminoglycosides with specific effect on aminoglycoside-6'- N-acetyltransferase/ 2'-O-phosphoryltransferase. The antimicrobial activities of different antibiotics, alone or in combination with baicalin, were investigated by the checkerboard method in 96-well microtitre plates. Fifty microlitres of the first antibiotic of the combination was serially diluted along the ordinate, while 50 μ l of baicalin was diluted along the abscissa. Each well was inoculated with 100 μ l of a bacterial inoculum of 5×10^5 CFU/ml, and the plates were incubated at 37°C for 24 h under aerobic conditions. The synergistic activities of the studied drugs were further evaluated by time-kill assay on the strains for 24 h at 37°C.

Results: In the checkerboard dilution test and time-kill assay, the sub-inhibitory concentrations of baicalin ranged from 16-64 μ g/ml could synergistically restore the antibacterial actions of ciprofloxacin (2 μ g/ml) and gentamicin (64 μ g/ml) at sub-inhibitory concentrations against the growth of the resistant strains SA-1199B and SA APH2"-AAC6' respectively. No synergistic effect of baicalin was observed with erythromycin against SA-MsrA.

Conclusion: Our results demonstrated that baicalin could reverse the multiple resistance of MRSA by inhibiting the NorA efflux pump and aminoglycoside modifying enzymatic activity.

The *In Vivo* and *In Vitro* Diabetic Wound Healing Effects of a 2-herb Formula and its Mechanisms of Action

Lau KM^{1*}, Liu CL^{1,2}, Tam CW¹, Lai KK¹, To MH¹, Kwok HF¹, Lau CP¹, Ko CH¹, Leung PC¹, Fung KP^{1,2} and Lau CBS¹.

¹*Institute of Chinese Medicine;* ²*School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong*

Diabetic complications, such as foot ulcer, impose major public health burdens worldwide. In our previous clinical studies, two Chinese medicine formulae F1 and F2 have saved over 80% limb salvage. A simplified 2-herb formula (NF3) comprising of Radix Astragali (RA, 黃耆) and Radix Rehmanniae (RR, 生地) in the ratio of 2:1 was used for further study.

The objective of the present study was to examine the ulcer healing effect of NF3 in diabetic rats, and its potential mechanisms of action in angiogenesis, anti-inflammation and fibroblast proliferation *in vitro*. Diabetic rat model with wound induction in the foot was used for studying the wound healing effect of oral administration of NF3. For studying angiogenesis, human umbilical vein endothelial cells (HUVEC) and human microvascular endothelial cells (HMEC-1) were used. MTT assay, migration scratch assay, and tube formation assay were assessed in both endothelial cells. For studying anti-inflammation, mouse macrophage cells (RAW264.7) were used for measuring the reduction of nitric oxide production. For studying fibroblast proliferation, human fibroblast cells (Hs27) were used.

Our *in vivo* results demonstrated a significant reduction of wound area at day 8 in NF3 (0.98 g/kg) group as compared to control ($p < 0.01$). Besides, NF3 significantly increased the cell migration in both HUVEC ($p < 0.01$, at 75 μ g/ml by 42%) and HMEC-1 ($p < 0.001$, at 75 μ g/ml by 104%) cells in migration scratch assay. NF3 also significantly increased the total tube length formed by HUVEC and HMEC-1 cells in Matrigel. In addition, significant inhibition of nitric oxide production ($p < 0.01$) was found in NF3-treated (1250-5000 μ g/ml) macrophage cells, suggesting its anti-inflammatory activity. Furthermore, NF3 (156-2500 μ g/ml) could significantly stimulate Hs27 proliferation in a dose dependent manner ($p < 0.05$).

In conclusion, the formula NF3 could enhance diabetic wound healing through the actions of angiogenesis, anti-inflammation and tissue regeneration.

Acknowledgement: This study is supported by HK UGC under the Area of Excellence project "Chinese Medicine Research and Further Development" (Ref. No. AoE/B-10/01).

The Anabolic Effect of Green Tea and its Polyphenol, Epigallocatechin on Bone Through the Differentiation of Mesenchymal Stem Cells

Chun Hay Ko^{1*}, Wing Sum Siu¹, Clara BS Lau¹, Kwok Pui Fung^{1,2}, Ping Chung Leung¹

¹Institute of Chinese Medicine; ²School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong.

Green tea, the most commonly consumed herb worldwide, has been recently demonstrated as a potent bone supportive agent. Our previous studies showed that green tea and its polyphenolic constituents could promote bone-forming osteoblast activities and inhibited the bone-resorpting osteoclast formation. The objective of the present study was to investigate whether green tea and its components can promote the osteogenic differentiation in bone progenitor mesenchymal stem cells (MSCs). In brief, the rat MSCs were isolated from the bone marrow of tibiae and femora. The cells were treated with two green tea extracts (caffeinated and decaffeinated forms) and six tea polyphenols (catechin, C; epicatechin, EC; gallic catechin, GC; epigallocatechin, EGC; gallic catechin gallate, GCG; epigallocatechin gallate, EGCG) under osteogenic induction. The alkaline phosphatase (ALP) activities and matrix calcium (Ca) deposition were assessed after 5 days and 10 days of treatment. The cell viability of each treatment group was determined by MTT assay. The difference between treatment and control groups were tested with the Kruskal-Wallis test, followed by post hoc Dunn test. Our results demonstrated that both green tea extracts could significantly increased ALP activities and Ca deposition dose-dependently in the concentrations without cytotoxicity (0-100µg/mL). Among all tea polyphenols, EGC was shown to be the most effective in promoting osteogenic differentiation. At 20µM, EGC increased ALP levels by 2.5 fold when compared with the respective control group. In conclusion, this is the first report of the bone-forming effects of green tea extracts and the polyphenol EGC. Our results provided scientific evidence to support the potential use of green tea in the treatment of bone degenerative diseases such as osteoporosis.

Acknowledgement: This study was supported by the Ming Lai Foundation and The International Association of Lions Clubs District 303, Hong Kong and Macau Tam Wah Ching Chinese Medicine Resource Centre.

Photoinactivation of Hypericin on MDR S Aureus And ESBL-Producing E.Coli Pathogens

Yow CMN¹, RWK Wu¹, HM Tang¹, Hamblin MR²

¹Department of Health Technology & Informatics, The Hong Kong Polytechnic University, Hong Kong

²Wellman Center for Photomedicine, Harvard Medical School, USA

Photodynamic inactivation using visible light source on Hypericin (HY) isolated from a natural *Hypericum perforatum*, can generate reactive oxygen species to eradicate the bacteria.

Here, the effect of HY mediated Photodynamic Therapy on *Staphylococcus aureus*, *E. coli* and their MDR strains were determined by minimum bactericidal concentration and SEM. A wild type strain each of *S. aureus* and *E. coli*, a clinical MDR isolated of MRSA and ESBL-producing *E. coli* were included.

For *S. aureus* strain, complete killing (99.99% = 6 log) for wild type and MRSA (MDR) was obtained with 4µg/mL Hypericin at 30 Jcm⁻². While 32.5% killing was obtained for both wild type *E. coli* and ESBL-producing *E. coli* with 20µg/mL at 30 Jcm⁻². Under Scanning Electron Microscopy, there was irregular and remarkable dented membrane changes in HY-treated *S. aureus* while no such changes were observed for *E. coli* strains.

This study offered new insights on HY mediated photoinactivation especially for the MDR pathogens.

This study was supported by PolyU grant (G-U422)

The Role of Reactive Oxygen Species in the Hepatoprotective Mechanism of Schisandrin B In Vivo: A Comparative Study with Curcumin and Menadione

Pou-Kuan Leong, Po Yee Chiu and Kam-Ming Ko

Division of Life Science, Biochemistry and Cell Biology Section, The Hong Kong University of Science & Technology, Hong Kong

Schisandrin B (Sch B) is a dibenzocyclooctadiene derivative isolated from the fruit of a traditional Chinese herb – *Schisandra chinensis*. Previous studies in our laboratory have shown that the hepatoprotection afforded by Sch B involves the enhancement in hepatic glutathione antioxidant status, particular in the mitochondrion. We hypothesize that reactive oxygen species (ROS) generated from the cytochrome P-450 (CYP)-catalyzed metabolism of Sch B may play an important role in the hepatoprotective effect. In present study, we compared the effects of Sch B with those of curcumin, a phytochemical, and menadione, a prooxidant, on ROS production in mouse liver microsomes and hepatic injury in carbon tetrachloride (CCl₄)-intoxicated mice. Results showed that when added at the same concentration of 20μM, the amount of ROS produced by curcumin was larger than those of menadione and then Sch B in liver microsomes. However, only Sch B and curcumin (2 mmol/kg, single oral dose) enhanced hepatic mitochondrial reduced glutathione (mtGSH) level and conferred hepatoprotection against CCl₄-induced toxicity *in vivo*, with the hepatoprotection afforded by Sch B being more potent. The inability of menadione (2 mmol/kg) to induce hepatoprotection may be due to the excessive amount of ROS production *in vivo*, presumably due to its high bioavailability. In contrast, the smaller extent of hepatoprotection by curcumin than that of Sch B may be due to the low bioavailability of oral administration. To test this postulation, we investigated the effect of long-term Sch B or curcumin treatment (20 mg/kg/day ×15 and 18.6 mg/kg/day ×15, respectively) on CCl₄ hepatotoxicity in mice. The result indicated that both Sch B and curcumin treatment enhanced hepatic mtGSH level and protected against CCl₄ hepatotoxicity to similar extents. In conclusion, ROS generated from microsomal enzyme-catalyzed xenobiotic metabolism is causally related to the enhancement in hepatic mtGSH level and the associated hepatoprotection.

Phytochemicals Ameliorates Oxidative Stress by Inducing Glutathione Antioxidant Response through ROS Production in H9c2 Cells

Na Chen*, Po Yee Chiu and Kam Ming Ko

Division of Life Science, Biochemistry and Cell Biology Section, The Hong Kong University of Science & Technology, Hong Kong

A number of phytochemicals have been reported to produce antioxidant action indirectly through activating the redox-sensitive Nrf2/ARE pathway that results in transcriptional induction of a series of cytoprotective proteins, including those involved in cellular glutathione antioxidant system. To investigate whether phytochemicals induce glutathione antioxidant response by producing reactive oxygen species (ROS), the differential abilities of phytochemicals, namely, (-)-schisandrin B [(-)-Sch B](from Schisandra berry), curcumin (from turmeric), resveratrol (from grapes) and epigallocatechin gallate (EGCG, from green tea), to produce ROS, as well as to increase reduced glutathione (GSH) level, a measure of glutathione antioxidant response, during the period of post-drug exposure were examined in H9c2 cells. Furthermore, the relative potencies of these phytochemicals in protecting against menadione cytotoxicity or tert-butylhydroperoxide (*t*-BHP)-induced GSH depletion were also investigated. The results indicated that phytochemicals at concentrations that can produce a threshold amount of ROS could induce glutathione antioxidant response in H9c2 cells. Consistently, the relative abilities of the tested compounds to protect against menadione toxicity were associated with the enhancement of cellular GSH under oxidative stress condition. In addition, the cytoprotection afforded by these phytochemicals were correlated with the suppression of *t*-BHP-induced GSH depletion and the subsequent glutathione reductase-mediated GSH recovery in H9c2 cells. In conclusion, the results support the postulation that phytochemicals induce glutathione antioxidant response and the associated cytoprotection through the intermediacy of ROS production in H9c2 cells.

Antimicrobial Activity of Fructus Mume Extract and its Main Acidity Compounds on *Streptococcus Mutans*

Yong Chen, Ricky Wong, C. Jayampath Seneviratne, Urban Hagg, Colman McGrath,
Lakshman P Samaranayake

Faculty of Dentistry, The University of Hong Kong, Hong Kong, Hong Kong

Objective: Fructus mume is a traditional Chinese medicine used for antiparasitic, and anti-inflammatory. This study aimed to evaluate the antimicrobial activity of Fructus mume extract and its three main acidity compounds citric acid, tartaric acid and oxalic acid against *Streptococcus mutans* in vitro.

Materials and methods: The main components of Fructus mume extract was analyzed by High Performance Liquid Chromatography (HPLC). The antimicrobial effect of Fructus mume water extract, 10% citric acid, 10% tartaric acid and 10% oxalic acid on the planktonic susceptibility of *Streptococcus mutans* were tested by agar diffusion assay and the microdilution method (MIC). The cell viability of *S.mutans* biofilm on Damon3 MX bracket (Ormco, USA) after exposed to Fructus mume extract was observed confocal laser scanning microscopy (CLSM).

Results: HPLC analysis revealed the main compounds of Fructus mume were organic acids. Standard agar diffusion assay showed that 1g/mL Fructus mume extract, 10% citric acid, 10% tartaric acid and 10% oxalic acid created $20\pm 0.5\text{mm}$, $13\pm 0.5\text{mm}$, $15\pm 0.5\text{mm}$ $17\pm 0.5\text{mm}$ inhibitory zones (Diameter) respectively. The antimicrobial MIC of Fructus mume extract, citric acid, tartaric acid and oxalic acid were 50 mg/mL, 2.5mg/mL, 2.5 mg/mL, 20 mg/mL, respectively. Microscope image showed Fructus mume extract obviously increased the amount of dead bacteria on the surface of *S.mutans* biofilm.

Conclusion: Fructus mume extract and its acidity compounds showed strong inhibitory effect on *Streptococcus mutans* in vitro which may support this TCM use as oral antimicrobial agent.

Keywords: Fructus mume, antimicrobial, Chinese medicine

Novel Mechanisms: Components of Dendrobium Species that Promote Aquaporin-5 (AQP-5) Expression

Lin X, Song JX, Stephen Sze CW, Tong Y, Kavin Wong CK, Chung WY, Mandy Choi YM, Kalin Zhang YB
School of Chinese Medicine, The University of Hong Kong, Hong Kong

Dendrobium species (Shihu) is a famous Chinese herb widely used in China and Asia [1]. The three species, *Dendrobium candidum*, *D. nobile*, and *D. fimbriatum* are listed in the Chinese Pharmacopoeia (2010). According to the theory of traditional Chinese medicine, the traditional function of Dendrobium species is to nourish yin and to promote the production of body fluids.

Recently, our clinical study found that extract from *D. candidum* could improve xerostomia in 16 patients with Sjögren's syndrome (SS), which is characterized by the deficient secretion of saliva due to the autoimmune destruction of the salivary glands, leading to dry mouth symptoms (xerostomia) [2]. Saliva and salivary gland biopsies from the labial glands of patients were collected and examined using immunoreactivity and immunohistochemistry techniques. Result showed higher labeling indices [i.e., percentage of acinus area immunoreactive for Aquaporin-5 (AQP-5)] in the biopsies of SS patients. This experiment implies that the AQP-5 expression in the labial glands of SS patients can be increased by the extract of *D. candidum*, thereby promoting the production of body fluids and resulting in the secretion of saliva to improve dry mouth symptoms.

To confirm further that the mechanisms of Dendrobium species in nourish yin and promote the production of body fluids, the autoallergic mouse model *in vivo* and the human salivary gland cell line A-253 of SS *in vitro* were established in our laboratory [3]. The polysaccharides of *D. candidum* were administrated as treatment. Results validated that the polysaccharides of *D. candidum* could reverse the pathological changes in the animal and cell model of SS. The mechanism study also proved that the polysaccharides of *D. candidum* could up-regulate the expression of AQP-5 and protect cells from apoptosis and increased AQP-5 expression in the organs related to body fluids. This finding can be an important pilot study to investigate the activity mechanisms of Chinese medicines that nourish yin and promote the production of body fluids.

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Polysaccharides of *Dendrobium Officinale* Restore the Abnormal Expression and Distribution of Aquaporin-5 in Sjögren's Syndrome Model

Xiang Lin, Yanbo Zhang

School of Chinese Medicine, The University of Hong Kong, Hong Kong

Aquaporin-5 (AQP-5), a major water channel protein expressed in salivary gland, plays a prominent role in Sjögren's syndrome (SS). Our previous study have demonstrated that AQP-5 is significantly down-regulated in the salivary glands of SS patients and in an autoimmune animal model of SS. Polysaccharides from *Dendrobium Officinale* (DP), a traditional herbal medicine with *yin*-tonifying efficacy, was found to increase the saliva secretion, prevent the inflammation and AQP-5 expression in both SS patients and this autoimmune animal model. However, the underlying mechanisms have not been investigated. Our present study showed that in mice model, the proinflammatory cytokine tumor necrosis factor α (TNF- α) decreases the AQP-5 mRNA and protein expression *in vivo* via activation of nuclear factor κ B (NF- κ B). Meanwhile, AQP-5 translocation from apical membranes to intracellular and basolateral membranes is also found. DP reverses the abnormal expression of AQP-5 induced by TNF- α and translocation induced by M₃ muscarinic receptor inhibitor. Our results suggest that inactivation of NF- κ B induced by TNF- α and the subsequent restoration of the expression and distribution of AQP-5 partially accounts for the efficacy of DP on SS and may provide a modern scientific explanation for the *yin*-nourishing function of DP described in traditional Chinese medicine.

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Resveratrol Improves Hippocampal Atrophy in Mice with Chronic Fatigue

Junji Moriya^a, Jun-Ichi Yamakawa^a, Hisae Hayasaki^b, Sinobu Matsui^b, Yoshiharu Motoo^c

^a Department of General Medicine, Kanazawa Medical University, Japan

^b Lifestyle-related Disease Center, Kanazawa Medical University, Japan

^c Department of Medical Oncology, Kanazawa Medical University, Japan

Neuroimaging indicates that structural and/or functional abnormalities exist in the central nervous system, especially in the hippocampus of patients with chronic fatigue syndrome (CFS). However, the pathophysiological mechanisms of CFS are still unclear, which is partly because of the lack of a suitable animal model.

In the present study, we established a mouse model of chronic fatigue by six repeated injections of *Brucella abortus* antigen into Balb/c mice, which was manifested as a significant reduction in daily spontaneous running activity, and hippocampal atrophy.

Thereafter, resveratrol (RSV), a polyphenolic activator of sirtuin 1 (Sirt1) was used for the treatment of this model. The daily spontaneous running activity was increased by more than 20%, and the hippocampus was enlarged after 4 weeks RSV therapy. Furthermore, RSV inhibited neuronal apoptosis and the expression of hippocampal acetylated-p53 in the chronic fatigue mice model, which may have contributed to the upregulated deacetylation of p53 by Sirt1. In addition, RSV improved neurogenesis and expression of brain-derived neurotrophic factor mRNA in the hippocampus. In conclusion, six repeated injections of *B. abortus* antigen induced hippocampal hypoactivity and atrophy in Balb/c mice.

It is speculated that RSV is an effective agent for improving fatigue symptoms and enlarging the atrophic hippocampus, by repressing apoptosis and promoting neurogenesis, which might be one possible mechanism for recovery from fatigue.

Polysaccharides of *Dendrobium Officinale* Restore the Abnormal Expression and Distribution of Aquaporin-5 in Sjögren's Syndrome Model

Xiang Lin, Yanbo Zhang

School of Chinese Medicine, The University of Hong Kong, Hong Kong

Aquaporin-5 (AQP-5), a major water channel protein expressed in salivary gland, plays a prominent role in Sjögren's syndrome (SS). Our previous study have demonstrated that AQP-5 is significantly down-regulated in the salivary glands of SS patients and in an autoimmune animal model of SS. Polysaccharides from *Dendrobium Officinale* (DP), a traditional herbal medicine with *yin*-tonifying efficacy, was found to increase the salivary secretion, prevent the inflammation and AQP-5 expression in both SS patients and this autoimmune animal model. However, the underlying mechanisms have not been investigated. Our present study showed that in mice model, the proinflammatory cytokine tumor necrosis factor α (TNF- α) decreases the AQP-5 mRNA and protein expression *in vivo* via activation of nuclear factor κ B (NF- κ B). Meanwhile, AQP-5 translocation from apical membranes to intracellular and basolateral membranes is also found. DP reverses the abnormal expression of AQP-5 induced by TNF- α and translocation induced by M₃ muscarinic receptor inhibitor. Our results suggest that inactivation of NF- κ B induced by TNF- α and the subsequent restoration of the expression and distribution of AQP-5 partially accounts for the efficacy of DP on SS and may provide a modern scientific explanation for the *yin*-nourishing function of DP described in traditional Chinese medicine.

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Resveratrol Improves Hippocampal Atrophy in Mice with Chronic Fatigue

Junji Moriya^a, Jun-Ichi Yamakawa^a, Hisae Hayasaki^b, Sinobu Matsui^b, Yoshiharu Motoo^c

^a Department of General Medicine, Kanazawa Medical University, Japan

^b Lifestyle-related Disease Center, Kanazawa Medical University, Japan

^c Department of Medical Oncology, Kanazawa Medical University, Japan

Neuroimaging indicates that structural and/or functional abnormalities exist in the central nervous system, especially in the hippocampus of patients with chronic fatigue syndrome (CFS). However, the pathophysiological mechanisms of CFS are still unclear, which is partly because of the lack of a suitable animal model.

In the present study, we established a mouse model of chronic fatigue by six repeated injections of *Brucella abortus* antigen into Balb/c mice, which was manifested as a significant reduction in daily spontaneous running activity, and hippocampal atrophy.

Thereafter, resveratrol (RSV), a polyphenolic activator of sirtuin 1 (Sirt1) was used for the treatment of this model. The daily spontaneous running activity was increased by more than 20%, and the hippocampus was enlarged after 4 weeks RSV therapy. Furthermore, RSV inhibited neuronal apoptosis and the expression of hippocampal acetylated-p53 in the chronic fatigue mice model, which may have contributed to the upregulated deacetylation of p53 by Sirt1. In addition, RSV improved neurogenesis and expression of brain-derived neurotrophic factor mRNA in the hippocampus. In conclusion, six repeated injections of *B. abortus* antigen induced hippocampal hypoactivity and atrophy in Balb/c mice.

It is speculated that RSV is an effective agent for improving fatigue symptoms and enlarging the atrophic hippocampus, by repressing apoptosis and promoting neurogenesis, which might be one possible mechanism for recovery from fatigue.

Platycodin D Regulates the Expression of Adipogenic Factors Related to AMPK-AKT Pathway in Adipocytes and Inhibits Fat Accumulation in High Fat Diet-induced Obese Mice

Eun Jeong Lee, Min Seok Kang, Yeong Shik Kim

College of Pharmacy, Seoul National University, Korea

Platycodin D (PD), a major compound isolated from the root of *Platycodon grandiflorum* A. DC. (Campanulaceae), has been reported for its effects on controlling obesity through down-regulating lipid accumulation *in vivo*. However, the inhibition mechanism of PD on lipid accumulation and obesity has not been fully understood. The current study investigated the molecular mechanism of PD, focusing on its ability to decrease the expression of adipogenic factors related to activated protein kinase (AMPK) in mediated differentiation inducer-induced 3T3-L1 adipocytes and its ability to inhibit fat accumulation in high fat diet-induced obese C57BL/6 mice *in vivo*. PD significantly reduced fat accumulation by inhibiting adipogenic signal transcription factors such as PPAR γ and cEBP α *in vitro*. The anti-adipogenic effect of PD was attributed to be associated with AMPK-reduced AKT pathway. PD reduced both body weight and fat mass and further improved lipid metabolism through increasing AMPK and reducing peroxisome proliferator-activated receptor γ (PPAR- γ) in adipose tissue. Hence, our results suggest that PD might serve as a potential lead compound for the treatment of obesity at least in part of AMPK activity.

Anti-inflammatory and Anti-angiogenic Effects of Resveratrol and its Analogs in Human Umbilical Vein Endothelial Cells and Zebrafish

Yan-Hui Deng, Emilia Conceição Leong, Deepa Alex, Simon Ming-Yuen Lee

Institute of Chinese Medical Sciences, University of Macau, Macau

Stilbene compounds are present naturally in many plants and their extensive biological activities have been identified. Resveratrol is a common stilbene with a protective role against atherosclerosis. To investigate whether different stilbene compounds, such as resveratrol (RSV), trans-3,5,4'-trimethoxystilbene (TMS) and polydatin (PD), have different anti-inflammatory effects, we determined the effect of pharmacological doses upon the adhesion of monocytic THP-1 cells to human umbilical vein endothelial cells (HUVEC), as well as the expression of adhesion molecules and the translocation of NF- κ B in HUVEC. Since there is cross talk between the pathways underlying anti-vascular inflammation and anti-angiogenesis, we further evaluated the anti-angiogenic activity of RSV and TMS *in vitro*, using human umbilical vein endothelial cells (HUVEC), and *in vivo*, using transgenic zebrafish assay. In zebrafish, TMS caused inter-segmental vessel (ISV) regression and downregulated VEGFR-2 mRNA expression. Also, TMS induced G2/M cell cycle arrest more specifically in endothelial cells of zebrafish. The results showed that TMS shows significantly more potent anti-angiogenic and anti-vascular inflammatory activities than RSV and PD in those assays. Therefore, it could be potentially developed as an alternative in the prevention and treatment of chronic inflammatory diseases such as atherosclerosis and cancer.

Calycosin From Radix Astragali Promotes Angiogenesis involving Estrogen Receptor and Mitogen-activated Protein Kinase (MAPK) Signaling Pathway in Zebrafish and HUVEC

Jing Yan Tang, Shang Li, Zhen Hua Li, Zai Jun Zhang, Guang Hu, Lorita Chi Veng Cheang, Deepa Alex, Maggie Pui Man Hoi, Simon Ming-Yuen Lee[#]

Institute of Chinese Medical Sciences, University of Macau, Macau

Background: Angiogenesis plays an important role in a wide range of physiological processes, and many diseases are associated with the dysregulation of angiogenesis. *Radix Astragali* is a Chinese medicinal herb commonly used for treating menopausal irregularity, cardiovascular disorders and metabolic syndrome. *Radix Astragali* has been shown to possess angiogenic effect in our previous study but its underlying mechanism remains unclear. The present study investigates the angiogenic effects of calycosin, a major isoflavonoid isolated from *Radix Astragali*, *in vitro* and *in vivo*.

Methodology: *Tg (fli1:EGFP)* and *Tg(fli1:nEGFP)* transgenic zebrafish embryos were treated with different concentrations of calycosin from 72 hpf to 96 hpf prior morphological observation and angiogenesis phenotypes assessment. Zebrafish embryos were exposed to calycosin from 72 hpf to 78 hpf before gene-expression analysis. The effects of VEGFR tyrosine kinase inhibitor on calycosin-induced angiogenesis were studied using 72 hpf *Tg (fli1:EGFP)* and *Tg(fli1:nEGFP)* zebrafish embryos. The pro-angiogenic effects of calycosin were compared with other selective estrogen receptor modulators (SERMs), raloxifene and tamoxifen, in 72 hpf *Tg (fli1:EGFP)* zebrafish embryos. The binding affinities of calycosin to estrogen receptors (ERs) were evaluated by cell-free and cell-based estrogen receptor binding assays. Human umbilical vein endothelial cell cultures (HUVEC) were pretreated with different concentrations of calycosin for 48 h then tested for cell viability and tube formation. The role of MAPK signaling in calycosin-induced angiogenesis was evaluated using western blotting.

Conclusion: Calycosin was shown to induce angiogenesis in HUVEC *in vitro* and zebrafish embryos *in vivo* via the up-regulation of vascular endothelial growth factor (VEGF), VEGFR1 and VEGFR2 mRNA expression. It was demonstrated that calycosin acted similar to SERMs by displaying selective potency and affinity to estrogen receptors ER α and ER β . Our results further indicated that calycosin promotes angiogenesis via activation of MAPK with the involvement of ERK1/2 and ER. Together, this study revealed, for the first time, that calycosin acts as a selective estrogen receptor modulator (SERM) to promote angiogenesis, at least in part through VEGF-VEGFR2 and MAPK signaling pathways.

Indirubin Shows Anti-angiogenic Activity in an In Vivo Zebrafish Model and an In Vitro HUVEC Model

In Kei Lam¹, Deepa Alex¹, ZhiXiu Lin^{2#} and Simon Ming Yuen Lee^{1#}

¹*Institute of Chinese Medical Sciences, University of Macau, Macau*

²*School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong*

Indirubin is an active ingredient of the traditional Chinese medicine, Dang Gui Long Hui Wan, commonly used for the treatment of chronic myelocytic leukemia (CML) and other inflammatory conditions. These anti-leukemic and anti-inflammatory activities may be mediated by anti-angiogenic action and valuable for the treatment of diseases with excessive angiogenesis such as CML and psoriasis. To investigate the anti-angiogenic activity of indirubin, we tested its inhibitory effect on blood vessel formation in zebrafish embryos and on endothelial cell proliferation in culture. The anti-angiogenic activity of indirubin was tested using transgenic zebrafish embryos with fluorescent vasculature and human umbilical vein endothelial cells (HUVECs). Apoptosis was analyzed with a Terminal Deoxynucleotidyl Transferase Mediated dUTP Nick End Labeling (TUNEL) assay. Indirubin dose-dependently inhibited intersegmental vessel formation in zebrafish embryos. It also inhibited HUVEC proliferation by the induction of cellular apoptosis and cell-cycle arrest at the G₀/G₁ phase. In short, the anti-angiogenic activity of indirubin may partly contribute to its anti-leukemic and anti-psoriatic properties and may be valuable for the treatment of diseases with excessive angiogenesis.

In vitro Study of Indigo Compound Recipe on JAK2 Mutated HEL Cell Line

Min Chen¹, Pengjun Jiang², Xuemei Sun²

¹Nanjing University of TCM, Nanjing

²Jiangsu Provincial Hospital of TCM, Nanjing

Objective: To study the effects of Indigo Compound Recipe with components from natural indigo, Kudzuvine Root and Zedoray Rhizome on proliferation, apoptosis and differentiation of HEL cell line with JAK2 mutation.

Methods: Effective anti-tumor components in Indigo Compound Recipe were concentrated with puriarity of over 80%. HEL cells were co-cultured with different concentrations of each component and proliferating inhibition rates over 24h or 48h were observed by MTT method. The apoptosis rates were demonstrated as positivity of Annexin V/PI by Flow cytometry. Differentiation status was evaluated by surface expression of CD13, CD41, GPA by Flow cytometry as well.

Results: Indigo concentrated decoction, the extraction of Kudzuvine Root and the Zedoray Rhizome all showed inhibitory effect on the proliferation of HEL Cell in a concentration and time dependent manner. The concentrated decoctions used in these experiments were 30-100ug/ml for Indigo, 800- 1600ug/ml for Kudzuvine Root and 60-80ug/ml for Zedoray Rhizome respectively. The inhibition effect on HEL Cells was enhanced with longer exposure time. After thirty minute of exposure of HEL cells to components within the above range, HEL cells were still alive, but cell proliferation was inhibited through apoptosis induction. However, high concentration of Zedoray Rhizome (>80ug/ml) caused cells death promptly. Further more, by comparison to the control group, apoptotic HEL cells were induced by these components in a time-dependent but not dose-dependent way. Myeloid differentiation demonstrated as expression rate of CD13 in 24h and megakaryocytic or erythroid differentiation showed as expression rates of CD41 or GPA in 48h were identified with the extraction of indigo and Zedoray Rhizome.

Conclusion: Proliferation inhibiting as well as apoptosis inducing of HEL cells with JAK2 mutation was demonstrated with all the three individual extract from Indigo Compound Recipe. And differentiation of HEL cells was induced by low concentration of extractions of indigo and Zedoray Rhizome.

Keywords: Indigo Compound Recipe, HEL Cell Line, JAK2 mutation.

Absorption and Metabolism of Flavonoids in Extract of Fructus Aurantii Immaturus

Yan Chen, Xiaobin Jia

Key Laboratory of New Drug Delivery System of Chinese Materia Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing

Si-Ni-San is a classic prescription in Treatise on Febrile Diseases of Zhang Zhongjing and is composed of four herbs, Bupleurum chinense, Paeoniae, Fructus Aurantii Immaturus and Glycyrrhiza uralensis Fructus. Aurantii Immaturus has effect on regulating vital energy and its main active compounds are flavonoids, such as naringin, hesperidin and neohesperidin. Recently the absorption and metabolism of these three flavonoids have been reported, however the absorption and metabolism of the water extract has been unknown.

In this paper, the absorption and metabolism of naringin, hesperidin and neohesperidin in Fructus Aurantii Immaturus water extract was investigated by rat intestinal perfusion model and the results were compared to the monomer components. In the perfusion model, absorption of naringin, hesperidin and neohesperidin in Fructus Aurantii Immaturus extract were higher than monomer components. In extract, Naringin ($20 \mu\text{mol}\cdot\text{L}^{-1}$) absorption was significantly ($p < 0.05$) increased in duodenum (41.1%), jejunum (39.9%) and ileum (32.57%); and also the absorption of Hesperidin ($20 \mu\text{mol}\cdot\text{L}^{-1}$) and Neohesperidin ($20 \mu\text{mol}\cdot\text{L}^{-1}$) were significantly ($p < 0.05$) increased in duodenum (45.83%, 53.83%), jejunum (47.17%, 47.61%) and ileum (30.85%, 32.05%).

To investigate the mechanism of increasing absorption, compatibility of these three flavonoids was observed. However, The results showed that it had no significant effect on intestinal absorption of each component ($p > 0.05$). Taken together, these results suggest that other components in extract of Fructus Aurantii Immaturus, such as alkaloids, might be involved in the absorption of flavonoids and the mechanism needs further study.

Chinese medicine has holistic and complex nature, so the monomer compounds can not be representative of the characteristics of overall medicine when study the absorption and metabolism of Chinese medicine.

Acknowledgments: This work is supported by state administration of traditional Chinese medicine of the people's republic of china

Glucuronidation of Wogonin and Other Four Analogical Flavones in Rat Liver Microsomes

Bin Chen, Xiao-bin Jia, Yan Chen

Key Laboratory of New Drug Delivery System of Chinese Meteria Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing

We characterized the specific glucuronidation of wogonin and other four analogical flavones primuletin, 7-hydroxyflavone, chrysin, and tectochrysin using rat liver microsomes. The results indicated at seven different concentrations there were significant differences in the glucuronidation of wogonin and other four analogical flavones by rat liver microsomes ($p < 0.05$, one way ANOVA). The rank order of the glucuronidation rates (nmol per min per mg of protein) was 7- hydroxyflavone > chrysin > wogonin > tectochrysin > primuletin. We found that these flavones had similar concentration-dependent patterns except tectochrysin using rat liver microsomes. Determination of kinetic parameters of glucuronidation of primuletin, 7- hydroxyflavone, chrysin, tectochrysin and wogonin show the result of differences in K_m and V_{max} values. In conclusion, the first systematic study of metabolism of wogonin and other four analogical flavones using rat liver microsomes shows that these flavones has similar metabolic patterns that are concentration-dependent and different rates of glucuronidation. The different substituent group or position of substituent group can impact their behavior of metabolism largely as well as the different metabolic models.

Kinetic Parameters	primuletin	7- hydroxyflavone	chrysin	tectochrysin	wogonin
$K_m(\mu M)$	2.815	9.625	3.333	—	1.326
$V_{max}(nmol/min/mg)$	2.849	351.500	102.800	—	25.470
$CL_{int}(ml/min/mg)$	1.012	36.519	30.843	—	1.9208
R^2	0.898	0.911	0.831	—	0.912
AIC	86.462	160.44	145.94	—	115.22

Table 1 Apparent kinetic parameters of metabolism of five flavones by rat liver microsomes, calculated based on curve fitting using Michaelis-Menten (MM) enzyme kinetics models as described in "Materials and Methods" section.

Eadie-Hofstee plot of tectochrysin by rat liver microsomes dose not conform to any known model. So, apparent kinetic parameters were not calculated.

Figure 1

Concentration-dependent glucuronidation of five flavones by rat liver microsomes

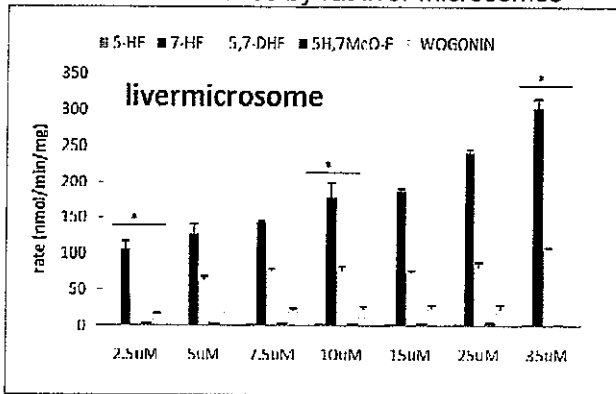
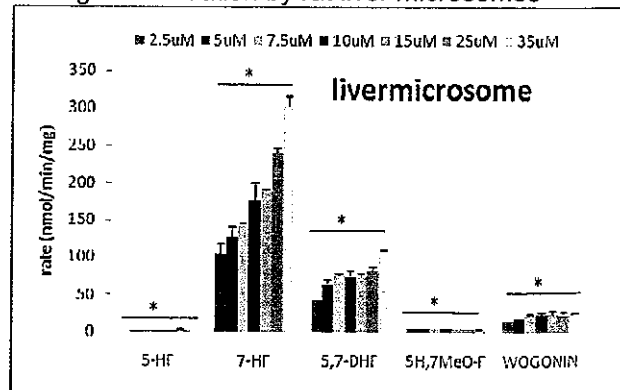


Figure 2

Impact of structural changes on flavone glucuronidation by rat liver microsomes



Keywords: Rat liver microsome, Glucuronidation, Wogonin, Flavones

Study on Processing Mechanism of Epimedium Based on Biological Transformation Combined with Intestinal Absorption Barrier

E Sun, Yan Chen, Xiao-Bin Jia, Xiao-Bin Tan, Fen-Xia Zhu

Key Laboratory of New Drug Delivery System of Chinese Materia Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing

In this study, the aim was to explain the Epimedium processing mechanism based on biological transformation combined with intestinal absorption barrier of active components. The change regularity of Epimedium flavonoids after processing was investigated with HPLC and UPLC. The biological transformation process of Epimedium flavonoids was studied under the effect of intestinal bacteria, intestinal enzyme and metabolism *in vivo*. Meanwhile, their transform products were identified using LC-MS with NMR. The absorption and transportation mechanism was approached by intestinal absorption models, such as *in situ* perfused rat intestinal model, Caco-2 cell model and so on. The results showed that the absorption and metabolism of Epimedium flavonoid glycosides existed difference. The secondary glycoside was absorbed more than poly-glycoside (baohuoside > icariin > EpimedinA, B, C). During the processing, heating can change the contents of Epimedium active flavanoids, and produced more absorbable active flavanoids such as icariin and baohuoside. It is concluded that the processing makes Epimedium produce more absorbable active flavanoids, which enhances the therapeutic effect of Epimedium. Study on the Epimedium processing mechanism from the view of biological transformation combined with intestinal absorption barrier provides new thought and new method for elucidating the processing mechanism of traditional Chinese medicine.

Acknowledgement: This research was supported by National Natural Science Foundation of China (Grant No.30572372, 30973944).

Preparation of Icaritin Liposomes and its Studies on Rat Intestinal Absorption *In Situ*

Zhang Zhenhai, Jia Dongsheng, Sun E, Jia Xiaobin*

Key Laboratory of New Drug Delivery System of Chinese Materia Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing

Icaritin (IT) could inhibit inducing osteoclast formation and bone resorption *in vitro* in dose-dependent manner. But IT has poor solubility in both oil and water. Investigation suggested that IT was hard to absorb in gastrointestinal tract. IT liposomes was prepared by modified ethanol injection method. L16 (4⁵) orthogonal design was used to optimize the formula with encapsulation efficiency as index. Based on the optimized condition, physico-chemical properties of IT liposomes was studied, including morphology, mean diameter and Zeta electric potential with a laser particle size analyzer. After the free drug was removed from the liposomes solution by Sephadex G-50 column, mini-column centrifugation method and super filtration, respectively. The entrapment efficiency (EE, %) of the liposomes was determined by HPLC and release degree was detected by reverse dialysis. The average intensity diameter, entrapment efficiency by the mini-column centrifugation method and drug loading of the optimized liposomes were (71.6 ± 6.9) nm (*n*=3), 91.49% and (9.15 ± 0.22) %, respectively. 72 h cumulative release of IT liposomes in 20% methanol PBS solution was over 90%. Intestinal absorption kinetics of IT and its liposomes were studied with perfused rat intestinal model. The results suggested that IT liposomes significantly increase coefficient of permeability and drug elimination percentage in different intestines (*p* ≤ 0.01). So liposomes as a drug carrier can enhance the oral absorption of IT in rat.

Acknowledgement: The authors would like to acknowledge the financial support of the National Natural Science Foundation of China (30572372, 30973944).

Effects of Danggui Buxue Tang on HIF-1 α and Angiogenesis in Fibrotic Rats

Jing Lu¹, Chenghai Liu^{1,2}

¹Institute of Liver Diseases, ShuGuang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai

²Shanghai TCM internal medicine E-institute, Key Lab of Liver & Kidney Diseases (Shanghai University of Traditional Chinese Medicine) Minster of Education, Shanghai

Backgrounds and Aim: Angiogenesis, which is a hypoxia-stimulated and growth factor-dependent process, plays a key role in development of cirrhosis. In our previous study, we confirmed that Danggui Buxue Tang (DBT) could attenuate liver fibrosis. Here we aimed to explore the mechanism of DBT action on liver fibrosis relating to liver tissue angiogenesis.

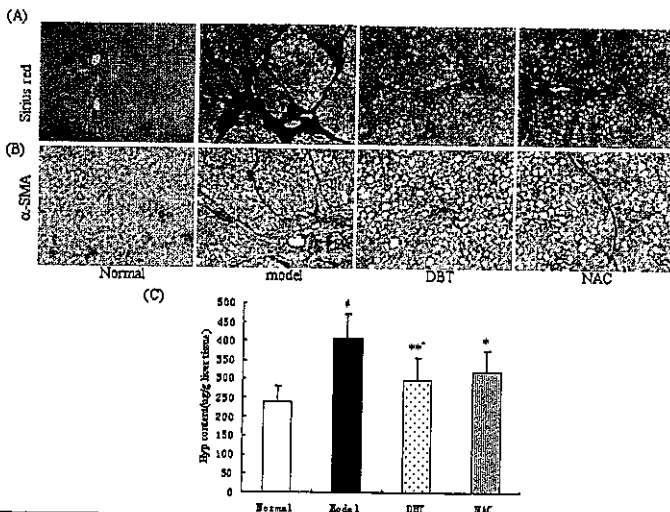
Methods : The Wistar rats were divided into 4 groups: Normal, model, DBT and NAC treatment group. Liver cirrhosis was induced by CCl₄ Multiple factors for 6 weeks. DBT treatment group were treated with DBT at a dose of 6g/kg body weight since beginning of CCl₄ intoxication until the end of experiment.

The hepatic collagen deposition was examined with Sirius red stain. Von Willebrand factor (vWF) protein expression was analyzed by immunofluorescence. α -SMA, HIF-1 α expression was analyzed by immunohistochemistry and Western blot. HIF-1 α mRNA was analyzed with real-time PCR. VEGF, TGF- β 1 and its receptors, ERK and p-ERK expression was analyzed by Western blot.

Results: DBT significantly attenuated liver cirrhosis, manifested by decreasing hepatic collagen deposition and α -SMA protein expression. vWF and VEGF protein expression was performed to analyze microvessel growth. In normal livers, vWF protein is expressed in great vessels of the portal tract and central veins, but not along sinusoids, VEGF protein is rarely or almost no expression; in model livers, vWF and VEGF protein expression was increased significantly; DBT decreased vWF and VEGF protein expression. Hypoxia is an important regulator of angiogenesis.

HIF-1 α in fibrosis liver was highly expressed, especially in liver acinus III zone; DBT significantly reduced HIF-1 α gene and protein expression. DBT decreased TGF- β Signal transduction pathway-- TGF β 1, its receptors and p-ERK protein expression.

Conclusion: DBT inhibited liver tissue angiogenesis and decreased HIF-1 α , TGF- β Signal transduction pathway protein expression; those are one of the mechanisms of DBT anti-fibrosis.



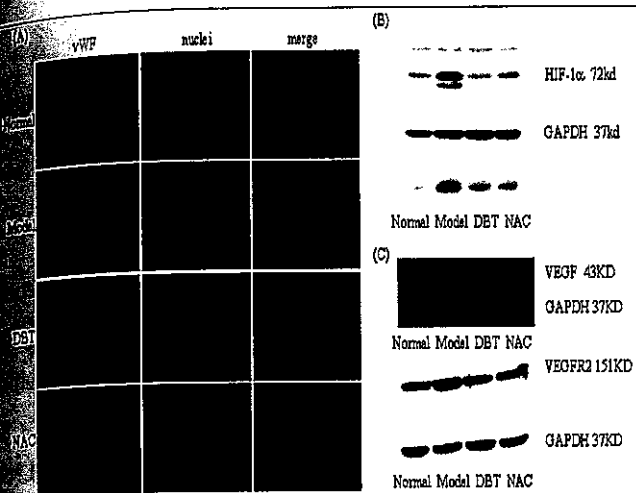


Figure 1: Effect of DBT on liver collagen deposition and α -SMA protein expression and Hyp content in CCl_4 induced liver fibrotic rats.

Figure2: Effect of DBT on vWF, HIF-1 α and VEGF/VEGFR2 protein expression in CCl_4 -induced liver fibrotic rats

Effects of Sorafenib on Gelatinase Activities and Hepatic Sinusoidal Endothelial Cell in Cirrhosis Mice

Jing Lu¹, Chenghai Liu^{1,2}

¹Institute of Liver Diseases, ShuGuang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai

²Shanghai TCM internal medicine E-institute, Key Lab of Liver & Kidney Diseases (Shanghai University of Traditional Chinese Medicine) Minister of Education, Shanghai

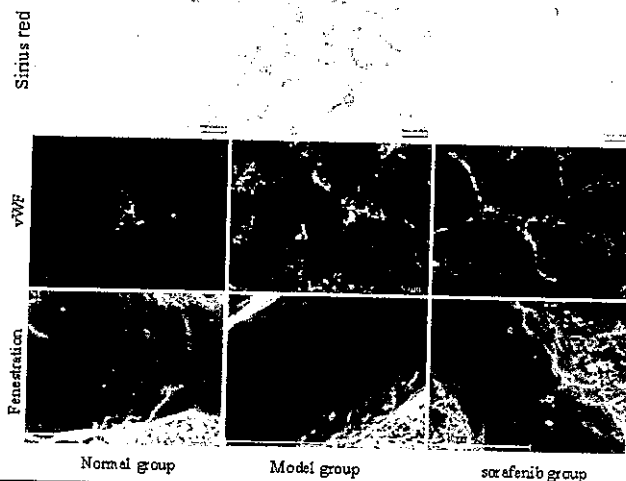
Background and Aim: Angiogenesis plays a key role in development of cirrhosis, and represents a potential therapeutic target. Receptor tyrosine kinase inhibitors have also begun to receive greater attention as a potential therapy in the treatment of cirrhosis, while the mechanistic basis for benefit is not fully elucidated; we aimed to evaluate the molecular effects of sorafenib, a multiple tyrosine kinase inhibitor, on gelatinase activities and sinusoidal endothelial cell in CCl₄ induced cirrhosis mice.

Methods: The C57BL/6 mice were divided into 3 groups: Normal, model and sorafenib treatment group. Liver cirrhosis was induced by CCl₄ subcutaneous injection for 8 weeks. Sorafenib treatment group were treated with sorafenib at a dose of 4mg/kg mice weight three days before the CCl₄ injection until the end of experiment.

The hepatic collagen deposition was examined by Sirius red stain. Hepatic hydroxyproline (Hyp) content was detected with Jamall's method. Hepatic sinusoidal endothelial cell phenotype conversion--Von Willebrand factor (vWF) protein expression was analyzed by immunofluorescence and Western blot. Endothelial fenestration was examined with scanning electronic microscope (SEM) and basement membrane was observed by transmission electron microscope (TEM). MMP-2/9 activities were measured with gelatin zymography and in situ zymography. Hypoxia-inducible factor1 α (HIF1 α), vascular endothelial growth factor (vegf), flt1 (vegf receptor 1), kdr(vegf receptor 2) mRNA and vegf protein expression were analyzed by Real time PCR and western blot respectively.

Results: Sorafenib significantly attenuated liver cirrhosis induced by CCl₄ in mice, manifested by decreasing hepatic collagen deposition, Hyp content. Sorafenib reduced hepatic sinusoidal endothelial injury, including decreasing vWF protein expression, improving hepatic sinusoidal endothelial fenestration. Sorafenib inhibited continuous basement membrane formation and MMP-2/9 activities. Sorafenib also decreased HIF1 α , vegfa, flt1, kdr mRNA and vegf protein expression.

Discussion / Conclusion: Sorafenib inhibited intrahepatic angiogenesis in cirrhosis mice through regulating MMP-2/9 activities and attenuating hepatic sinusoidal endothelial cell injury.



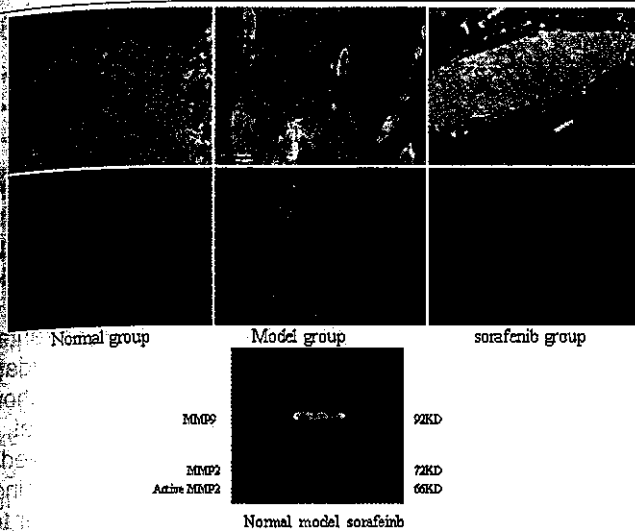


Figure 1. Effects of Sorafenib on collagen deposition (Sirius red staining), vWF expression, Hepatic sinusoidal endothelial fenestration (SEM).

Figure2. Effect of Sorafenib on continuous basement membrane formation (TEM) and MMP 2/9 activities (gelatin zymography)

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Effect of Xiaozhang Cataplastm on Ascites and Dose-Effect Relationship in Cirrhotic Mice with Ascites

Zhen Zeng, Chenghai Liu

Institute of Liver Diseases, ShuGuang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai

Background and Aim: Xiaozhang Cataplastm had adjuvant effect to reduce ascites as umbilicus plaster in cirrhotic patients on clinical. To investigate the change of ascites and dose-effect relationship by Xiaozhang Cataplastm plastered on the navel of mice with dimethylnitrosamine (DMN)-induced cirrhosis and obvious ascites.

Methods: Sixty ICR mice were randomly divided into 6 groups. Groups 1 (n=10/group) served as normal control groups, in which the mice were given no DMN. Groups 2 to 6 (n=10/group), the animals were given intraperitoneal injection of DMN with a dosage of 10 μ g /kg of body weight per day for 3 consecutive days per week for 4 weeks. At the end of week 4, Group 2 were served as model control given no Cataplastm. Group 3 were served as model comfort group receiving placebo, Xiaozhang Cataplastm were given to group 4-6(3 level dose,high/normal/low) by umbilical sticking by dressing change every day for one week. At the end of week 5, body weight, abdominal circumference, volume of ascites, the liver function including serum ALT activity, Alb and total bilirubin (TBil) level, Compared the differences with these three doses of Xiaozhang Cataplastm in ascites subsidise .

Results: During the process of DMN intoxication, two mice died in model groups, and two mice died in groups 6(low). Compared with normal control group mice, volume of ascites, body weight, liver weight, Alb content decreased significantly ($p < 0.05$). However, spleen weight, abdominal circumference, ALT level and TBil level obviously rosed ($p < 0.05$). Compared with group 3 (model placebo group), group 4 had reduced volume of ascites, abdominal circumference obviously, ($p < 0.05$) and increased Alb content level slightly ($p < 0.05$).

Conclusion : Xiaozhang cataplastm showed good effects in promoting subsidise of mice liver Cirrhosis with ascites induced by dimethylnitrosamine, The most influence obviously at high doses with this Cataplastm.

Glucuronidation, a New Metabolic Pathway of Pyrrolizidine

Yu-Qi He, Chang-Hong Wang, Zheng-Tao Wang

Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai

Pyrrolizidine alkaloids (PAs) cause significant hepatotoxicity to human and animals via metabolic activation by liver P450 enzymes. Metabolic processes of PAs were studied for several decades including metabolic activation, hydroxylation, N-oxidation and hydrolysis. However, the phase II metabolisms, in particular glucuronidation of intact PAs, have not been investigated, although glucuronidation plays an important role in the elimination and detoxification of xenobiotics. In this study, three important problems about PAs glucuronidation have been investigated. First, via the glucuronidation study of SEN – a representative hepatotoxic pyrrolizidine alkaloid (PA), glucuronidation type on PAs was identified as N-glucuronidation. Second, glucuronidation of SEN was not only catalyzed by human but also other animal species and showed significant species difference. Rabbit, cattle, sheep, pig and human had the higher activity than mouse, rat, dog and guinea pig on SEN glucuronidation. Kinetics of SEN glucuronidation in human, pig and rabbit followed the Michaelis-Menten equation of one site binding model while in cattle and sheep followed that of two sites binding model. Third, glucuronidation was demonstrated to be a metabolic process of not only SEN but also other toxic PAs including monocrotaline, adonifoline and isoline.

Andrographolide Inhibits the Expression and Function of CYP3A4 in $1\alpha, 25$ Dihydroxyvitamin D₃ Treated Caco-2 Cells

Feng Qiu, Xiao-Long Hou, Kyoko Takahashi, Lixia Chen, Ning Kang, Junichi Azuma, Xinsheng Yao

Department of Natural Products Chemistry and Department of Biochemistry and Molecular Biology, Shenyang Pharmaceutical University, Shenyang

Andrographolide is one of principal constituents of a famous traditional herbal medicine *Andrographis paniculata* (Burm) Nees which has been commonly used in China, India and some southeast Asian countries for a long time. The preparations containing andrographolide have been widely applied in clinic in China for the treatment of infectious diseases. In this study, the effects of andrographolide on CYP3A4 isoform in $1\alpha, 25$ dihydroxyvitamin D₃ treated Caco-2 cells were investigated. Andrographolide, which were found to be non-cytotoxic towards the caco-2 cells at concentrations ranging from 1 to 100 μ M, exhibited an moderate inhibition on nifedipine and testosterone-mediated CYP3A4 catalytic activities, whose IC₅₀ is 44 and 34 μ M, respectively. Further investigation showed that andrographolide inhibited expression of CYP3A4 protein and mRNA with a dose-dependent manner. These results indicated that andrographolide could inhibit the function and expression of major human drug metabolizing enzyme CYP3A4 and suggested that the preparations containing andrographolide should be examined for potential pharmacokinetic drug interactions in vivo.

Keywords: *Andrographis paniculata*, andrographolide, CYP3A4, Caco-2, $1\alpha, 25$ dihydroxyvitamin D₃

The Effects of Buyanghuanwutang on Pulmonary Fibrosis Induced by the Bleomycin in Rats

Wang Fei, Wu Wenbin

Clinical Medicine College, Chengdu university of Traditional Chinese Medicine, Chengdu

Objectives: To evaluate the therapeutic effect and mechanism of Buyanghuanwutang which is created by WANG-Qing-ren in treating pulmonary interstitial fibrosis (PIF) induced by bleomycin in rats. **Methods:** Pulmonary fibrosis were made with 78 SD rats by intra-tracheal injection of bleomycin A5 and allocated into 4 groups randomly, untreated model group (n=18), metretion group (n=18), Byhut group I (n=18) and Byhut group II (n=18). Both groups were put to death on the 7th, 14th, 28th day. Other six rats were control group and put to death on the 28th day. Bronchoalveolar lavage were collects and the total and differential cells were counted, ELISA was applied for TNF- α quantitation and transforming growth factor β 1 (TGF- β 1) mRNA expression and protein level of the lung tissue were measured. **Results:** The total cells count, TNF- α release, TGF- β 1 mRNA expression and protein content from AM in model group were significantly increased compared with control group ($p < 0.05 \sim 0.01$) while these in Byhut group I and Byhut group II were significantly decreased compared with model group (both $p < 0.05$). **Conclusion:** Buyanghuanwutang has definite effect in treating PIF. The mechanism may be through inhibiting the release of TNF- α , the TGF- β 1 mRNA expression and protein, decreasing collagen protein, so as to ameliorate the inflammation and fibrosis.

Keywords: Buyanghuanwutang, pulmonary fibrosis, bronchoalveolar lavage fluid, tumor necrosis factor

The Effect of Xin-San-Guo-Tang Compound Tibetan on Polycythemia induced by Chronic Hypoxia in Rat

Wu Wenbin, Meng Xianli

College of Pharmacy, Chengdu university of Traditional Chinese Medicine, Sichuan

Abstract: Objective To observe the effects of Xin-San-Guo-Tang on rats with High altitude polycythemia. **Methods:** 50 male SD rats were randomized 5 groups (1) normoxia group (2) plateau model group (3) Rhodiola group (4) high dose Xin-San-Guo-Tang group (5) low dose Xin-San-Guo-Tang group. In addition to normoxia group, the rats were exposed to intermittent hypoxia which simulating altitude of 5 000 m for 22 h/d for 40 days to build polycythemia model. Observed at 41 d of Xin-San-Guo-Tang soup with chronic hypoxia-induced polycythemia in rats blood count, blood gas analysis and serum erythropoietin effect. **Results:** The erythrocyte count, hemoglobin and hematocrit were advanced in (2), while PH value, PaO₂, SaO₂, PaCO₂ were decrease, serom EPO were increased. The erythrocyte count, hemoglobin and hematocrit in high and low dose of Xin-San-Guo-Tang were significantly lower than plateau group, PaO₂, SaO₂, PaCO₂ were advanced than plateau group. And the high dose group was more effective than the low dose group, while still higher than normoxia group. **Conclusion:** The Tibetan medicine Xin-San-Guo-Tang could prevent and treat high altitude polycythemia.

Keywords: San-Guo-Tang; hypoxia; high altitude polycythemia

The Absorption of Mercury Of "Zuo Ta" in the Small Intestinal

Yong Zeng, Ping Wang, Xianli Meng, Yi Zhang

Department of Pharmacology, Chengdu University of Traditional Chinese Medicine, Sichuan

A high performance liquid chromatographic method was established for the determination of Hg in "ZUO TA" using diethyldithiocarbamate (DEDTC) chelate. A Diamonsil™ C₁₈ (250×4.6mm, 5μm) column was used with a mixture of methanol- water (containing 22μmol/L DEDTC) (90:10, V/V) as the mobile phase, with the speed at 1.0 ml/min, the temperature in 30°C and a detector set at 272nm. The calibration curve was linear ($r=0.9997$) in the range of 21.0ug/ml~210.0ug/m of Hg²⁺. The recoveries were 102.59%, with RSD of 1.66%. The HPLC method is proved to be precise, reproducibility and reliable. It is suitable for determination of Hg.

To study the Hg absorption rate at different segment of rats and to determine if specific absorption site existed, everted gut sac was used to compare the absorption of Hg in "ZUO TA" and mercury chloride in jejunum, ileum, duodenum and colon. The concentration of Hg in the intestinal medium was determined by HPLC method. The highest absorption extent of Hg in mercury chloride was observed in jejunum, ileum, duodenum and colon. While, no significant absorption of Hg in "ZUO TA" was observed in each segment of the rats' intestine, except determining a very low concentration of Hg after 120 min. Using the HPLC method, there in no Hg chromatography peak of the portal vein samples collecting from the manhood rat after administrating "ZUO TA" at high dose over 90s of man at several times.

The study shows: the Hg in "ZUO TA" will not detected in the intestines absorption at a once administration of "ZUO TA"

Prenylflavones from *Epimedium* for Breast and Prostate Cance

E.L. Yong*, Tiong Chi Tze, Zhang Shi Jun

Department of Obstetrics & Gynecology, National University Hospital, National University of Singapore, Singapore

Prenylflavones are a new class of flavonoids originally isolated from beer. Glycosylated prenylflavones are abundant in the Traditional Chinese Medicinal plant *Epimedium* (Berberidaceae). Oral ingestion of these glycosylated prenylflavones gives rise to the aglycone metabolites icaritin (ICT) and desmethylicaritin (DICT) in sera.

Our data indicate that that ICT and DICT can dose-dependently inhibit the proliferation of estrogen-responsive MCF-7 breast and prostate cancer cells. Mechanistic studies indicate effects were due to the dose-dependent suppression of estrogen and androgen receptor proteins, due to increased proteasome degradation of the steroid receptors.

In order to bring prenylflavones to the clinic, we have performed extensive studies on an extract for *Epimedium* that is enriched for prenylflavones. Phylogenetic, taxonomic, and chemical profiling of 37 specimens from 19 *Epimedium* species, including *E. koreanum*, have been performed. Using well defined cultivars, a standardized extract of *Epimedium* has been produced. Sensitive and rapid methods to measure serum levels of ICT and DICT have been developed. Pharmacokinetic and pharmacodynamic studies in animal models show that ICT and DICT levels in serum are high and can exert biological effects in sera when prenylflavones are administered.

An open-label, 2-period, randomized, crossover study was conducted whereby healthy human subjects were randomized to receive either a single dose of the estrogenic prodrug estradiol valerate, or a water decoction of *Epimedium* prepared according to traditional methods. None of the subjects exhibited any adverse events related to ingestion of *Epimedium* extract. Ex-vivo evaluation indicate that administration of *Epimedium* decoction led to a 6% increase in ER α adjusted GMR of AUC over baseline ($P<0.05$). There was a trend for reduction in MCF-7 breast cancer cell growth. These anti-proliferative effects of *Epimedium* prenylflavones on breast and prostate cancer cells suggest their possible use as anti-cancer medications.

Effects of Resveratrol on Pressure Overload-induced Cardiac Hypertrophy and Apoptosis in a Rat Abdominal Aortic Constriction Model

Mei-Lin Shen^a, Kung-Wei Lee^{bcd}, Ching-Huei Lin^a, Chih-Yang Huang^{ae†}

^aSchool of Chinese Medicine, China Medical University, Taichung

^bGraduate Institute of Clinical Medical Science, China Medical University, Taichung

^cFine Heart Clinic, Changhua

^dDivision of Cardiology, Department of Medicine, China Medical University Hospital, Taichung

^eGraduate Institute of Basic Medical Sciences, China Medical University, Taichung

[†]Department of Health and Nutrition Biotechnology, Asia University, Taichung

Epidemiological studies had showed that consumption of resveratrol rich diets is related to decrease in risk of cardiovascular diseases in certain sub-groups in the French population. However, the aim of this study investigated the effect of RES on pressure overload-induced cardiac hypertrophy and apoptosis. Cardiac hypertrophy was established in a rat abdominal aortic constriction (AC) model. After AC was conducted, rats were randomly divided into three groups. Resveratrol was dissolved into ethanol and then treated with 10, 20 and 30 mg/kg/day doses by intraperitoneal injection for 30 days. The control group was treated with equal volumes of ethanol. The results implied that RES administrations decreased cardiac hypertrophy and inhibited fibrosis (HE and Trichome staining). Echocardiograms results indicated that it significantly improved LV systolic and diastolic function. This study indicates that RES is a potent cardioprotective agent in AC rats.

Sesamin Reduces Cardiac Hypertrophy in a Rat Coronary Artery Ligation Model

Ching-Huei Lin^a, Mei-Lin Shen^a, Chien-Wei Rolis Hou^b, Chih-Yang Huang^{acd†}

^aSchool of Chinese Medicine, China Medical University, Taichung

^bDepartment of biotechnology, yuanpei university, HsinChu

^cGraduate Institute of Basic Medical Sciences, China Medical University, Taichung

^dDepartment of Health and Nutrition Biotechnology, Asia University, Taichung

Cardioprotective effects of sesamin, a novel antioxidant and free radical scavenger, were studied in a rat model of coronary artery ligation. After the procedure, the surviving rats were randomly allocated to three groups and treated with sesamin (0.1 and 1 mg/kg/day) or vehicles (intraperitoneal injection) for four weeks. Our samples were examined by histological analysis, TTC staining, western blot, and immunofluorescence assay. The SE-1 group had significantly reduced sizes of infarction, cell size and fibrosis. Otherwise, the SE-1 group also had significantly decreased expression levels of BNP and TGF- β 1 proteins. In this study, our results indicated that SE is a potential agent of cardioprotection in coronary artery ligation rats. Its cardioprotective effects may be due to a reduction of BNP and TGF- β 1.

Evaluation of Vasorelaxation and Antioxidant Activity of *Rhodiola Rosea*

Li-Min Liu¹, Tzong-Der Way², Sheng-Chu Kuo¹, Daih-Huang Kuo³

¹Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung

²School of Biological Science and Technology, China Medical University, Taichung

³Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung

Rhodiola rosea L. is a herb for anti-fatigue, anti-aging, heart protection, anti-hypoxia, and other related activity. The study was investigated the vasorelaxation effect and potential mechanism of *Rhodiola rosea* and their fraction extracts in isolated porcine coronary artery model, and evaluated antioxidant activity and related free radical-scavenging effects. The methanolic extract (ME) and its five fractions of n-hexane (HxF), dichloromethane (DcF), ethyl acetate (EaF), n-butanol (BtF) and water (WtF) were prepared for vasorelaxation evaluation. Contents of total phenols (gallic acid) and total flavonoids (Rutin), DPPH free radical-scavenging assay and Xanthine oxidase inhibitory activity were evaluated among these fractions.

The results revealed that ME possessed significant relaxation effect in porcine coronary artery at the concentration of 1mg/ml. HxF and WtF showed strong vasorelaxation activity. So we performed mechanism studies by HxF. The result indicated that HxF relax coronary artery by endothelium-dependent pathway, promotion of NO synthesis, activation of cGMP-independent pathway and inhibition of calcium influx.

The contents of total phenols and total flavonoids were DcF and EaF. In DPPH free radical-scavenging assay, IC₅₀ value of each fraction was 101.3µg/mL, 35.7µg/mL, 28.1µg/mL, 45.4µg/mL and 39.3µg/mL respectively. DcF and EaF were more obvious than other in Xanthine oxidase inhibitory activity. The relationship between antioxidant activity and contents of gallic acid and rutin was evaluated. Correlation coefficients (R²) of DPPH free radical-scavenging assay and total phenols and total flavonoids each were 0.7945 ($p < 0.05$) and 0.1324 ($p < 0.01$). Similarly, R² values for Xanthine oxidase inhibitory activity and contents of total phenols and total flavonoids were 0.5941 ($p < 0.01$) and 0.0288 ($p < 0.001$). So we could suggest these fractions positive relationship was exhibited between antioxidant activity (DPPH free radical-scavenging and Xanthine oxidase inhibitory activity) and antioxidant material contents (gallic acid).

Laser-induced Carotid Artery Injury Model in Rat to Study the Effects of Ferulic Acid

Chin-Hsien Chang, Yen-Lin Chang and Hen-Hong Chang*

Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taipei

Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Taipei

Aim: The purpose of this study was to establish a novel, laser-based microplatform for inducing carotid artery thrombosis in rats. Our method uses a rat model of laser-induced cerebral embolism to evaluate stroke treatment. Based on different cerebral embolism models, we altered dye dosage and laser light source to create different embolic conditions. Our study provides a new research platform for the development of novel therapeutic modalities and new drug development.

Method: WKY rats were anaesthetized, catheter inserted into the femoral vein. Next, the neck skin was incised and the junction of the internal carotid artery and the external carotid artery identified. DPSS Green Laser was used to irradiate the middle of carotid arteries for 10 min and rose Bengal dye (60 mg/kg) injected in the femoral vein to induce thrombosis. After surgery, rats were fed with ferulic acid daily. On Day 0 and After Day 1, week 1, week 3 and week 4, the rats were sacrificed and carotid arteries were removed and carotid artery sections with H&E staining and vessel thickness was analyzed using Image software.

Results: Our data showed that control group rats exposed to 600 seconds of 532 nm DPSS green laser light together with 60 mg/kg rose bengal demonstrated significant hyperplasia in the carotid artery after 28 days. Hyperplasia of carotid arteries was significantly decreased in the rats IP ferulic acid (80mg/Kg) compared with the control group.

Keywords: stroke, carotid artery, thrombosis, laser, ferulic acid

Anti-Inflammation Effects of Andrographolide Attenuates Hepatic Angiogenesis and Fibrogenesis in Thioacetamide-Induced Liver Injury Mice

Ya-Shu Chang, Hen-Hong Chang, Tzung-Yan Lee

Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Taipei

Background and Aim: Liver fibrosis is a wound healing process in most chronic liver diseases occurrence and simultaneous associated with inflammation and angiogenesis. Andrographolide (ANDRO), the major active component from *Andrographis paniculata*, has shown to possess antioxidant and anti-inflammatory effects. The aim of this study was to investigate mechanisms of ANDRO against thioacetamide (TAA)-induced mice with hepatic fibrosis.

Methods: All experimental groups except the normal control group received TAA (100 mg/kg) twice a week for either 1 week or 4 weeks, and co-treated with ANDRO (20 and 100 mg/kg BW) for 4 weeks. Effects of ANDRO treatment were monitored in the livers of fibrosis mice by measuring angiogenesis, inflammatory infiltrate and fibrosis markers.

Results: The study demonstrated that ANDRO reduces TAA-induced liver inflammation and oxidative stress by reducing neutrophil infiltration and down-regulation of inflammatory mediator including, COX-2, p-cPLA2, NF- κ B protein levels, IL-6, IL-1 β , TNF- α , INF- γ mRNA expression, lipid peroxidation and up-regulated SMP30 protein levels. Histological data showed a significant ameliorate hepatic fibrosis after treatment with ANDRO. ANDRO also dose-dependently suppressed protein expression of α -SMA and TGF- β R1. Doubling immunofluorescence stain showed that the anti-fibrotic mechanism of ANDRO may be related to the modulation of hepatic stellate cells activation. In addition, ANDRO treatment resulted in a significant decrease in HIF-1 α and its target genes expression, including VEGF and PDGF. Other evidences showed that the expression of VEGFR1 was adjacent to areas of α -SMA positive cells with fibrotic septa, and the VEGFR1 is co-expressed with VCAM-1 in the liver tissue section.

Conclusions: This study indicates that ANDRO modulates hepatic inflammation to reduce angiogenesis and liver fibrosis in thioacetamide-induced mice.

2-Methoxystypandrone Represses RANKL-induced Osteoclastogenesis

Wen-Fei Chiou, Jyh-Fei Liao and Chien-Chih Chen

Division of Basic Chinese Medicine Research, National Research Institute of Chinese Medicine, Taipei

Department of Pharmacology, National Yang-Ming University, Taipei

Department of Biotechnology, Hungkuang University, Taichung

2-Methoxystypandrone (2-MS) is a naphthoquinone isolated from *Polygonum cuspidatum*, a Chinese herb used to remedy bone diseases. It is of interest to determine whether 2-MS has antagonistic actions against osteoclast development and bone resorption. In this study, we examined the effect and the molecular mechanisms of action of 2-MS on the receptor activator of nuclear factor κ B (NF- κ B) ligand (RANKL)-activated RAW264.7 cells, a murine preosteoblast cell line. 2-MS (1-10 μ M) concentration-dependently inhibited RANKL-induced tartrate-resistance acid phosphatase (TRAP, an early marker of osteoclast differentiation) activity. The number of multinuclear osteoclasts, actin rings and resorption pit formations were also markedly inhibited by 2-MS via targeting osteoclast differentiation at an early stage and without significant cytotoxicity. The anti-resorpting effect of 2-MS was strengthened by decreasing dendritic cell-specific transmembrane protein and matrix metalloproteinase-9 (MMP-9) mRNA expressions. RANKL increased MMP-9 gelatinolytic activity was also attenuated by concurrent, but not by subsequent addition of 2-MS. 2-MS prominently inhibited not only the RANKL-triggered nuclear translocations of NF- κ B, c-Fos and nuclear factor of activated T cells c1 (NFATc1), but also the follow-up NFATc1 induction. The upstream I κ B degradation and MAPKs phosphorylation were also substantially repressed. RANKL facilitated the association of tumor necrosis factor receptor associated factor 6 and transforming growth factor β -activated kinase 1 (TRAF6-TAK1), a signal complex important for osteoclastogenesis by RANKL. Results showed such signaling molecules formation was destroyed by 2-MS. In conclusion, the anti-osteoclastogenic mechanisms of 2-MS might be caused by the interruption of RANKL-induced association of TRAF6-TAK1 complex then abrogate downstream I κ B-mediated NF- κ B and MAPKs-mediated c-Fos activation pathways to repress NFATc1 and certain gene expressions essential for bone resorption.

Lipucan[®] Stimulates Glucose Uptake through Enhanced GLUT4 Expression and Translocation

Yu-Chuan Huang^a, Wen-Liang Chang^b, Tsu-Chung Chang^c, Hang-Ching Lin^d and Muh-Hwan Su^{d*}

^aInstitute of Preventive Medicine; ^bSchool of Pharmacy; ^cDepartment of Biochemistry, National Defense Medical Center, Taipei

^dDepartment of Research and Development, Sinphar Group, Taipei

Background: Lipucan[®] is a botanical extract and partially purified from a traditional Chinese medicine—the dried sclerotium of the fungus *Poria cocos* (Schw.) Wolf (Fam. Polyporaceae). Six lanostane type triterpenoids were isolated from Lipucan[®]. It is interested in looking for the effect and mechanism of Lipucan[®] on glucose uptake.

Methods and Materials: Lipucan[®] was manufactured by Sinphar Pharmaceutical Co., Ltd., Sinphar Group. Extraction and Isolation of triterpenoids from Lipucan[®] were done in R & D Center, Sinphar Group. 3T3-L1 pre-adipocytes, related glucose uptake, glucose transporter transcripts, Western blot analysis, and translocation assay were performed in the laboratory of Department of Biochemistry, National Defense Medical Center, Taipei, Taiwan. Statistical analysis was performed using the SPSS 11.0 for Window software. The analysis of variance (ANOVA) was used to determine the statistical significance of differences between the values for the various experimental and control groups.

Results: It was demonstrated that Lipucan[®] stimulated glucose uptake in 3T3-L1 adipocytes. The rate of basal glucose uptake increased significantly in a dose-dependent manner from 3.32 ± 0.26 to a maximum about 3.61 ± 0.13 nmole/min (150 % of control, $P < 0.01$) at dose of 3 ng/ml to 0.3 μ g/ml of Lipucan[®]. To identify the active component and investigate the underlying stimulus activity on glucose uptake, six lanostane-type triterpenoids were isolated from Lipucan[®] using silica gel chromatography and preparative HPLC. Among them, pachymic acid, tumulosic acid, and polyporenic acid C at a concentration of 0.01 μ M markedly stimulated the rate of glucose transport. The effect of pachymic acid on the expression profile of glucose transporters in differentiated 3T3-L1 adipocytes was also analyzed. The possible mechanism will be presented in the meeting.

Conclusion: We demonstrated the insulin-like activities of Lipucan[®] in stimulating glucose uptake, GLUT4 gene expression and translocation, and promoting triglyceride accumulation in adipocytes. Our study provides important insights into the underlying mechanism of hypoglycemic activity of Lipucan[®].

The Importance Of The Metabolic Regulation Of Critical Dioxygenases In Cancer And Other Chronic Diseases By Botanicals

S Barnes^{*1}, P Vayalil¹, A Piras², GP Page¹, M Crowley¹, T Whitsett¹, CA Lamartiniere¹, H Kim¹

¹University of Alabama at Birmingham, USA¹

²University of Cagliari, Cagliari, Italy²

Gene expression changes that occur in the mammary gland prior to (at weaning) and after puberty (50 days of age) were examined by microarray analysis on rats on an isoflavone-free diet. Gene-ontology analysis revealed a 2-fold reduction on the Krebs cycle pathway at 50 days. Western blot analysis revealed that the levels of isocitrate dehydrogenase (IDH) and succinate dehydrogenase B (SDHB) were substantially lower in 50-day vs in 21-day mammary gland. The Krebs cycle not only provides energy in the form of ATP, but also the keto acids for the synthesis of macromolecules. The metabolic product of IDH is α -ketoglutarate, a cofactor with oxygen in the hydroxylation of hypoxia-induced factor 1 α (HIF-1 α), the so-called oxygen sensor, as well as other dioxygenases. Immunohistochemical and quantitative immunofluorescence analysis of the mammary gland detected low levels HIF-1 α in the mammary gland pre-pubertally; post-pubertally (50 days), it was detected at higher levels and localized to the epithelial cells lining the ducts of the mammary gland. In 50-day glands from rats fed GEN or RES, however, a 50% lower signal for HIF-1 α was detected. These data suggest that the chemopreventive activity of GEN and RES is due in part to an impact on HIF-1 α signaling resulting from effects on Krebs cycle enzyme gene expression. This observation may extend to other cancers and chronic diseases. Interestingly, the most consistent gene mutation in patients with brain gliomal cancer occurs in IDH1 and IDH2. In the latter case, the mutation changes the enzymatic product to 2-hydroxyglutarate. Another dioxygenase that is of great significance is JMDH2a, a JmJC domain-containing protein, which demethylates Lys9 on histone 3 and regulates heterochromatin. This study may also provide a metabolic rationale for the effect of botanicals in chronic disease.

Acknowledgements: Supported by U54 CA100949

The Yin and Yang Actions of North American Ginseng Root in Modulating the Immune Function of Macrophages

Edmund MK Lui, Chike Godwin Azike, Daniel Zigler, Hou Jirui and Pei Hua

Ontario Ginseng Innovation and Research Consortium, Department of Physiology and Pharmacology, University of Western Ontario, Canada

Previous studies by different investigators have demonstrated both immuno-stimulatory and anti-inflammatory (immuno-suppressive) effects of ginseng. The objective of this study was to investigate the mechanism underlying these apparent paradoxical effects by examining the immunomodulatory effect of aqueous (AQ) and alcoholic (AL) extracts prepared from 4 year old Ontario grown North American ginseng roots in RAW 264.7 murine macrophages. Our results showed that AQ extract alone unregulated production of nitric oxide (NO) and TNF- α , while AL extract has no such effect. On the other hand, the AL extract suppressed the LPS-stimulated NO and TNF- α production when given 2hr before LPS challenge, suggesting an acute direct anti-inflammatory action. This effect was not, however, observed with the AQ extract. The indirect anti-inflammatory property was evaluated by incubating macrophage for 24 hr with the extract before LPS challenge; and both types of extracts exerted an indirect inhibitory effect. These data suggest that AQ extract was proinflammatory in nature but also possessed indirect anti-inflammatory effect by reducing the responsiveness of macrophages to inflammatory stimulus. In contrast, AL extract had both direct and indirect anti-inflammatory effect. The observed immuno-stimulatory and anti-inflammatory effects of ginseng can be considered as the yin and yang actions of ginseng.

The immunostimulatory action of AQ extracts on cancer cells was examined with a co-culture system of macrophage and-melanoma cells. AQ was not cytotoxic to melanoma cells alone, but macrophages treated with ginseng were cytotoxic in co-culture. The observed anti-cancer activity was attributed in part to the release of soluble mediators. AQ extracts also stimulated Kupffer cells isolated from mouse liver.

Phytochemical analysis revealed differences between the two types of extracts: polysaccharides (PS) were present in the AQ extract but not AL extract, while the latter contained higher levels of ginsenosides. Our data also showed that PS has a central role in mediating the immunomodulatory effect of the AQ extract.

Funding from the Ontario Research fund, ministry of Research & Innovation.

The Study of RNAi System Inhibited the Expression of IL-1RI in Human Rheumatoid Arthritis Fibroblast-like Synovial cell

Junhua Zhuang, Xiao Chen, Xianzhang Huang

The Second Clinical School, Guangzhou University of TCM, Guangdong
School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

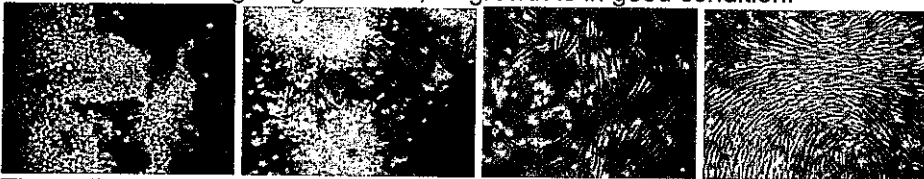
Objective: Construct the RNAi system of IL-1RI which can inhibit the expression of IL-1RI in human rheumatoid arthritis fibroblast-like synovial cell significantly.

Method:

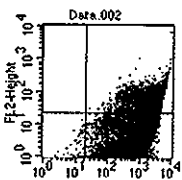
1. Culture and identify the fibroblast-like synovial cell of human rheumatoid arthritis in vitro.
2. Design, synthesize and screen for the special IL-1RIsiRNA and Use the technology of RNAi to inhibit the expression of IL-1RI gene in FLS.

Results:

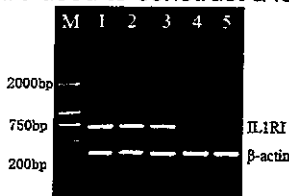
1. Using tissue culture and the digestion were successfully cultured fibroblast-like synovial cells, The cell can be transmitted to eighth generation, Its growth is in good condition.



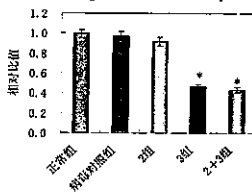
The cell's purity is as high as 99% in the fourth generation. The percentage of CD90 + cells was 99.04%. CD90 can be used as FLS cell surface markers.



2. We have designed three IL-1RI gene-specific interference sequences, The NO 2 and NO3 sequences can be used to construct a lentiviral vector system successfully.



The NO3 system can play significantly effect to inhibit the expression of IL-1RI gene.



Conclusion:

1. We have cultivated the fibroblast-like synovial cell of human rheumatoid arthritis in vitro successfully.
2. We have constructed the RNAi system of IL-1RI which can inhibit the expression of IL-1RI in human rheumatoid arthritis fibroblast-like synovial cell significantly. It suggests that this experiment is a beneficial research for curing the human rheumatoid arthritis.

Schisandra Sphenanthera Extract (SchE) is a Promising Tacrolimus-Sparing Agent for Renal Transplant Recipients

Jia-li Li¹, Hui-chang Bi¹, Xue-ding Wang¹, Chang-xi Wang², Min Huang^{1*}

¹School of Pharmaceutical Sciences, Sun Yat-sen University, Guangdong

²Kidney Transplant Department, Transplant Center, First Affiliated Hospital, Sun Yat-sen University, Guangdong

Background: Tacrolimus is the first-line immunosuppressant widely used after renal transplantation, however, its high price makes a huge financial burden for patients. Thus, tacrolimus-sparing agents that could decrease its dose required to maintain desired therapeutic concentration range are in high demand. A preparation of ethanolic extract of *Schisandra sphenanthera* (SchE), which contains 7.5 mg schisantherin A per tablet, is often used as a hepatoprotective agent when drug-induced hepatitis occurs in transplant recipients. We found that SchE could significantly increase trough concentrations of tacrolimus in renal transplant recipients. Therefore, the aim of this study was to assess the effect of SchE as a promising tacrolimus-sparing agent on the pharmacokinetics of tacrolimus in renal transplant recipients. **Methods:** 12 renal transplant recipients who had took tacrolimus for 2 weeks and reached stable concentration level were enrolled in this study. 10 blood samples were taken over a 12-h period. Then SchE (0.54g, tid) was coadministered with tacrolimus for 15 d, another 10 blood samples were taken on last day over a 12-h period. **Results:** After coadministration of SchE, tacrolimus dose-adjusted C_0 , C_{max} , AUC_{0-12h} were increased by 290.9% ($P<0.001$), 180.1% ($P<0.001$) and 212.1% ($P<0.001$), respectively. Time to peak concentration (t_{max}) was significantly delayed from 1.6 ± 1.0 h to 2.6 ± 1.3 h ($P=0.010$). Tacrolimus daily dose was decreased by 52.7% ($P<0.001$). **Conclusion:** SchE can significantly increase tacrolimus concentrations and reduce tacrolimus doses in renal transplant recipients. Thus, frequent and strict therapeutic drug monitoring of blood tacrolimus concentration and dose adjustment are highly recommended when coadministering SchE in clinical use. It also suggests that SchE may be a promising tacrolimus-sparing agent for its potential therapeutic and financial saving benefits.

Acknowledgement: The work was supported by the National Major Projects for science and technology development from Science and Technology Ministry of China (grant number 2009ZX09304-003 and 2008ZX09312)

Amelioration Collagen-induced Arthritis in Rats by Sinomenine through Regulation of MMPs, TIMPs, and Cytokines

Yuen Fan Wong, Hua Zhou, Jue Wang, Xiong Cai, Liang Liu *

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by chronic inflammation of the synovium in joint cavities. This inflammation is characterized by the presence of macrophages, lymphocytes, and synovial fibroblasts, and it leads to the progressive destruction of articular cartilage, ligament, and bone. Recent studies have revealed that proinflammatory cytokines, such as IL-1 β and IL-6, play a central role in the perpetuation of synovial inflammation. Cartilage destruction is due to proteolysis by metalloproteinases (MMPs) in remodeling of the extracellular matrix. MMP-2 and -9 play important roles in arthritic diseases. Elevated levels of these enzymes have been detected in arthritic synovial fluid. All connective tissue cells produce endogenous tissue inhibitors of metalloproteinases (TIMPs) which bind tightly and irreversibly to active MMPs as well as pro-MMPs. An imbalance in the ratio of local concentrations of active MMPs/TIMPs is an important determinant of the extent of connective tissue turnover. The stem of the Chinese medicinal plant, *Sinomenium acutum* Rehder & Wilson (Family Menispermaceae), has been used to treat various rheumatic and arthritic diseases, of which the major bioactive component is sinomenine. We investigated the nature and molecular mechanisms of the anti-arthritic effect of sinomenine on collagen-induced arthritis (CIA) in female Wistar rats. The results showed that sinomenine markedly suppressed the incidence and disease progression of established CIA, showing as dramatic reduction of paw swelling, ESR, and arthritic scores. Sinomenine suppressed the production of proinflammatory cytokines IL-1 β and IL-6 in serum, inhibited the protein expressions and activities of MMP-2 and MMP-9, and elevated the protein expressions and activities of TIMP-1 and TIMP-3 in rat paw tissues. Taken together, sinomenine would be beneficial for protection of the arthritic joints through inhibition of proinflammatory cytokines and normalization of the MMPs/TIMPs balance and thus could be an ideal therapeutic agent for the treatment of RA.

A Simplified Method for Quantifying Cell Migration/ Wound Healing in 96-well Plates

Patrick Y.K. Yue, N.K. Mak, Ricky N.S. Wong*

Department of Biology, Hong Kong Baptist University, Hong Kong

Studying of cell migration and its underlying mechanisms are of great significance in various fields of research including basic biology and pharmaceutical development. Cell migration plays a key role in both normal and pathological physiological conditions. The cell migration or scratch wounding assay is an easy and economical *in vitro* method to assess a large numbers of testing compounds. Even though this simple assay has been used for decades, researchers are trying to modify such experimental protocols and the "wounding" device. In this study, an eight-channel mechanical "wounder" was designed for performing cell migration assay particularly in a 96-well culture plate format. With special designs of guiding-bar, adjustable-pin and disposable tips, the "wounder" restricted the scratch area at the center of each well and ensured a perfect contact between each pin and the well surface in a contamination-free manner. Taken together, this mechanical "wounder" produced a uniform denudation of cell monolayer in each well with a wound size of 600 μm . Using this modified wounding device, the effects of epidermal growth factor and DL- α -difluoromethylornithine on re-epithelialization of IEC-6 cells; and serum on the wound healing of HUVECs were demonstrated. This "wounder" facilitates the cell migration study in small research laboratory, especially, that advanced imaging system is not required. (This work is under patent pending – publication no. 101339104).

Novel Anti-Inflammatory and Analgesic Effects of Plumbagin through Inhibition of Nuclear Factor- κ B Activation

Pei Luo, Yuen Fan Wong, Lin Ge, Liang Liu, Hua Zhou*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) (PL), a naturally occurring yellow pigment, is found in the plants of the Plumbaginaceae, Droseraceae, Ancistrocladaceae, and Dioncophyllaceae families. It has been reported that PL exhibits anti-carcinogenic activity. However, there is no report on the anti-inflammatory and analgesic effects of plumbagin *in vivo*. We for the first time reported the anti-inflammatory and analgesic effects of PL orally administrated in a range of dosages from 5 to 20 mg/kg. The result showed that PL significantly and dose-dependently suppressed the paw edema of rats induced by carrageenan or various pro-inflammatory mediators, including histamine, serotonin, bradykinin and prostaglandin E2 (PGE2). It also reduced the number of writhing episodes of mice induced by intraperitoneal injection of acetic acid. Mechanistic studies showed that PL effectively decreased the production of pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α . It also inhibited the expression of pro-inflammatory mediators iNOS and COX-2 while it did not inhibit the expression of COX-1. Further studies demonstrated that PL suppressed I κ B α phosphorylation and degradation and thus inhibited the phosphorylation of p65 subunit of NF- κ B. This study suggests that PL has potential to be developed into an anti-inflammatory agent for treating inflammatory diseases.

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Immunomodulatory Activities of *Ganoderma Sinense*

Yue GGL^{1*}, Sze DMY², Chan BCL¹, Ng MCH², Fan K², Cheng L¹, Fung KP^{1,3}, Leung PC¹ and Lau CBS¹.

¹Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong

²Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong

³School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

Various species of the *Ganoderma* genus are common ingredients in many health supplements in Asia. For example, *Ganoderma lucidum* had previously been shown to possess chemopreventive and immunomodulatory properties. Our present study aimed to evaluate the immunomodulatory activities of another *Ganoderma* species, *Ganoderma sinense* (GS), using human peripheral blood mononuclear cells (PBMC). Our results showed that the polysaccharide-enriched fraction of GS hot water extract (100 – 400 µg/ml) exhibited significant stimulatory effects on PBMC proliferation as shown in [methyl-³H]-thymidine incorporation assay. When the fruiting bodies of GS were divided into pileus and stipe parts and were separately extracted with hot water, the polysaccharide-enriched fraction isolated from GS stipe (50 – 400 µg/ml) showed the most immunostimulating effects in PBMC. The productions of TNF-α, IL-10 and TGF-β were significantly enhanced in the GS stipe polysaccharide-enriched fraction-treated PBMC (n=12). In addition, the proportion of CD14⁺ monocyte subpopulation within the PBMC was specifically increased. The composition of monosaccharides of the active fraction was determined by GC-MS and gel permeation chromatography. Our study demonstrated for the first time the immunostimulatory effects of GS stipe polysaccharide-enriched fraction on PBMC. The findings revealed the potential use of *Ganoderma sinense* (especially including the stipes of fruiting bodies) as an adjuvant supplement for cancer patients, whose immune activities were suppressed during chemotherapies.

Investigation of the Immunomodulatory Effects of Radix Astragali Targeting Dendritic Cells

Michelle Chun-Har Ng¹, Kei Fan², and Daniel Man-Yuen Sze¹

¹Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong

²Faculty of Pharmacy, The University of Sydney, Australia

Dendritic cells (DC) play pivotal role in the initiation, programming and regulation of tumour-specific immune responses. However, cancer-associated microenvironment has been shown to adversely affect this DC-related immune-surveillance system. Eventually DCs become defective and are not able to up-regulate the co-stimulatory molecules of CD40 and CD80 and thus fail to effectively prime the naive T-cells. We here hypothesized that compounds derived from Traditional Chinese Medicines (TCM) may up-regulate CD40 and CD80 expression on DC leading to an effective anti-cancer immunity. Highly prevalent prostate cancer is the second most frequently diagnosed cancers among men in economically developed countries. Recently, a 2-herb Shengqifuzhen injection with Radix Astragali (Huangqi, HQ) and Radix *Codonopsis* (Dangshen, DS), has been found to be useful for improving clinical outcomes in the treatment of end-stage prostate cancer patients in a major prostate clinic in the 2nd Affiliated Hospital of the University of Chinese Medicine Guangzhou China. Based on such clinical relevance, this study aims: 1.) to investigate the effect of the 2-herb combinations on CD40 and CD80 expression on DC using our established THP-1-based DC standardized functionality platform; and 2.) to correlate the DC biological results with the corresponding HPLC-DAD chromatographic profiles. HQ and DS were mixed in three different ratios, which are 3:1, 1:1 and 1:3; and extracted by 3 different solvent systems -- deionized water; methanol; and 1:1 water-methanol mixture. Sixteen extracts were prepared for both chromatographic analysis and DC assay. The flow cytometric data suggested that CD80 and CD40 expression on THP-1 with 3-hr water extract was found to be up-regulated in a dose-response manner and in lesser extent for the 0.5-hr water extracts. There was no observed up-regulation of the CD80 and CD40 DC co-stimulatory markers using the methanol extracts; while reduced expression of these two markers observed in corresponding 1:1 water-methanol mixture extracts.

Differential Effects of Radix Paeoniae Rubra on Cytokine and Chemokine Expression Inducible by Mycobacterium

Liangjie Wang¹, Cindy LH Yang¹, Terry CT Or¹, James CB Li^{1,2}, Allan SY Lau^{1,2,3}

¹ Molecular Chinese Medicine Laboratory, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

² Cytokine Biology Group, Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong

Mycobacterium tuberculosis (Mtb) is still a major cause of morbidity and mortality in the world. Upon initial infection with *Mycobacterium*, macrophages secrete multiple cytokines and chemokines, including IL-6, IL-8 and TNF- α , to mediate host immune responses against the pathogen. We previously showed mycobacteria also induce the production of IL-10, an anti-inflammatory cytokine, via PKR activation contributing to mycobacterial evasion of host immunity.

Radix Paeoniae Rubra (RPR) is a commonly used traditional Chinese medicine (TCM) with potential anti-inflammatory, hepatoprotective and neuroprotective effects. It has been used by TCM practitioners to treat patients with tuberculosis. In this study, we investigated the immunoregulatory effects of RPR on mycobacterial infection in human macrophages.

The interaction of *Bacillus Calmette-Guérin* (BCG) with primary human blood macrophages (PBMac) was used as a model. Using a bioactivity guided fractionation scheme, a subfraction (RPR-EA-S1) with potent immunoregulatory effects was obtained after a series of fractionation procedures.

The qPCR and ELISA results showed that RPR-EA-S1 enhanced IL-8 expression and did not affect the expressions of IL-6 and TNF- α in BCG-stimulated macrophages. However, it could specifically inhibit BCG-induced IL-10 production in a dose and time-dependent manner. To delineate the underlying mechanisms, Western blots were performed and showed that RPR-EA-S1 did not affect the phosphorylation of MAPK, Akt or GSK3 β . Instead, it suppressed the degradation of I κ B α in the cytoplasm and inhibited the translocation of transcription factor NF- κ B1 p50 to the nucleus. Thus, the inhibitory effects of RPR-EA-S1 on IL-10 expression in BCG-activated PBMac may be due to the reduced nuclear translocation of NF- κ B1 p50. Such suppression of IL-10 induction may create a less favorable environment for mycobacteria and potentially play a positive role in mycobacterial killing. Taken together, our results indicate that RPR may have a role for its use as an adjuvant for anti-Mtb therapy.

(LW is the recipient of a HKU postgraduate research studentship. The program was funded by grants to ASL from Prof. Francis SK Lau and Mr William Au Research Fund.)

Recent Progress on Elucidation of Immunomodulating Activity and its Action Mechanism of Kampo (Japanese Herbal) Medicines

Haruki Yamada*, Hiroaki Kiyohara and Takayuki Nagai

Kitasato Institute for Life Sciences, Kitasato University, Japan

Kampo medicines have been used as the formulation consisting of several component herbs, and regulate the homeostasis of the whole body by affecting body systems such as neural, endocrine and immunological systems. Some Kampo medicines are known to recover systemic fatigue, and general weakness, enhance the defense system against infectious diseases and improve allergic diseases through regulation of immunological systems. Because Kampo medicines are taken orally, and the active ingredients of the medicines may first affect intestine, it is suggested that a part of efficacies of Kampo medicines causes through mucosal immune systems. Hochuekkito (HET: Qu-Zhang-Yi-Qi-Tang) has been used as a tonic and improves the function of digestive system and defense system against various infections. HET enhanced the antigen specific IgA immune response in murine mucosal immune system through increased population of L-selectin positive B lymphocytes. HET also modulated the function of the epithelial cells in murine intestine, and certain polysaccharides in HET were identified to involve in the activity. Shoseiryuto (SST: Xiao-Qing-Long-Tang) has been used for the treatment of allergic bronchial asthma and common cold etc., clinically. SST reduced the airway hyperresponsiveness and OVA specific IgE antibody level in bronchoalveolar wash of OVA-sensitized airway inflammation model mice. SST also showed oral adjuvant activity for nasally administrated influenza vaccine, and enhanced antiviral IgA antibody level in nasal and bronchoalveolar washes. The activity was caused by the combination effects of two stereoisomers of certain hydroxyl fatty acid (pinellic acid). These observations may be useful for the clinical use of Kampo medicines as the evidence-based medicine, and its drug development due to new concept.

Bioactivity Guided Evaluation of Sesquiterpenes as an Anti-inflammatory Activity Isolated from *Cyperus Rotundus* L: Effect of Chemical Structures and Cellular Signaling Pathway

Salman Khan, Eun Myoung Shin, IL Kyun Lee, Kang Ro Lee, Yeong Shik Kim*

College of Pharmacy, Seoul National University, Korea

The functioning of the immune system is finely balanced by the activities of pro-inflammatory and anti-inflammatory mediators or cytokines. Unregulated activities of these mediators can lead to the development of serious inflammatory diseases. A variety of safe and effective anti-inflammatory agents are available, with many more drugs under development. Of the natural compounds, the sesquiterpenes (nootkatone, α -cyperone, valencene and β -selinene) isolated from *Cyperus rotundus* L. have received much attention because of their potential anti-inflammatory effects. However limited studies have been reported regarding the influence of sesquiterpenes structure on anti-inflammatory action. In the present study, the anti-inflammatory potential of four structurally divergent sesquiterpenes was evaluated on lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, murine macrophages. Among four sesquiterpenes, α -cyperone and nootkatone, showed stronger anti-inflammatory and potent NF- κ B inhibition effect on LPS-stimulated RAW 264.7 cells. Molecular analysis revealed that various inflammatory mediators (iNOS and COX-2) were reduced significantly and correlated with the down-regulation of NF- κ B signaling pathway. Additionally, electrophoretic mobility shift assay (EMSA) elucidated that nootkatone and α -cyperone dramatically suppressed LPS-induced NF- κ B-DNA binding activity using 32 P-labeled NF- κ B probe. Hence, our data suggest that α -cyperone and nootkatone are potential therapeutic agents for inflammation associated diseases.

Suppression of LPS-induced Inflammatory and NF- κ B Responses in RAW 264.7 Macrophages by Anomalin Isolated from the Root of *Saposhnikovia Divaricata*

Salman Khan, Eun Myoung Shin, Yoo Hyun Jung, Jinwoong Kim, Yeong Shik Kim*

College of Pharmacy, Seoul National University, Korea

Treatment of inflammatory diseases today is largely based on interrupting the synthesis or action of mediators that drive the host's response to injury. On the basis of this concept most of the drugs have been developed. In our continuing search for the novel anti-inflammatory agents from the traditional medicinal plants, *Saposhnikovia divaricata* (Umbelliferae) ethanol (70%) extract of were brought under investigation in terms of LPS stimulated murine macrophages, RAW264.7 cell line. Anomalin, a pyranocoumarin compound isolated from the root of *S. divaricata*, was found to be exhibit potent anti-inflammatory activity in tested experimental models. To be more specific, anomalin, inhibited LPS-induced inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) protein and mRNA expressions in dose dependent manners, which leads to the inhibition of nitric oxide (NO) production. In addition, the immunoblotting showed that total extract and anomalin, suppressed LPS-induced phosphorylation and degradation of I κ B α . Molecular analysis revealed that several pro-inflammatory cytokines (TNF- α , IL-6 β , and IL-1 β) were reduced by anomalin, and correlated with the down regulation of nuclear factor- κ B (NF- κ B) signaling pathways. To further identify the mechanisms underlying its anti-inflammatory activity, electrophoretic mobility shift assay (EMSA) using ³²P-labeled NF- κ B probe was conducted. The activation of LPS induced NF- κ B DNA binding was significantly inhibited by anomalin. The present data suggest that anomalin is a major anti-inflammatory agent in total extract of *S. divaricata* and can be a potential therapeutic candidate for the inflammatory disorders.

Novel Labdan Diterpene Derivative Isolated from *Leonurus Japonicum* as a Function of Anti-inflammatory Activity in LPS-stimulated RAW 264.7 Cells

Salman Khan, Eun Myoung Shin, Sa Wang Baek, Jinwoong Kim, Yeong Shik Kim*

College of Pharmacy, Seoul National University, Korea

Inflammation is a dynamic process with pro-inflammatory mediators and cytokines. A number of drugs have been developed to treat inflammation including agents that reduce the activity of specific mediators and cytokines. The nitric oxide (NO) and prostaglandin E₂ (PGE₂) play a decisive role in various physiological and pathophysiological conditions. The phytochemical investigation of *Leonurus japonicum* led to the isolation of novel labdan diterpene derivative, 15, 16-epoxy-3-hydroxyabda-8, 13(16), 14-trien-7-one. 15, 16-epoxy-3-hydroxyabda-8, 13(16), 14-trien-7-one, exhibited potent inhibitory effect on the iNOS and COX-2 protein expression using LPS-stimulated RAW 264.7 cells, macrophages. As a mechanism of anti-inflammatory action shown by 15, 16-epoxy-3-hydroxyabda-8, 13(16), 14-trien-7-one, suppression of nuclear factor (NF)- κ B activation has been documented. Furthermore, the labdan compound exhibited significant inhibitory effect on LPS-mediated NF- κ B-DNA binding and AP-1-DNA binding activity using electrophoretic mobility shift assay (EMSA) ³²P-labeled NF- κ B probe. Taken together, labdan diterpene 15, 16-epoxy-3-hydroxyabda-8, 13(16), 14-trien-7-one, suppressed LPS-induced NF- κ B activation, resulting down-regulation of iNOS and COX-2 protein expression, which was attributable to its inhibitory action of LPS-induced NO and PGE₂ production. These results may contribute to the anti-inflammatory activity of 15, 16-epoxy-3-hydroxyabda-8, 13(16), 14-trien-7-one.

Panax Notoginseng Reduces Atherosclerotic Lesions in apoE-deficient Mice and Inhibits TNF α -induced Endothelial Adhesion, Molecular Expression and Monocyte Adhesion

Jian-Bo Wan^{1,2}, Nan Wang¹, Jing-Dong Wang², Cheng-Wei He², Zai-Jun Zhang¹, Simon Ming-Yuen Lee¹, Jing X. Kang², Yi-Tao Wang¹

¹Institute of Chinese Medical Sciences, University of Macau, Macau

²Department of Medicine, Massachusetts General Hospital and Harvard Medical School, USA

It is widely appreciated that inflammatory mechanism is associated with atherogenesis. *Panax notoginseng* has been shown to possess anti-inflammatory activity, implying the possibility of antiatherosclerotic effects. Here, the antiatherogenic effect of total saponins from *P. notoginseng* (PNS) was examined with apoE deficient mice fed a western-type diet. PNS was administered orally to two treatment groups at a dosage of 4.0 and 12.0 mg/day/mouse, respectively. After 8 weeks, as compared to the control group, both PNS-treated groups decreased significantly the extent of atherosclerotic lesion by 61.4% and 66.2%, respectively, ($P < 0.01$) in the aortic area. Moreover, we found that PNS exhibited potent pro-angiogenic activity in human endothelial cell and zebrafish embryo. The PNS was separated into 20(S)-protopanaxatriol saponins (PTS) and 20(S)-protopanaxadiol saponins (PDS). We compared the anti-vascular inflammatory effects of the three saponin fractions (PNS, PDS and PTS) and two purified ginsenosides, Rb1 (a major constituent in PDS) and Rg1 (a major constituent in PTS), on adhesion of monocytes to activated endothelial cells and the expression of TNF α -induced endothelial adhesion molecules. Interestingly, PDS appeared to be more potent than PNS and PTS against vascular inflammation. Although Rb1 was a major constituent in PDS fraction, it was significantly less active than PDS, suggesting that synergetic interaction among different ginsenosides in PDS fraction may contribute to its higher potency. The mechanism of these anti-vascular inflammatory effects was found to involve inhibition of NF- κ B pathway activation. Thus, the antiatherogenic effect of PNS was associated with beneficial alterations in vascular inflammation as well as angiogenic pathways.

The Effects of Mitragynine on the Expression of COX-1 and COX-2 in LPS-stimulated RAW264.7 Macrophage Cells

Utar Zulkhurnain, M.I.A Majid, M.I Adenan, Mei Lan Tan

Malaysian Institute of Pharmaceuticals and Nutraceuticals, Malaysia

Areca nut (commonly known as betel nut) chewing has been shown to be associated with metabolic syndrome and cardiovascular disease (CVD). The mechanism by which betel nut ingestion could lead to development of CVD is not precisely known, however, dyslipidemia and hyperhomocysteinemia and hypertriglyceridemia could be some of the potential risk factors. Present study was undertaken to investigate the effects of different dosages of betel nut on components of metabolic syndrome, such as hypertriglyceridemia, low HDL-cholesterol, obesity, fasting hyperglycemia, inflammation, and hyperhomocysteinemia in a rat model.

Twenty-five adult female Sprague-Dawley rats, aged 10-12 weeks were divided into four equal groups, with the exception of group-1. Group-1 served as the control group ($n=7$) and received water, whereas groups 2, 3 and 4 were given water suspension of areca nut orally in dosages 30mg, 60mg and 90mg, respectively for period of 5 weeks. At the end of 5 weeks, animals were sacrificed and blood was collected.

Plasma/serum were analyzed for glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, homocysteine, folate, vitamin B12 and N-acetyl- β -D-glucosaminidase (NAG). When the mean concentration values in four groups were compared using one way ANOVA followed by Tukey's HSD-test, there was a significant increase in the concentrations of total cholesterol ($p=0.038$) and triglycerides $p=0.05$ in the group receiving 30mg/day betel nut compared to the control group. However, administration of higher doses of areca nut had no significant effect on the serum levels of glucose, HDL-cholesterol, LDL-cholesterol, and NAG (a marker of inflammation).

Plasma homocysteine concentration in the group-4 receiving 90mg/day areca nut was significantly decreased compared to the control group ($p=0.036$) and this appeared to be due to increased level of folate in this group. Mean vitamin B12 levels were not found to be significantly different in all the groups. Low dosages of betel nut are associated with greater risk of hypercholesterolemia and hypertriglyceridemia.

Anti-Malarial Drug Artesunate Attenuates Allergic Airway Inflammation and Inhibits Phosphoinositide 3-kinase Pathway in Mouse Asthma Model

W.S. Fred Wong, Chang Cheng, Eugene W.X. Ho

Department of Pharmacology and Department of Epidemiology & Public Health, National University of Singapore, Singapore

Phosphoinositide 3-kinase (PI3K)/Akt pathway is linked to the development of asthma. Anti-malarial drug artesunate is a semi-synthetic derivative of artemisinin, the principal active component of a medicinal plant *Artemisia annua*, and has been shown to inhibit PI3K/Akt activity. We hypothesized that artesunate may attenuate allergic asthma via inhibition of the PI3K/Akt signaling pathway. Female BALB/c mice sensitized and challenged with OVA developed airway inflammation. Bronchoalveolar lavage fluid was assessed for total and differential cell counts, and cytokine and chemokine levels. Serum IgE levels were determined. Lung tissues were examined for cell infiltration and mucus hypersecretion, and the expression of inflammatory biomarkers. Airway hyperresponsiveness was monitored by direct airway resistance analysis. Artesunate dose-dependently inhibited OVA-induced increases in total cell count, eosinophil count, IL-4, IL-5, IL-13 and eotaxin levels in bronchoalveolar lavage fluid, and reduced serum level of ovalbumin-specific IgE. It attenuated OVA-induced lung tissue eosinophilia and airway mucus production, mRNA expression of E-selectin, chitinases, IL-17, IL-33, Muc5ac and inducible nitric oxide synthase in lung tissues, and airway hyperresponsiveness to methacholine. In normal human bronchial epithelial cells, artesunate blocked epidermal growth factor-induced phosphorylation of Akt and its downstream substrates tuberin, p70S6 kinase and 4E-binding protein 1, and transactivation of NF- κ B. Similarly, artesunate blocked the phosphorylation of Akt and its downstream substrates in lung tissues from OVA-challenged mice. Our findings implicate a potential therapeutic value of artesunate in the treatment of asthma and it may act by inhibiting PI3K/Akt pathway. (This work was supported by BMRC 06/1/21/19/443 to WSWF)

Identification of the Immunomodulatory Ingredients in Semen Cuscutae

Shuo-Ju Liu¹, Meng-Ja Yang¹, Hui-Chi Huang¹, Wen-Te Chang¹, Meng-Chiou Lee¹, Ching-Liang Chu^{2,3}, and Ming-Kuem Lin¹

School of Chinese Medicine Resources, China Medical University, Taichung

Semen Cuscutae, also known as Cuscuta seed or Tu-Si-Zi, is one of the commonly used Chinese medicinal materials. It has been using as a tonic for nourishing the liver and kidneys. However, the immunoregulatory effect of Semen Cuscutae is rarely studied. Dendritic cells (DCs) play a critical role in initiating immune response. Thus, DCs are regarded as a major target of immunomodulator for controlling immune responses. In this study, we examined the effect of Semen Cuscutae on mouse bone marrow-derived DC activation. We found that the methanol extract of Semen Cuscutae (MESC) induced the pro-inflammatory cytokine production and promoted DC maturation by enhancing the expression of maturation markers. Also, the n-butanol and methanol layers of the fractionation of MESC potentially suppressed LPS-induced DC activation. To identify the active ingredients, we are further purifying compounds from MESC by using positive phase open column and reverse phase C18 column-HPLC equipment. Remarkably, our study demonstrates the immunomodulatory activity of Semen Cuscutae to DC for the first time and then extends its biological function. It is interesting to reveal the molecular mechanism for the DC immunomodulation by finding the active component for further application in pharmaceutical industry.



***Carthamus Tinctorius* L Attenuates Lipopolysaccharide-Induced Cardiomyocyte Fibrosis Via Down-Regulating the Expressions of uPA, MMP-2, MMP-9**

Yun-Chen Tien^a, Wen-Huang Peng^a, Chih-Yang Huang^{bcd}

^aGraduate Institute of Chinese Pharmaceutical Sciences, College of Pharmacy, China Medical University, Taichung

^bDepartment of Chinese Medicine, China Medical University, Taichung

^cGraduate Institute of Basic Medical Sciences, China Medical University, Taichung

^dDepartment of Health and Nutrition Biotechnology, Asia University, Taichung

Upregulation of urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA) and matrix metalloproteinases (MMPs) is associated with the development of myocardial infarction (MI), dilated cardiomyopathy, cardiac fibrosis and heart failure (HF). Evidences suggest that lipopolysaccharide (LPS) participates in the inflammatory response in the cardiovascular system. Flos Carthami, the flower of *Carthamus tinctorius* L. (Compositae) is an important traditional Chinese medicine used for the treatment of heart disease and inflammation. This study investigated the effect of Flos Carthami (FC_{EIOH}) ethanolic extract on LPS-induced fibrosis in H9c2 cardiomyoblast cells and primary cardiomyocytes. H9c2 cells induced fibrosis with LPS administration (1µg/ml). The H9c2 cells were divided into five groups: Control, LPS (1µg/ml) treatment, and three different dose FC_{EIOH} (31.25, 62.5, 125, and 250µg/ml) treatment. We detected fibrosis proteins uPA, tPA, MMP-2 and MMP-9. We found that FC_{EIOH} could down-regulated the expression of uPA, tPA, MMP-2 and MMP-9 in LPS-induced cardiomyocyte fibrosis. However, FC_{EIOH} (31.25, 62.5, 125, and 250µg/ml) treatment showed no effects on the expression of tissue inhibitor of metalloproteinase-1, -2, -3 and -4 TIMP-1, -2, -3 and -4.

Stimulatory Effect of *Echinacea purpurea* Extract on Trafficking Activity in Mouse Dendritic Cells: Revealed by Genomic and Proteomic Analyses

Shu-Yi Yin, Lie-Fen Shyur and Ning-Sun Yang

Agricultural Biotechnology Research Center, Academia Sinica, Taipei

Several *Echinacea* species have been used as nutraceuticals or botanical drugs for "immunostimulation", but scientific evidence supporting their therapeutic use is still controversial. In this study, a phytochemical mixture extracted from the butanol fraction (BF) of a stem and leaf (S+L) extract of *E. purpurea* ([BF/S+L/Ep]) containing stringently defined bioactive phytochemicals was obtained using standardized and published procedures. The transcriptomic and proteomic effects of this phytoextract on mouse bone marrow-derived dendritic cells (BMDCs) were analyzed using primary cultures. Treatment of BMDCs with [BF/S+L/Ep] did not significantly influence the phenotypic maturation activity of dendritic cells (DCs). Affymetrix DNA microarray and bioinformatics analyses of genes differentially expressed in DCs treated with [BF/S+L/Ep] for 4 or 12 h revealed that the majority of responsive genes were related to cell adhesion or motility (*Cdh10*, *Itga6*, *Cdh1*, *Gja1* and *Mmp8*), or were chemokines (*Cxcl2*, *Cxcl7*) or signaling molecules (*Nrxn1*, *Pkce* and *Acss1*). TRANSPATH database analyses of gene expression and related signaling pathways in treated-DCs predicted the JNK, p38, ERK1/2 or MAPKAPK pathways as the putative targets of [BF/S+L/Ep]. In parallel, proteomic analysis showed that the expressions of metabolic-, cytoskeleton- or NF-κB signaling-related proteins were regulated by treatment with [BF/S+L/Ep]. *In vivo* cell trafficking assay further showed that DCs treated with [BF/S+L/Ep] were able to migrate more effectively to peripheral lymph node and spleen tissues than DCs treated as control groups. Results from this study suggest that [BF/S+L/Ep] modulates DC mobility and related cellular physiology in the mouse immune system. Moreover, the signaling networks and molecules highlighted here are potential targets for nutritional or clinical application of *Echinacea* or other candidate medicinal plants.

The Inhibitory Effect of Phytocompound Cytopiloyne on Cytokine Expression and its Signal Transduction Mechanism in Dendritic Cells

Wen-Chi Wei^{1,2}, Wen-Chin Yang¹, Lie-Fen Shyur¹, Jyh-Horng Sheu², Vanisree Staniforth¹, Swey-Shen Chen¹ and Ning-Sun Yang^{1,4,5*}

¹Agricultural Biotechnology Research Center, Academia Sinica, Taipei

²Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taipei

³Department of Allergy and Immunology, IgE Therapeutics, Inc. USA; Department of Molecular Biology, TSRI, La Jolla, USA

⁴Department of Life Science, National Taiwan University, Taipei and ⁵Department of Life Science, National Central University, Taipei

Cytopiloyne, a polyacetylenic glucoside from *Bidens pilosa*, was shown in our previous study to control and prevent type I diabetes in non-obese diabetic mice, via suppression of the differentiation of Th0 cells into Th1 cells and the promotion of their differentiation into Th2 cells. In this study, we investigated the immunomodulatory effect of cytopiloyne on the maturation and function of human monocyte-derived dendritic cells (DCs). Cytopiloyne reduced the expression of LPS-induced DC maturation makers (CD40, CD80 and CD86). In an allogeneic mixed lymphocyte reaction (MLR) assay, we observed that cytopiloyne treatment shifted the cell polarizing capacity of DCs from Th1 to Th2 pathway. Importantly, cytopiloyne exhibited a unique capability to inhibit the LPS-induced expression of IL-12 and IL-10 but not of IL-6 and TNF- α in test DCs. Further studies to delineate the mechanistic basis of cytopiloyne's action revealed that cytopiloyne exerted its inhibitory effect on cytokine expression by interference with the rictor-mTOR-mediated signaling network involving the activation of Akt and persistent phase of NF- κ B and Erk1/2 activation. Together, our results demonstrate that cytopiloyne effectively suppresses the expression of IL-12 in human DCs via specific signaling pathways, and thereby effects the differentiation of Th1 cells. This phytocompound may therefore be further explored as a useful modulator of human immune cell functions with potential health care applications.

Anti-inflammatory Effect of Specific Herbal Extracts in Skin and Colon-associated Inflammatory Disease Models

Chin-Chun Wen, Yuh-Ting Huang, Li-Ting Huang, Sheng-Chu Kuo, Shu-Hui Lin and Ning-Sun Yang*

¹Agricultural Biotechnology Research Center, Academia Sinica, Taipei

²Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung

Recently, international and other Asian countries pay high attention to the science-based application of traditional medicinal herbs. Our previous *in vivo* and *in vitro* data showed that WCH, the hot water extract of a commonly used traditional Chinese medicinal (TCM) herb in Taiwan, can confer high anti-inflammatory effect. Recent studies also point out that inflammation is strongly associated with carcinogenesis. Therefore, this study is aimed to further explore the effect of WCH on colitis-associated colon cancer (CAC), and inhibitive effects of WC extracts prepared by different extraction methods in inflammation-related animal models. In our sub-acute toxicity study, both high dose (1000 mg/kg body weight) and low dose (100 mg/kg body weight) treatment of WCH showed no toxicity in tested mice. The WC extracts prepared by different extraction methods showed different levels of protection on body weight, histological section and histological score in acute colitis mice model, with the best results obtained in WCH treated mice. HPLC was used to analyze the chemical composition of WCH extracts. Fresh WC extract showed two specific peaks in the fingerprint pattern but dry WC extract didn't. We also set up the azoxymethane / dextran sulphate sodium-induced CAC mice model, and found that WCH-treated group showed a higher survival rate than the non-treated group. In addition, we used the WC ethanol extract in a mouse skin inflammatory model induced by chemical substances or UV-B irradiation. Results indicated that WC alcohol extract inhibited the expression of MMP-9 and COX-2. In short, WC extracts prepared by different extraction methods showed good anti-inflammatory effect in different inflammation-associated animal models.

Specific Herbal Extract (PE) Suppresses NF- κ B-mediated Inflammation in Murine Macrophages and in Mouse Skin

Chin-Chun Wen, Yuh-Ting Huang, Li-Ting Huang, Wen-Ching Huang, Cheng-Han Kuo, Pei-Wen Hsiao, Jin-Bin Wu, Pei-Wen Hsiao, Sheng-Chu Kuo and Ning-Sun Yang*

Agricultural Biotechnology Research Center, Academia Sinica, Taipei
Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung

With appreciation on the profound effects and the long history of traditional Chinese medicines (TCM), evidence-based application of traditional medicinal herbs has attracted much attention from international communities. Recent research finding suggest that inflammation is associated with a spectrum of infectious, heart and malignant diseases. Various inflammatory cells and pro-inflammatory cytokines (e.g., TNF- α , IL-6, IL-1 β , IL-12) have been identified as key mediators for the inflammation and the NF- κ B signaling system is recognized as a paradigm in response to various inflammatory or danger signals. In this study, we investigated a specific traditional medicinal herb, alias named as PE for possible anti-inflammatory effect in murine macrophages and in mouse skin. Our data revealed that specific, partially purified fractions (PE-15 and PE-21) could inhibit strongly NF- κ B and TNF- α gene expression in the murine macrophage cell line (RAW 264.7 cell line) as a response to LPS stimulation, likely via the IKK α / β pathway, leading to a decreased level of iNOS-mediated nitric oxide (NO) release, and MMP-9 and COX-2 expression. Moreover, our *in vivo* animal model studies showed that PE-15 and PE-21 can suppress NF- κ B and TNF- α reporter gene expression induced by the particle bombardment of (physical stress) treatment in mouse skin. With further purification for PE-15 and PE-21, two similar phytochemicals (PE15A and PE21A) were isolated and they conferred inhibition of NF- κ B and TNF- α reporter gene expression *in vitro* and *in vivo*. Taken together, we suggest that PE fractions (PE-15 and PE-21) and two phytochemicals (PE15A and PE21A) have the potential to be further evaluated as candidate therapeutic agents for skin-inflammation and other inflammatory diseases.

The TCM Five-pattern Personality Characteristics of Depressed Patients

Xue-yu Lv, Wei-dong Wang

Guang'an men Hospital, China Academy of Chinese Medical Science, Beijing

Objective: to explore the TCM five-pattern personality characteristics of depressed patients and assess the scientific nature of Five-Pattern Personality Inventory in clinic practice. **Method:** The study was carried out at psychology department of Guang'an Men Hospital China Academy of Chinese Medical Science between September 2009 and March 2010. Inclusion criteria: (1) accordance with the depression diagnostic criteria of CCMD-3; (2) age between 18 and 65 years; (3) Cultural degree more than junior middle school ; (4) volunteer to join. Exclusion criteria:(1) not accordance with the depression diagnostic criteria of CCMD-3; (2) age < 18 or > 65 years; (3) Cultural degree less than junior middle school; (4) not willing to join. Rejection criterion: those whose data are incomplete for analysis. According to criterions above, 88 depressed patients are enrolled and. The TCM personality inventory Five-Pattern Personality Inventory and relevant depression scale data of patients were collected. The data are analyzed by SPSS 13.0. **Result:** The original score of Taiyang, Shaoyang and Yin-yang balanced dimensions of patients are significantly less than national norms ($P < 0.01$), the score of Taiyin dimension is significantly higher than national norms ($P < 0.01$); the score of Shaoyin dimensions is not different from national norms ($P > 0.05$); the score of Taiyin dimensions are positively correlated with score of MADRS, depression and anxiety factor of MMPI ($P < 0.05$ or 0.01). However, there is no significant correlation between scores of Taiyin dimension and SAS as well as SDS ($P > 0.05$); the analysis of standardized T score indicates the same result. **Conclusion:** the personality characteristics of TCM five-pattern of depressed patients are obvious: Taiyin dimension is stronger, and Taiyang, Shaoyang and Yin-yang balanced dimensions are weaker; and it proves that Five-Pattern Personality Inventory has certain value for clinic practice.

Risk Factors of Gastrointestinal and Hepatic Adverse Drug Reactions in the Treatment of Rheumatoid Arthritis

Miao Jiang¹, Aiping Lu^{1,2}

¹Institute of Basic Research In Clinical Medicine, China Academy of Traditional Chinese Medicine, Beijing
²E-institute of Shanghai Municipal Education Commission, Shanghai TCM University, Shanghai

Objective: To explore the risk factors on the gastrointestinal adverse drug reactions (GI ADRs) and hepatic ADRs in the treatment of rheumatoid arthritis (RA) with conventional Western Medicine (WM) and traditional Chinese Medicine (CM) therapy.

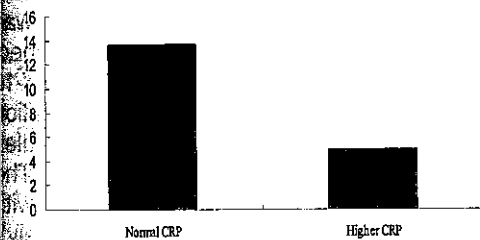
Methods: A multi-center, randomized-clinical trial was conducted on RA patients in China. After 12 and 24 weeks of treatment, the efficacy and safety of WM therapy and CM therapy were evaluated. The Chi-square and logistic regression were conducted to analyze the correlations between the biological parameters and the ADRs.

Results: 505 patients were recruited from 9 centers and randomly assigned into WM therapy group (n=251) or CM group (n=254). 397 of them completed the 24 week treatment (194 in WM and 203 in CM group). Total ADRs incidence and withdrawal rates were similar in two groups. For the patients treated with WM, Logistic regression analysis shows that CRP level is negatively related to GI ADRs ($p < 0.05$), and IgG level is positively related to hepatic ADRs ($p < 0.01$) in the patients treated with WM. In the patients treated with CM, no laboratory measurements were found related with GI ADRs and hepatic ADRs.

Conclusion: CRP in normal scale and higher IgG level were supposed to be the risk factors for GI and hepatic ADRs respectively in the RA patients treated with WM therapy.

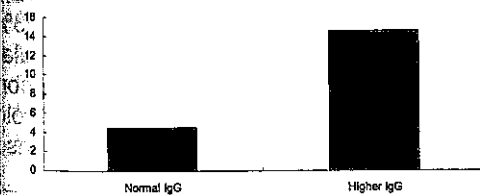
Keywords: Adverse Drug Reaction, Risk Factor, CRP, IgG, Rheumatoid Arthritis

Figure 1 Comparison on the GI ADRs in the patients with normal CRP level and higher CRP level.



GI ADRs	CRP level		Chi Test	
	Normal	Higher	Chi square value	P value
Without	112	117	4.5568	0.0328
With	16	6		

Figure 2 Comparison on the hepatic ADRs in the patients with normal IgG level and higher IgG level.



Hepatic ADRs	IgG level		Chi Test	
	<16.85	>16.85	Chi square value	P value
Without	128	100	7.5829	0.0059
With	6	17		

Impact of Doctor's Identification on TCM Syndrome (Pattern) Differentiation

Mengyu LIU, Aiping LU, Jin PENG, Xiaoqiu WU

Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing

Differential Treatment based on Traditional Chinese medicine (TCM) pattern or syndrome differentiation) is the basic principles of Traditional Chinese medicine in diagnosis and treatment of disease. The typical eight principal syndromes have recognized as the standards, but the diversified TCM syndrome identified by individual doctor makes the TCM syndrome differentiation more complicated and diversified. Different doctors might identify the different TCM syndromes for the same patient, even one doctor might identify different TCM pattern in same patient in different time. Physical survey found that the doctors' identification on the combined TCM patterns in a disease showed lower consistency than on a single TCM pattern. Similarly one doctor's identification on a single TCM pattern could be in good consistency in different period of the identification. Thus multiple concurrent symptoms which are the key parts for TCM pattern differentiation could easily lead to be different in recognition process.

Keywords: TCM Syndrome, compound syndrome types, single syndrome types

Extraarticular Symptoms could Influence ACR Response in the Treatment of Rheumatoid Arthritis with Biomedicine

Chi Zhang, Qinglin Zha, Yiting He³, Miao Jiang⁴, Cheng Lu⁵, Aiping Lu⁶

*Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing
University Journal editorial board, Jiangxi University of Traditional Chinese Medicine, Jiangxi
Guangdong Traditional Chinese Medicine Hospital, Guangdong*

Background: Extraarticular symptoms are important in the pattern differentiation of Traditional Chinese Medicine (TCM), and the study is aimed to find the associations between extraarticular symptoms and ACR Response in 194 cases of rheumatoid arthritis (RA) treated with biomedicine. **Methods:** The data set was obtained from a randomized clinical trial. 194 RA patients were treated with the biomedical therapy (diclofenec, methotrexate and sulfasalazine). ACR20 response at 24 weeks was used for the efficacy evaluation. 18 symptoms (including 13 extraarticular symptoms) that TCM practitioners focus on were collected for exploration on the association between the symptoms and the efficacy of the biomedical therapy with association rules method. **Results:** After treatment for 24 weeks, ACR20 response with the biomedicine was 69.59%. The association rules analysis on each symptom showed that the soreness in waist was more associated with ACR20 response, while with lower support (selected sample size based, 20.10% and 14.95% respectively); cold intolerance and cold joint were found to be associated with ACR20 response with higher support (48.97% and 53.61% respectively), and the confidences (predicted effective rate) were 73.08% and 71.23% respectively. The associations on combination symptoms (among them, there was at least one extraarticular symptom) and ACR20 response indicated that cold intolerance or cold joint with higher confidence and support were most important extraarticular symptoms. **Conclusions:** The RA patients with "cold intolerance" and "cold joints", which are extraarticular symptoms that TCM practitioners focus on, could show higher ACR20 response when treated with the biomedical approach.

The Effect of Tangshen Formula on Patients with Type 2 diabetic Kidney Disease: a Multicentric Randomized Placebo-Controlled Trial

Liping Yang, Ping Li, Yiping Chen, Jing Hong, Xiuping Jin, Jing Li Xiaoguang Lu, Xin Yang

Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing

Objective: To evaluate the efficacy and safety of Chinese herbal medicine Tangshen formula (TSF) in treatment of type 2 diabetic kidney disease (DKD). **Methods:** Stratified blocked randomization method was adopted to allocate 192 seeds into the treatment arm and placebo arm, patients number ratio 2:1, with allocation concealment conducted in the drug dispensing procedure. 181 patients with type 2 DKD were enrolled in the trial. After a 2-week of run-in period, during which patients' blood pressure was controlled under 140/90mmHg and their fasting blood glucose under 7.8mmol/L, then patients were randomized to receive 6 months of treatment with TSF (n=122) (16g daily) or placebo (n=59), in addition to basic western medicine treatment. The primary outcomes were urinary albumin excretion rate (UAER) for microalbuminuric patients and 24-hour urinary protein (24h-Upro) for macroalbuminuric patients, measured every 3 months. **Results:** The baseline characteristics of the two groups were comparable. In the microalbuminuric cohort, repeated measures data analysis revealed that there was significant reduction of UAER in TSF group (compared with placebo group, $P=0.0323$). In the macroalbuminuric cohort, 24h-Upro was significantly decreased in treatment group (compared with placebo group, $P=0.0153$). There was a reduction of serum creatinine level ($P=0.0192$), and elevation of eGFR ($P=0.0216$) in the treatment group compared with placebo. There was significant elevation of HDL in patients with macroalbuminuria ($P=0.0107$) in the treatment group compared with placebo. No adverse effect was found in the trial. **Conclusion:** TSF may have beneficial effects in patients with DKD who are receiving recommended therapy, and there is synergistic effect of TSF with western medication. However, longer periods of follow-up with endpoint outcomes evaluation is still needed.

Excretion of Urinary Glycosaminoglycans in Type 2 Diabetic Nephropathy

Tingting Zhao, Xiaoguang Lu, Ping Li, Jingzhen Guo, Zhiguo Li

Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing

Glycosaminoglycans (GAGs) are a group of complex anionic heteropolysaccharide that in plasma membranes and extra-cellular matrix, with the exception of hyaluronic acid, are linked to core protein-forming proteoglycans (PG). GAGs play a pivotal role in the filtration and selective permeability of the glomerular basement membrane and alterations in GAGs metabolism have been found to play a role in diabetic vascular complications. In order to investigate the metabolism of GAGs during the process of diabetic kidney disease, the concentration GAGs in the first void of morning urinary were analyzed in four groups of type 2 diabetic patients (n = 146, 40 to 75 years old): without nephropathy; with microalbuminuria; with macroalbuminuria; ending-stage of nephropathy. An increased urinary GCR (ratio of GAG to urinary creatinine) in diabetic patients was detected in subjects with nephropathy progression (3.75 ± 3.87 , 4.68 ± 3.81 , 9.24 ± 5.93 mg/ μ mol, respectively). Urinary GCR in female patients with microalbuminuria and macroalbuminuria (5.76 ± 4.84 , 7.00 ± 3.15 mg/ μ mol) are significantly higher than that of the same stage in males (2.29 ± 1.29 , 3.95 ± 4.02 mg/ μ mol). However, the difference of GCR between control group (1.24 ± 0.74 mg/ μ mol) and normal albuminuric group of diabetic patients (1.90 ± 1.01 mg/ μ mol) was not statistically significant. Significant correlation between GCR and serum creatinine, GCR and urea nitrogen, GCR and GFR were found. In particular, there was significant correlation between GCR and HbA1c for male patients. It was concluded that the excretion of urinary GAG may be useful for determining the early stage of diabetic nephropathy and the progression of the disease.

Acknowledgment: this work was supported by National key project of basic research P.R. China (No. 2005CB523503), and project of National natural fund P.R. China (No. 30801539).

Clinical Study on BuShenQiangDuZhiLiu Decoction in the Treatment of Ankylosing Spndylitis

Yiting He

Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangdong

Using BuShenQiangDuZhiLiu decoction to cure 30 AS patients that is renal deficiency and cold syndrome, after 24weeks of treatment in contrast pretreatment, the improvement rate of ASAS20, ASAS50 and ASAS70 is 10.00%, 66.67% and 10.00% respectively. There was significant difference for Clinical symptoms and syndromes such as back pain, duration of back early morning stiffness (EMS), Bath Ankylosing Spondylitis Disease Activity Index(BADAI),global assessment of patients and doctors , the Schober's test, occiput to wall and finger to floor distance. There was also significant difference for index of inflammation including ESR and CPR, and for index of bone metabolism such as OPG, RANKL. The results presented that BuShenQiangDuZhiLiu decoction has some effects on improving the clinical symptoms and syndromes and preventing destruction of bone.

Review and Comparison of Commonly Used Casual Relationship Standard of Adverse Drug Reactions in TCM Clinical Trials

Xinfeng Guo, Jianting Li, Quan Zhu

DME Center and Preclinical Medicine School, Guangzhou University of Chinese Medicine, Guangdong

Background: Accurate report of the adverse drug reaction and scientific analysis of the casual relationship are basic skills of a qualified physician, but some adverse drug reactions are various and atypical that cannot be readily distinguished from the disease per se or other complications, the determination of causal relationship is really difficult.

Objectives: To review and compare some commonly used criteria in causal relationship judgment in TCM clinical trials.

Results: The most commonly used is the Naranjo criteria. The drug adverse reaction monitoring center of SFDA in China has established relevant criteria, which can be used in specific context. The Naranjo criterion comprises of five items in the SFDA criteria, and adopts weighted scoring method. Its scientific property has gained accredit from most investigators. However, it is merely used for adverse reactions of pharmacological related type, and items including concentration monitoring, dose relationship and placebo effect are not applicable in trials of TCM preparations, and unpharmacological related type of adverse reactions. The five items of SFDA are applicable to adverse reactions of both ADR types and TCM compound. However, cases that cannot be classified may occasionally occur because of the scoring method it uses.

Conclusions: Studies should be carried out to determine how to modify the SFDA criteria in casual relationship judgment in TCM clinical trials.

Efficacy of a Chinese Herbal Proprietary Medicine (Hemp Seed Pill) for Functional Constipation. A randomized, Double-blind, Placebo Controlled Clinical Trial

Chung-Wah Cheng, Zhao-xiang Bian, Li-xing Zhu, Justin Wu, Joseph JY Sung

School of Chinese Medicine, Department of Mathematics, Hong Kong Baptist University, Hong Kong
Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

Objectives: Functional constipation (FC) is a common clinical complaint. Despite lacking consolidated evidence, Chinese herbal medicine (CHM) becomes one of popular alternative treatments. The aim of this study was to assess the efficacy and safety of a CHM proprietary, Hemp Seed Pill (HSP), in an optimal dose with a rigid study design. **METHODS:** This was a prospective, randomized, double-blind, placebo controlled study for FC patients (Rome III criteria) in Excessive Syndromes defined by traditional Chinese medicine (TCM) theory. The whole study last for 18 weeks, including two weeks run-in, eight weeks treatment and eight weeks follow-up period. The optimal dose selected was first determined from three dosages (2.5g bid, 5.0g bid and 7.5g bid), for which 5.0g bid was a recommended dosage, in a separated study. The primary end point was the responder rate for complete spontaneous bowel movement (CSBM) during treatment. Participants with a mean increase of CSBM ≥ 1 /week compared with their baseline were defined as responders. Secondary outcome measures included responder rate during follow-up, individual and global symptom assessment, and reported adverse effects. **RESULTS:** The dose in 7.5g bid, which showed better therapeutic effect than that of 2.5g bid and 5.0g bid among 96 subjects (32 per arm), was selected to compare with placebo. 120 subjects were randomized (60 per arms). Responder rates for the HSP group and placebo group were 43.3% and 8.3% during treatment, and 30.0% and 15.0% in the follow-up period, respectively ($p < 0.05$). HSP benefited on the increase of CSBM, relieving the severity of constipation and straining of evacuation, and effectively reduced the use of rescue therapy when comparing with placebo during treatment. No serious adverse effect was reported. **CONCLUSIONS:** HSP (7.5g bid) is safe and effective for alleviating FC for subjects in Excessive Syndromes by comparing with placebo. Specific dose determination study may be crucial for all CHM studies.

This study was supported by Health and Health research Fund of HKSAR.

Systematic Review on the Efficacy and Safety of Herbal Medicines for Vascular Dementia

Sui Cheung Man, Siva Sundara Kumar Durairajan, Jia Hong Lu, Liang Feng Liu, Li Xia Xie, Wan Fung Kum, Yan Wang and Min Li*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Background: Vascular dementia (VaD) is the second most common form of dementia, and the most frequent cause of dementia in the elderly. It is characterized by executive dysfunction and behavioral psychological symptoms such as apathy, anxiety, depression, suicidal thoughts, etc. Current orthodox medication (OM) treatment is limited, and some patients resort to herbal medications (HM). We present a systematic review of existing research that aims to assess the efficacy and safety of herbal medications (HM), as either monotherapy or adjunct to orthodox medications (mainly comprised of cholinesterase inhibitors, OM) for vascular dementia (VaD).

Methods: We identified trials from the electronic databases and hand search of a list of Chinese and English journals. All published studies reporting randomized, controlled clinical trials on VaD, comparing HM as monotherapy or adjuvant therapy, with placebo or OM as controls, were included. There was no restriction on the ethnicity, gender, age, or disease duration of the participants in the trials. Two independent reviewers assessed the trials for their eligibility. Risk of bias assessment of the trials was performed according to the revised CONSORT statement.

Results: Forty-seven studies testing different HM are included. Out of the 43 HM monotherapy studies, 37 reported HM to be significantly better than OM or placebo; six reported similar efficacy between HM and OM. All four HM adjuvant studies reported significant efficacy. No major adverse events for HM were reported. Chinese ethnicity was dominant among the participants; gender distribution was unclear. Heterogeneity in diagnostic criteria, interventions and outcome measures hindered comprehensive data analysis. Thirty most commonly used herbs were identified for future studies.

Conclusion: Studies comparing HM with OM suggest that HM can be a safe and effective treatment for VaD, either alone or in conjunction with OM. However, methodological flaws in the design of the studies limited the extent to which the results could be interpreted. Further investigation on the thirty most commonly used herbs may lead to a discovery of new and effective treatment for VaD. Further multi-center trials with large sample size, high methodological qualities and standardized HM ingredients are necessary for clinical recommendations on the use of HM in treating VaD.

Developmental Toxicity of Chinese Herbs: A Review of Experimental Studies

Ping Xiang Deng¹, Yan Ting Chen¹, Xiao Ying Tian¹, King Fai Cheng², Zhong Zhen Zhao¹, Min Xu^{1,*}

¹ *School of Chinese Medicine, Hong Kong Baptist University, Hong Kong*

² *Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong*

Natural herbs and herbal products are used by pregnant women worldwide but there is insufficient evidence to document their safety in embryo development. Although herbal therapy has been recognized as a unique way to treat pregnant disorders since ancient time, more and more herbs or their compounds have been found to cause adverse embryonic and maternal effects by *in vivo* and *in vitro* experimental studies. In view of that it is difficult to identify developmental toxicity of herbal medicines directly by human trials; experimental data from *in vivo* and *in vitro* models would provide an invaluable reference for herbal users. This review aimed to summarize currently available experimental studies on developmental toxicity related to Chinese herbs used commonly in clinics. It is necessary to remind clinicians and patients to carefully use unsafe herbs and herbal preparations or compounds. Authors emphasize that to systematically identify development toxicity of herbal medicines following internationally acceptable guidelines should be a priority in further researches.

Keywords: Developmental toxicity, herbal medicine, herbal product, review

Herbal TMGT Decoction in the Treatment of Hypertension: A Meta-analysis

Wai Keung Darwin Doo¹, Vivian Wong², Teresa Ngan², Xin Lin Chen³, Xiao Ying Tian¹, Dong Ying Xu^{1,4}, Xiao Ping Xu¹, Wei Xu¹, Min Xu^{1,*}

¹ Hong Kong Baptist University, Hong Kong

² The Hospital Authority of Hong Kong, Hong Kong

³ Guangzhou University of Chinese Medicine, Guangdong

⁴ Guangxi University of Chinese Medicine, Nanning

Introduction: Herbal therapy has been widely applied to treat hypertension clinically. The aims of this study were to count traditional Chinese herbal formulae (TF) used in hypertension treatments, and assess the efficacy and safety of most chosen TF in hypertension treatments.

Method: Electronic databases and hand-search materials were used for screening randomized controlled trials (RCTs). Jadad's scale was used to assess the quality of eligible trials. Clinical data were estimated by odd ratio or weight mean difference with 95% confidence interval in meta-analyses.

Result: (1) Total 127 RCTs with oral TF treatments were identified, and Tian-Ma-Gou-Teng (TMGT) decoction was the most chosen TF in them. (2) For the treatment with TMGT decoction, 29 RCTs (involved 2419 patients) met the inclusive criteria and only 1 trial (3.45%) was assessed as high-quality trial. (3) Compared with Western medications (WM), TMGT decoction was more effective to increase the number of improved patients (6 trials, $P=0.003$), degrade systolic blood pressure (13 trials, $P=0.16$) and diastolic blood pressure (13 trials, $P=0.81$), amend total cholesterol (3 trials, $P<0.00001$), triglyceride (3 trials, $P<0.00001$), endothelin (4 trials, $P=0.72$). (4) TMGT decoction plus WM was more effective than WM alone to increase the number of improved patients (13 trials, $P<0.00001$), degrade systolic blood pressure (13 trials, $P=0.10$) and diastolic blood pressure (13 trials, $P<0.00001$), amend total cholesterol (3 trials, $P<0.00001$) and triglyceride (3 trials, $P<0.00001$). (8) Adverse events occurred during the treatment of TMGT decoction were less than those caused by WM.

Conclusion: The results of this meta-analysis suggest that TMGT decoction should be an effective and safe herbal remedy in the treatment of hypertension, although further clinical trials with high-quality study design should be performed to verify the evidence in this study.

Keywords: TMGT decoction, herbal medicine, hypertension, meta-analysis

Herbal XFZY Decoction in the Treatment of Coronary Artery Disease: A Meta-analysis

Pang Nin To¹, Vivian Wong², Teresa Ngan², Xin Lin Chen³, Xiao Ying Tian¹, Dong Ying Xu^{1,4}, Wei Xu¹, Min Xu^{1,*}

¹ Hong Kong Baptist University, Hong Kong

² The Hospital Authority of Hong Kong, Hong Kong

³ Guangzhou University of Chinese Medicine, Guangdong

³ Guangxi University of Chinese Medicine, Nanning

Introduction: Herbal therapy has been widely applied to treat coronary artery disease (CAD) clinically. The aim of this study was to assess the efficacy and safety of a herbal formula Xue-Fu-Zhu-Yu (XFZY) decoction in the treatment of CAD.

Method: Randomized controlled trials (RCTs) to examine the efficacy and/or safety of XFZY decoction in CAD treatments were valid. Jadad's scale was used to assess the quality of eligible trials. Data were estimated by odd ratio or weight mean difference with 95% confidence interval in meta-analyses.

Result: (1) 42 RCTs met the inclusive criteria and totally involved 3772 patients, and 7 RCTs in them were assessed as high-quality trials (16.67%). (2) Compared with Western medications (WM), XFZY decoction was more effective to improve angina pectoris (13 trials, $P < 0.00001$), amend the electrocardiogram (13 trials, $P < 0.00001$), improve nitroglycerin discontinuation or decrease rate (1 trial, $P = 0.01$), reduce total cholesterol (4 trials, $P = 0.09$) and triglyceride (4 trials, $P < 0.00001$). (3) XFZY decoction plus WM was more effective than WM alone to improve angina pectoris (27 trials, $P < 0.00001$), amend the electrocardiogram (18 trials, $P < 0.00001$), reduce the frequency of angina pectoris attacks (3 trials, $P < 0.00001$) and duration of angina pectoris (3 trials, $P = 0.0009$), improve nitroglycerin discontinuation or decrease rate (4 trials, $P < 0.00001$), total cholesterol (2 trials, $P = 0.08$) and triglyceride (2 trials, $P < 0.0001$). (4) There was no significant adverse events occurred during the treatment of XFZY decoction.

Conclusion: XFZY decoction should be an effective and safe herbal remedy in the treatment of CAD. However, further clinical trials with high-quality study design should be performed to verify the evidence in this study.

Keywords: XFZY decoction, herbal medicine, coronary artery disease, meta-analysis

The Safety Assessment of Radix Dipsaci in Embryonic Development of Mice

Kak Lam¹, Xiao Ying Tian¹, Yan Ting Chen¹, Hiu Lai Kong¹, King Fai Cheng², Ping Xiang Deng¹, Nan Ding¹, Meng Yun Xing¹, Xiao Ping Xu¹, Min Xu^{1,*}

¹ School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

² Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong

Introduction: Radix Dipsaci (RD) has been clinically applied to treat pregnant disorders for thousands of years. Our previous survey indicated that RD is the first choice herb in current clinical trials for preventing and treating threatened and habitual miscarriages. The aim of this study was to evaluate the safety of RD water extract on embryonic development in mice.

Method: The RD water extract was prepared as its usual type in clinics, and quantitatively authenticated by HPLC analyses. A segment II study was carried out according to international guidelines. Pregnant mice were randomly assigned into four groups (n=17 per group) to receive 0 (equal volume distilled water), 2, 8, 32 g/kg/day of RD extract at the gestation day (Gd) 6 to 15 respectively. Other 17 pregnant mice were treated with Vitamin A of 200,000 IU/kg at Gd 7, 9 and 11 as positive controls. Fetal and maternal evaluations were conducted on Gd 18, and skeletal examinations were done after the Alizarin red-S staining.

Result: (1) Tested RD samples contained Asperosaponin VI (>3.0%) and fulfilled the quality standard of Chinese Pharmacopoeia (2005). (2) There was no significant difference in fetal parameters including numbers of dead and resorpted fetuses; percentages of post-implantation loss, external and skeletal malformations, etc. among four-dose groups (0-32 g); although the rate of resorpted and malformed fetuses was significantly higher in positive controlled mice. (3) In maternal parameters the relative body weight gain of high-dose group (32 g) was significantly lower than those in other dose groups (0-8 g, $P < 0.01$), while its kidney index was significantly higher than that in negative control group (0 g, $P < 0.01$).

Conclusion: The daily treatment of RD water extract with a dosage at or below 32 g/kg (17 times to the daily dosage of adult human) would not cause significant fetal developmental toxicity. But RD water extract at high dosage might induce potential maternal toxicity.

Keywords: Radix Dipsaci, embryonic development, embryotoxicity, mouse

Recent Situation of Manipulation of Osteoarthritis of Knee

En Zhu

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Osteoarthritis of knee is also known degenerative osteoarthritis, which is a common disease of elderly. As China entered the aging society, incidence of this disease rise gradually. Therefore prevention and control of this disease more effectively becomes more and more valued. This paper has sum up and classified different manipulation approaches of this disease. In order to explain to the readers the recent situation of the clinical treatment, different approaches will be discussed one by one.

Conclusion: Focus of current clinical approach is mainly to relieve symptoms, improve knee joint repairmen, and to slow down disease progression. But highly effective approach is absent. The mechanism of manipulation is as follows: 1. Manipulation promotes localised circulation. 2. Manipulation promotes microcirculation. 3. Manipulation promotes venous return, reduce patellar intraosseous pressure, promote intra-articular tissue repair. 4. Manipulation promotes mechanical balance of knee. 5. Manipulation enhances joint movement.

Using Chinese Version of MYMOP in Chinese Medicine Evaluation: Validity, Responsiveness and Minimally Important Change

Vincent CH Chung^a, Vivian Wong^b, Lau Chun Hong^a, Henny Hu^b, Lam Tat Hing^c, Lin Xiao Zhong^c, Samuel Y Wong^a, Sian M Griffiths^a

^a School of Public Health and Primary Care, Chinese University of Hong Kong, Hong Kong

^b Chinese Medicine Department, Hospital Authority Head Office, Hong Kong

^c Yan Chai Hospital cum The Chinese University of Hong Kong Chinese Medicine Training and Research Centre, Hong Kong

Background: Measure Yourself Medical Outcome Profile (MYMOP) is a patient generated outcome instrument applicable in the evaluation both allopathic and complementary medicine treatment. This study aims to adapt MYMOP into Chinese, and to assess its validity, responsiveness and minimally important change values in a sample of patients using Chinese medicine (CM) services.

Methods: A Chinese version of MYMOP (CMYMOP) is developed by forward-backward-forward translational strategy, expert panel assessment and pilot testing amongst patients. 272 patients aged 18 or above with subjective symptoms in the past 2 weeks were recruited at a CM clinic, and were invited to complete a set of questionnaire containing CMYMOP and SF-36. Follow up were performed at 2nd and 4th week after consultation, using the same set of questionnaire plus a global rating of change question. Criterion validity of CMYMOP was assessed its correlation with SF-36 at baseline, and responsiveness was evaluated by calculating the Cohen effect size (ES) of change at two follow ups. Minimally important difference (MID) value were estimated via anchor based method, while minimally detectable difference (MDC) figures were calculated by distribution based method.

Results: Criterion validity of CMYMOP was demonstrated by negative correlation between CMYMOP Profile scores and all SF-36 domain and summary scores at baseline. For responsiveness between baseline and 4th week follow up, ES of CMYMOP Symptom 1, Activity and Profile reached the moderate change threshold (ES>0.5), while Symptom 2 and Wellbeing reached the weak change threshold (ES>0.2). None of the SF-36 scores reached the moderate change threshold, implying CMYMOP's stronger responsiveness in CM setting. At 2nd week follow up, MID values for Symptom 1, Symptom 2, Wellbeing and Profile items were 0.894, 0.580, 0.263 and 0.516 respectively. For Activity item, MDC figure of 0.808 was adopted to estimate MID.

Conclusions: The findings support the validity and responsiveness of CMYMOP for capturing patient centred clinical changes within 2 weeks in a CM clinical setting. Further researches are warranted (1) to estimate Activity item MID, (2) to assess the test-retest reliability of CMYMOP, and (3) to perform further MID evaluation using multiple, item specific anchor questions.

Evaluation of the Pharmacological Activities of Traditional Chinese Medicine in the Amelioration of Symptoms of Allergic Asthma

Yue GGL¹, Chan BCL¹, To MH¹, Kwok HF¹, Hon EKL², Fung KP^{1,3}, Leung PC¹ and Lau CBS¹

¹Institute of Chinese Medicine; ²Department of Pediatrics; ³School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

The use of health supplements containing traditional Chinese medicine (TCM) for the prevention and the relief of symptoms of respiratory tract disorders has increased in recent years. Two TCM formulae have been shown to improve acute cough symptoms and possess *in vitro* anti-allergic activities in our previous studies. In the present study, twelve individual herbs from these formulae were selected for *in vitro* pharmacological evaluation, including anti-tussive, anti-inflammatory and immunomodulatory activities. The effects of the TCM extracts on agonist-induced concentration-responses were studied using rat tracheal rings *ex vivo*. The anti-inflammatory and immunomodulatory activities of the TCM extracts were evaluated using mouse macrophage cell line RAW264.1 and human peripheral blood mononuclear cells (PBMC), respectively.

Among the extracts tested, those of Rhizoma Cynanchi Stauntonii and Radix Glycyrrhizae showed potent attenuation on the acetylcholine- and carbachol-induced contractions in rat tracheal tissues (n=6-8). For the *in vitro* anti-inflammation assay, three extracts (Radix Glycyrrhizae and two different extracts of Herba Schizonepetae) showed significant inhibitory effects on LPS-induced nitric oxide production but no cytotoxic effects on the RAW264.1 cells (n=4). The effects of the TCM extracts on human PBMC proliferation and cytokine production were evaluated using [methyl-³H]-thymidine incorporation assay and ELISA, respectively. The extracts of Rhizoma Cynanchi Stauntonii, Radix Astragali, Pericarpium Citri Reticulatae, Radix Scutellariae and Radix Glycyrrhizae all showed significant inhibitory effects on phytohaemagglutinin-induced proliferation without cytotoxicity in PBMC (n=8-12). These extracts also showed inhibitory effects on TNF- α , IFN- γ and IL-2 production in a concentration-dependent manner.

The results obtained from this study provided scientific evidence towards the use of these herbs in respiratory tract disorders and also gave us a better understanding of the mechanism of action in ameliorating the symptoms.

Acknowledgement: This project is financially supported by Food and Health Bureau, Health and Health Services Research Fund no. 06070401.

Development of Biomarker for the Assessment of Pyrrolizidine Alkaloid-Containing Herbal Medicine Induced Hepatotoxicity

Na Li,¹ Fan Zhang,² Jiang Zheng,² Ge Lin^{1*}

¹School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong;
²Center for Developmental Therapeutics, Seattle Children's Research Institute, Division of Gastroenterology, Department of Pediatrics, University of Washington, USA

The safety of herbal medicines has attracted more public attentions in recent decades with the growing awareness of incidences in natural product poisonings. Among many natural toxins, pyrrolizidine alkaloids (PA) and PA-containing herbal medicines are one of the most significant groups, because PAs are diversely distributed across the plant kingdom with about 660 found in over 6,000 plants worldwide. Furthermore, most of the naturally occurring PAs are hepatotoxic and can induce hepatic sinusoidal obstruction syndrome (HSOS) and/or liver cancer. Yearly, numerous incidents of PA poisoning make a significant impact on public health. However, to date no suitable and specific methods are available for the diagnosis and assessment of PA intoxication.

Regardless of different structures of hepatotoxic PAs, they all induced hepatotoxicity via the hepatic metabolic activation to generate reactive metabolites (pyrroles) followed by the formation of pyrrole-macromolecular (such as proteins) adducts in the liver. Therefore, we hypothesize that pyrrole-protein adduction may be a potential biomarker of PA-induced toxicity. The present study reports the development of an immunoassay to detect pyrrole-protein adduction in the liver samples obtained from rats treated with PAs or PA-containing herbs. An antibody against pyrrole moiety in pyrrole-protein adducts was firstly raised, which specifically recognized pyrrole moiety in BSA-pyrrole but not BSA. Using the antibody, significant differences in the immune responses, which recognized pyrrole-protein adduction, were observed between the control and PA-treated rat liver samples by Western blot and ELISA assays. Moreover, dose-related immune response was demonstrated in the treatments with different PA dosages. The results also demonstrated that the formation of pyrrole-protein adducts correlated with hepatotoxicity in rats exposed to PAs. Our findings suggested that pyrrole-protein adduction has a potential to be developed as a mechanism-based biomarker for the assessment of PA-induced hepatotoxicity. This research was supported by Hong Kong Jockey Club Charities Trust Fund (JCICM-15-07).

A Double-Blind, Randomized, Placebo-Controlled Trial to Evaluate the Effect of Multi-herb Mixture, NM_KIDS_042 on the Height Growth in Elementary School Children

Mi-Yeon Kim, Jin-Hee Park, Donghun Lee, Sung Hyun Lee, Yoon Jung Kim, Jaemyoung Shim, Yun-Young Kim, Gyutae Chang, Jin-Yong Lee, Ryowon Choue, Hocheol Kim

College of Oriental Medicine, Graduate School of East-West Medical Science and Research Institute of Clinical Nutrition, Kyung Hee University, Korea
NeuMed Inc, Korea

Objectives: Although growing interest of increasing height in children, few studies have evaluated efficacy of herb extracts on height growth. Our objective was to evaluate efficacy of the multi-herb mixture (NM_KIDS_042) on height growth during supplementation period for 12 weeks in elementary school children.

Methods: We conducted a double-blinded, randomized, placebo-controlled study and screened during February to July 2008 in children who aged 7-12 years and height below 25th percentile by age and gender of 2007 Korean national growth chart. We measured the change of anthropometrics, serum GH, IGF-1 and IGFBP-3 levels, and nutrient intakes, also compared the variables between placebo and NM_KIDS_042 group.

Results: One hundred-three participants were screened from the elementary school children in Korea. Of these, 99 subjects supplemented and were randomly assigned placebo and NM_KIDS_042, with 9 subjects withdrew. Height growth had a significant increase in the NM_KIDS_042 group of 2.2 ± 0.7 cm than in the placebo group of 1.9 ± 0.7 cm ($P = .047$) during 12 weeks, and mean height percentile in the NM_KIDS_042 group of 19.2 ± 7.7 and 21.4 ± 9.1 ($P < .001$) significantly increased from baseline to end of supplementation. Fat free mass in the both group was increased ($P < .001$) and fat mass in the NM_KIDS_042 group was decreased ($P = .023$). A significant difference of IGFBP-3 level, but not IGF-1 level, between placebo and NM_KIDS_042 group was noted at end of supplementation, 3064.7 ± 723.6 ng/ml and 3386.7 ± 737.0 ng/ml ($P = .039$), respectively.

Conclusions: NM_KIDS_042 is increasing height and have a positive effect on body composition in elementary school children regardless of short period for 12 weeks, and more effective regimen in improving height growth.

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Studies on the Curative Effects of Wu Xing Jian Gu Exercise to Patients with Osteoporosis

Xiao Shi, YuChao Liu, FuLong Li

Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai

Wu Xing Jian Gu exercise was based on Chinese traditional sports and modern rehabilitation, and integrated the characteristics of osteoporosis, which was divided into eight parts and lasted 25 minutes. By stretching upper limbs, lifting the foot, stretching the chest and other actions to strengthen and improve the muscle, the sense of balance, the flexibility and stability in spines, meanwhile to reduce the incidence of fall and fracture as well as to enhance the quality of life.

We selected 94 patients with primary osteoporosis to observe the curative effects of Wu Xing Jian Gu exercise, which was lasted 90 days. They were randomly divided into basic treatment group, which was given vitamin D plus calcium, and combined treatment group, which was given vitamin D plus calcium and the rehabilitation exercises of Wu Xing Jian Gu exercise. After the treatment, the 25-hydroxy vitamin D was increased and the bone alkaline phosphatase was decreased in the two groups ($P < 0.05$), the latter one was better than the former one in increasing 25-hydroxy vitamin D ($P < 0.05$). Both of them could affect the bone metabolism, the combined treatment was better than the basic treatment. The scores of the scale of TCM for primary osteoporosis patients, Osteoporosis Quality of Life Scale and the fall scale were all decreased in the two groups ($P < 0.05$), and the latter one was better than the former one, especially in the emotional and the physiological functions, while appetite in physical function in OQOLS. Both of them could change quality of life and the sense of balance, reduce the risk of falling, the combined treatment was better than the basic treatment. Wu Xing Jian Gu exercise has curative effects to patients with osteoporosis.

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Mi-Yeon Kim, Jin-Hee Park, Donghun Lee, Sung Hyun Lee, Yoon Jung Kim, Jaemyoung Shim, Yun-Young Kim, Gyutae Chang, Jin-Yong Lee, Ryowon Choue, Hocheol Kim

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Some Methodological Issues in Clinical Trials for the Treatment of Syndromes in Traditional Chinese Medicine

Hai Hong

Institute of Advanced Studies, Nanyang Technological University, Singapore

In traditional Chinese medicine (TCM), the diagnosis and treatment of illnesses centres around the identification and treatment of syndromes (*zheng* 证) as distinct from diseases (*bing* 病) and from symptoms (*zhenghou* 症候). Although well defined within classical TCM theory, syndromes are difficult to specify in quantitative observational terms for clinical trial purposes. Other methodological difficulties relate to the application of randomized controlled trials (RCTs) to the treatment of syndromes, such as a wide variety of diseases exhibiting the same syndrome and the tendency for syndromes to evolve and transform within the period of clinical trials. This paper will take a preliminary look at some of these methodological difficulties.

Comparison of Chinese Herbal Formula and Laxatives for Residents with Chronic Constipation in Long-term Care: A Randomized, Double-blind, Placebo-controlled Trial

Chien-Hsun Huang, Jui-Shan Lin, and Yi-Chang Su

School of Chinese Medicine, China Medical University, Taichung

Department of Community and Family Medicine, National Taiwan University Hospital Yun-Lin Branch, Taichung

Background: Constipation is a prevalent condition in institutionalized adults with a number of co-morbidities, but many of these patients as well as their healthcare providers are dissatisfied with current laxative therapy. This study evaluated the therapeutic efficacy and safety of an ancient herbal formula of traditional Chinese medicine (TCM) for the residents with constipation in long-term care.

Methods: In this randomized, double-blind, placebo-controlled trial, we randomized 90 residents from three long-term care units to receive 8 weeks of treatment with TCM or placebo and then followed up the subjects for an additional 4 weeks.

Results: The mean numbers of weakly spontaneous bowel movement in the TCM group were greater than in the placebo group during the treatment phase of 8 weeks ($P < .05$); the greatest difference was during weeks 1-4 (6.2 ± 2.2 vs 3.4 ± 2.1 , $P < .001$). Smaller mean numbers of weekly rectal treatments were observed with TCM compared with placebo during weeks 1-8 ($P < .05$). The mean numbers of weekly rescue laxative tablets of magnesium oxide were significantly less in the TCM group than in the placebo group during entire 12-week period ($P < .01$), with the greatest difference during weeks 5-8 (14.4 ± 16.3 vs 33.4 ± 23.5 , $P < .001$). No significant safety concerns were noted.

Conclusions: The six-herb TCM formulation is safe and effective, which may provide a complementary therapy on constipation in long-term care. Further clinical trials may be necessary for comparisons of efficacy or cost-effectiveness with current laxatives.

An Twelve-week, Randomized, Double-blind Study to Evaluate the Efficacy and Tolerability of Danshen (Salvia Miltiorrhiza) as Add-on Therapy in Taiwanese Patients with Essential Hypertension

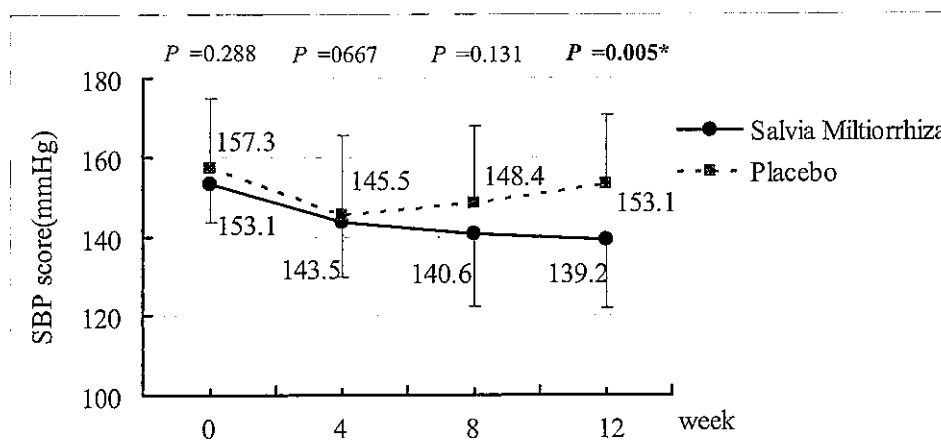
James Cheng-Chung Wei; Tsung-Yuan Young; Balance C.M. Chen; Ming-Yung Lee; Kwo-Chang Ueng
 Chinese Medicine Clinical Trial Center, Chung Shan Medical University Hospital, Taichung

Aim: To evaluate the efficacy and tolerability of Danshen extract formula in patients with uncontrolled hypertension.

Methods: This was a randomized, double-blind, 12 weeks, active-controlled, parallel-group study that was conducted in Chung Shan Medical University Hospital. Taiwanese men and women between 18 and 80 years of age with either with sitting diastolic BP ≥ 90 or systolic BP ≥ 140 despite current anti-hypertensive treatment for at least 8 weeks. Patients with any of the following were excluded: severe hypertension (ie, a DBP > 110 mm Hg or SBP > 180 mmHg), secondary hypertension; clinically significant hepatic or renal dysfunction; coagulopathy. All subjects were allocated randomly in 1:1 ratio to receive fixed-dose Danshen extract mixture 500 mg or placebo bid for 12 weeks. Any other drugs that might affect BP were prohibited during the trial period. Primary endpoint was control rate of BP defined as the proportion of subjects with a final sitting SBP < 140 mm Hg and DBP < 90 mm Hg at week 12. Secondary endpoints consisted of response rate defined by either reduction of SBP > 10 or DBP > 5 mmHg, mean changes of SBP, DBP, and heart rate.

Results: By ITT analysis, significant reduction of SBP at week 12 was noted in Danshen group compared to placebo. (13.8 vs 4.2 mmHg, $p = 0.005$). Control rate was 46.7% in Danshen group and 16% in placebo group. ($p = 0.016$). Decrease of pulse rate was also noted in Danshen group (-3.2 vs +2.7 /min, $p = 0.028$). Plasma fibrinogen level decreased significantly in Danshen group, compared to placebo. (-11.1 vs +3.8 mg/dl, $p = 0.025$). Adverse events were not statistically different between two groups.

Conclusion: Danshen (Salvia Miltiorrhiza) extract reduced systolic blood pressure, pulse rate and plasma fibrinogen level and was well tolerated.



Effect of Green Tea Extract on Obese Women: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

Chung-Hua Hsu

*Institute of Traditional Medicine, National Yang-Ming University, Taipei
Branch of Chinese Medicine, Taipei City Hospital, Taipei*

Background: To examine the effect of green tea extract (GTE) on obese women and to explore the relationship between GTE and obesity-related hormone peptides.

Methods: A randomized, double-blind, placebo-controlled clinical trial was conducted from July 2006 through June 2007 in Taipei Hospital, Taiwan. Seventy-eight of 100 obese women aged between 16 and 60 years, BMI > 27 kg/m² and who had not received any other weight control maneuvers within the last 3 months completed this study. The subjects were randomly divided into Groups A and B. Group A (n = 41) received GTE while Group B (n = 37) took cellulose as a placebo, one capsule (400 mg) three times each day for 12 weeks. The body weight (BW), body mass index (BMI) and waist circumflex (WC) were measured in the beginning of the study and after 12 weeks of treatment with GTE. The data were compared and expressed as % reduction.

Results: There was only a 0.3% reduction in BW (0.15 kg) after 12 weeks of treatment with GTE. There was no statistical difference in % reduction in BW, BMI and WC between the GTE and placebo groups. Within group comparison revealed that the GTE group had significant reduction in LDL-cholesterol and triglyceride, and marked increase in level of HDL-cholesterol, adiponectin and ghrelin. On the other hand, the placebo group showed significant reduction in triglyceride only, and marked increase in the level of ghrelin alone.

Conclusions: This study showed no statistical difference in % reduction in BW, BMI and WC between the GTE and placebo groups after 12 weeks of treatment. The intake of GTE (491 mg catechins containing 302 mg EGCG) for 12 weeks is considered safe as evidenced by the results.

An Open, Single Arm, Multi-center, 12-Week Phase IV Study to Evaluate the Efficacy and Safety Profiles of Memoregain® in Subjects with Mild to Moderate Vascular Dementia

Simon Hu, Peter Huang, Yi-Ta Lee and Muh-Hwan Su

Department of Research and Development, Sinphar Group, Taipei

Background: Memoregain® is a botanical drug extract and partially purified from a Chinese traditional medicinal herb—*Cistanche tubulosa*. Memoregain® was approved for the indication of vascular dementia (VD) in mainland China 5 years ago. Via this large sample size phase IV study, we aimed to re-confirm the efficacy in general and special populations, and to identify less common adverse reactions in patients diagnosed with mild to moderate VD.

Methods: This is an open, single arm, multi-center, 12-week phase IV study. Around 2000 mild to moderate VD patients aged 40-85 years were planned to be recruited in this study. All eligible subjects were treated with 2 Memoregain® 0.3 g capsules tid for 12 weeks. Efficacy assessments including Mini-Mental State Examination (MMSE), Traditional Chinese Medicine (TCM) syndrome score, Alzheimer Disease Assessment Scale—Cognitive Subscale (ADAS-cog), Activity of Daily Living scale (ADL), and Blessed Behavioral Scale (BBS), safety evaluations including adverse event recording, Electrocardiography (ECG) and other Lab routine tests were performed during the study period.

Results: Till May 2010, 81 complete case report forms (CRFs) were retrieved from sites. Six of the 81 subjects had early withdrawn from the study and not performed post-treatment evaluations. The efficacy analysis results were made based on the 75 complete subjects, while the safety evaluations were based on all the 81 treated subjects. All of the efficacy evaluations showed subjects with significant improvement after 12 weeks of treatment. The mean (95% CI) improvement for MMSE, TCM syndrome and ADAS-cog were 3.4 (2.8, 4.0), 4.8 (3.7, 5.9) and 6.2 (4.9, 7.6), respectively. The mean (95% CI) reduction of ADL and BBS were 2.7 (1.5, 3.9) and 2.3 (1.6, 2.9). During the study period, 7 adverse events were reported in 6 subjects, only 1 mild hypersomnia event was judged as at least possibly related to the study drug.

Conclusions: The preliminary analysis showed that Memoregain® was safe and well tolerated for the treatment of mild to moderate VD patients. Both cognition and daily life abilities of VD patients were demonstrated with significant improvement after 12 weeks of Memoregain® treatment.

Treatment of Osteoarthritis with HLXLD: A Translational TCM Study in the US

David Y-W. Lee¹, Lixing Lao², Kamal Moudgil², Harry Fong³, and Brian Berman²

¹Harvard Medical School/McLean Hospital, Bio-Organic & Natural Products Laboratory, USA

²University of Maryland, School of Medicine, Center for Integrated Medicine, USA

³University of Illinois at Chicago, College of Pharmacy, USA

Osteoarthritis (OA) is the most common form of arthritis and is a major cause of morbidity, physical limitation, and increased health care, especially in the elderly. Currently, no therapies have definitively been effective to cure OA, therefore, the management of OA patients is primarily focused on reduction of symptoms, particularly pain and limitation of function. In general, pharmacological therapies for OA of the knee can be categorized into six main groups: (1) simple analgesics; (2) non-steroidal anti-inflammatory drugs (NSAIDs), including; (3) intra-articular (IA) therapy, including glucocorticoids and hyaluronic acid; (4) topical analgesics (e.g., capsaicin), (5) nutritional supplements; and (6) investigational biological agents. Unfortunately, the above therapies may be also associated with significant adverse effects.

Although many persons with OA in the United States use CAM therapies. The scientific basis for the use of CAM in the treatment of OA, however, is relatively sparse. The goal of this translational research is to conduct a preclinical and clinical trial to determine mechanism of action, safety and efficacy of a Traditional Chinese Medicine (TCM) called Hou-Lou-Xiao-Lin Dan (HLXLD), as an alternative treatment in patients with OA of the knee. The primary hypothesis to be tested in this trial is that patients randomized to receive HLXLD will have significantly more improvement than those randomized to placebo herb as measured by the WOMAC Osteoarthritis Index. Based on the highly encouraging results generated from animal and preliminary Phase I studies, we believe that HLXLD stands an excellent chance to be developed as a safe and effective alternative therapy for management of OA. (Funded by NIH/NCCAM P01-AT-002605 to Brian Berman)

Keywords: Osteoarthritis, Translational research, inflammation, TCM, HLXLD, CAM therapy, NSAID, Arthritis.

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<p align="center">Clinical Study of Elemene Injection Combined with Radiotherapy in Treatment for Non-small-cell Lung Carcinoma Patients with Brain Metastasis (BM-NSCLC)</p> <p align="center">Wei Hou and Yongming Zhou</p> <p align="center"><i>Department of Oncology, Guang'an men Hospital, China Academy of Chinese Medical Sciences, Beijing</i></p> <p>Objectives: To investigate the function of herbal medicine elemene injectio Combined with Radiotherapy for non-small-cell lung carcinoma Patients with Brain Metastasis (BM-NSCLC) in aspects of promoting the efficacy of radiotherapy, reducing the side-effects of radiotherapy, improving life quality.</p> <p>Methods: In this study 96 BM-NSCLC cases were divided into experimental group and control group randomly. 48 cases in the experimental group, who were given elemene injectio combined with radiotherapy, and 48 cases in the control group, who were given radiotherapy only. The course of treatment was 4 weeks. The clinical symptoms, Karnofsky score and quality of life were respectively compared before and after treatment.</p> <p>Results: The effective rate of the experimental group (CR+PR) is 12.76%, and the control group is 8.69%. The difference between the two groups have no statistical significance (P=0.178). The main symptoms in two groups were improved, there was significant statistical difference (P=0.000). Comparing the life quality (Karnofsky scores) of the two groups, the improvement rate of the experimental group was 46.80%, while the control group was 13.04% (P=0.000). There were no significant differences of the blood routine test, the hepatic and renal function between the two groups before and after treatment.</p> <p>Conclusions: Elemene injection can increase the efficacy of radiotherapy, improve the Quality of life for BM-NSCLC Patients, without toxicity or side effects.</p> <p>Keywords: Elemene Injection, non-small-cell lung carcinoma, Brain metastases, Radiotherapy, Clinical research</p>
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<p align="center">Evidence-based Medical Study of TCM on Non Small Cell Lung Cancer</p> <p align="center">Hong-Sheng Lin</p> <p align="center"><i>Department of Oncology, Guang'an men Hospital, China Academy of Chinese Medical Sciences, Beijing</i></p> <p>Led by Oncology Department of Guang'an men Hospital, China Academy of Chinese Medical Sciences, we have held multi-center large-sample clinical trials in the treatment of Non Small Cell Lung Cancer (NSCLC) since 2000, including the randomized controlled trials (RCT) of Capital Improvement Program "TCM Study on Prolonging the Survival Time of Post-operative NSCLC Patients", National Tenth Five-Year Plan "TCM Treatment on the Advanced NSCLC", and the ongoing cohort study of National Eleventh Five-Year Plan "Comprehensive Therapeutic Regimen on NSCLC". The previous RCT showed that TCM with the main treatment principle of strengthening body resistance and consolidating the root can improve the 1-year survival rate (integrative group: 89.19%, 83.15%, western group: 78.98%) and 2-year survival rate (integrative group: 64.86%, 52.17%, western group: 47.16%), obviously improve the clinical symptoms, and decrease the tendency of relapse and metastasis in the treatment of stage IIIA post-operative NSCLC. The RCT in the treatment of stage IIIA-IV NSCLC patients showed that the median survival time in integrative group was 12.03 months, while it was 8.46 months in the group of western medicine; there was no statistical difference in the chemotherapy completion rate between two groups; the improvement of TCM symptoms, physical status of integrative group were better than those of western group, there were statistical differences between two groups. The results demonstrated the clinical therapeutic effects and its characteristics of TCM in the treatment of NSCLC, which provides favourable evidence and realible data in the clinical application.</p>

A Comparative Study on the Methods of TCM and Western Medicine Therapeutic Evaluation of Advanced Non-small Cell Lung Cancer

Pei-tong¹ Zhang, Ming-wei Yu², Zong-yan Yang¹

Department of Oncology, Guang'an men Hospital, China Academy of Chinese Medical Sciences, Beijing
Department of Oncology, Beijing Chinese Medical Hospital, Beijing

Objective To compare the differences and characteristics in TCM and Western medicine Therapeutic Evaluation methods Applications. **Methods** A total of 200 cases of advanced non-small cell lung cancer from 3 subcenters were brought into the study, among which there were 104 cases in TCM group and 96 cases in chemotherapy group. And their short-term therapeutic effects were observed by "the clinic efficacy appraisal standard of therapy for advanced lung cancer with TCM" simultaneously and by the follow-up Western medical solid tumor's effect evaluation criterion, including clinical symptoms, tumor body, cassette score, weight and immune function evaluation. **Results** According to WHO solid tumor's effect evaluation criterion, the efficacy of chemotherapy group was much better than that of TCM group. While, according to the clinic efficacy appraisal standard of therapy for advanced lung cancer with TCM, the efficacy of TCM group was better than that of chemotherapy group, without statistical difference ($P=0.05$), however, there was a very strong trend of appearing difference. There was difference in the results of the two evaluation methods. **Conclusion** Compared with WHO solid tumor's effect evaluation criterion, "the clinic efficacy appraisal standard of therapy for advanced lung cancer with TCM" can reflect more features and advantages of TCM for cancer treatment, having value for further study.

Keywords: therapeutic evaluation; non-small cell lung cancer; TCM

The Distribution of Traditional Chinese Medicine Constitution and Prognosis Analysis in Primary Liver Cancer

LONG Shun-qin, HU Xue-jun, YANG Xiao-bing, WU Wan-yin, DENG Hong, CHAI Xiao-shu, HE Wen-feng, HUANG Li, ZHOU Yu-shu, LIAO Gui-ya, OUYANG Yu-shu, CAI Jiao-zhi

Department of Cancer, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangdong

Objective: To investigate the distribution of Chinese medicine constitution and to analyze prognostic factors among cases with the primary liver cancer (PLC). **Methods:** Standardized classification measurement questionnaire of nine constitutions in Chinese Medicine, were used to investigate the Chinese medicine constitution of 151 primary liver cancer patients. Clinical follow-up of the patients was conducted to calculate the survival time. The impacts of Chinese medicine constitution and other factors for prognosis of PLC were analyzed, too. **Results:** The constitution in Chinese medicine was as follows: Gentleness type, Qi-deficiency type, Yang-deficiency type, Wet-heat type, Qi-depression type, Blood-stasis type, Special diathesis type, Yin-deficiency type and Phlegm-wetness type. The frequencies of which were 37 (24.5%), 36 (23.8%), 25 (16.6%), 21 (13.9%), 14 (9.3%), 10 (6.6%), 4 (2.6%), 2 (1.3%) and 2 (1.3%), respectively. Univariate and multivariate analysis indicated that Qi-deficiency type, TNM staging, AFP level and CLIP score were independent prognostic factors for survival time, and Qi-deficiency type was important independent factor which might be worth of a further investigation for predicting prognosis. **Conclusion:** The constitution in Chinese medicine of the patients with PLC was mostly Gentleness type, Qi-deficiency type, Yang-deficiency type and Wet-heat type. Qi-deficiency type was a strong independent prognostic factor of survival time in PLC.

Keywords: Chinese medicine constitution patterns; Primary Liver Cancer; COX regression

Clinical Research on External High Frequency Thermotherapy plus TCM Differentiation for Treating Advanced Non-small Cell Lung Cancer

Wu Wan-yin, Yang Xiao-bing, Deng Hong, Long Shun-qin, Sun Liang-sheng, He Wen-feng, Zhou Yu-shu, Liao Gui-ya, Chen Shi-min, Shan Shi-pu

Department of Cancer, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangdong

Objective: To observe the clinical efficacy and clinical benefit response of external high frequency thermotherapy combined with TCM Differentiation in the treatment of patients with advanced non-small cell lung cancer (NSCLC). **Methods:** The study adopted a prospective, small sample and randomized controlled method and divided the advanced non-small cell lung cancer patients into two groups according to table of random digit, one with the treatment of hyperthermia combined with TCM Differentiation (the therapeutic group), the other with TCM Differentiation (the control group). The efficacies were evaluated after three to four cycles. **Results:** Sixty-six patients accomplished the study, There was little significant difference on sex, age, pathology and stage of the disease ($P > 0.05$). After the patients underwent different treatments, no patients got complete response or partial response in both groups. In the therapeutic group, the disease control rate was 72.2 percent, and 10 got progression disease (28.8 percent), as the control group was 63.3 percent, and 11 got progression disease (36.7 percent), There was significant difference according to statistical comparisons ($P < 0.05$), it suggested that the combining regimen had superiority on the disease control rate. As to long-term efficacy, the median survival time (MST) of treatment group was 7.5 months, time to progress (TTP) was 5.5 months, and one-year survival rate were 21.4 percent. As far as the control group was concerned, the result was 6.8 months, 4.5 months and 16.6 percent respectively. There was significant difference ($P < 0.05$) on TTP, but no difference on MST or one-year survival rate. **Conclusions:** Hyperthermia combines with TCM Differentiation has better tolerance and short term efficacy in the treatment of patients with advanced NSCLC.

Keywords: Non-small cell lung cancer, External high frequency thermotherapy, TCM Differentiation, Efficacy

Effect and Advantage of Orally Taking Chinese Herbal Medicine for Treatment of Lung Cancer

Xiao-Ying Tian and Liang Liu*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Lung cancer is one of the most common cancers and of the leading causes of human death worldwide. Most of patients with lung cancer are diagnosed at advanced stages as it is difficult to be detected in the early stage. Radio/chemotherapy serves as the major method for treatment of lung cancer in conventional medicine but without satisfactory efficacy and even with a number of adverse effects. So, it is very popular in mainland China to employ integrated treatment of radio/chemotherapy and Chinese herbal medicine. On analysis of the published reports in the past two decades, we found that orally taking Chinese herbal medicine could significantly improve quality of life and body resistance, relive clinical painfulness, reduce adverse effects of radio/chemotherapy, and prolong life-span of the patient alive with carcinoma in the body. However, in the reported literatures design of the research projects needs to be more standardized and quality and standards be enhanced. And, molecular mechanisms of the herbal treatments are expectedly elucidated.

Keywords: Oral treatment of Chinese herbal medicine; Traditional Chinese medicine; Lung cancer; Integrative Chinese and western medicine; Review.

Correlational Study on the Five Personalities and Breast Cancer

Jian-ping Chen, Jade Tsui Yin Li, Wai-song Yeung

School of Chinese Medicine, The University of Hong Kong, Hong Kong

Objective: To understand the relationship between emotions/personality and breast cancer using the five phases theory of the traditional Chinese medicine approach, with a view to inspire TCM treatment and prevention for breast cancer. **Methods:** Breast cancer subjects and healthy individuals were recruited and completion of questionnaires. Comparison of subjects' general information was carried out to ensure the comparability of the two groups, followed by statistical analysis of their responses by SPSS 11.5. Patient consent for participating in this research had been obtained. **Results:** (i) Earth-type subjects dominate the normal sample. The difference between the breast cancer group and normal group is of statistical significance ($p < 0.05$). (ii) Water-type subjects of the breast cancer group show eminent negative emotion tendencies after confirmed diagnosis of breast cancer. **Conclusions:** A protective indicator of personality for breast cancer is found in earth-type subjects, though correlation between other types of emotion and personality and the onset of breast cancer is not observed. These findings provide grounds for future research in this field.

Keywords: Breast neoplasms; personality; Medicine, Chinese Traditional; questionnaires

Phase II Clinical Efficacy and Safety of SR-T100 in the Treatment of Actinic Keratosis

Kou-Wha Kuo and Hamm-Ming Sheu

G&E Herbal Biotechnology Co., Ltd, Tainan

Department of Dermatology, National Cheng Kung University Hospital, Tainan

SR-T100[®] gel is a developing topical medicine for treating AK (Actinic Keratosis). AK is one of the most frequent diagnosed skin cancer diseases by US dermatologists. 75% patients of AK are usually subjected for cryotherapy by dermatologists. However, the recurrent rate after cryotherapy is larger than 70%. So far, SR-T100[®] gel has completed phase II clinical trial in Taiwan. The result shows a high efficacy rate with very mild side effect. Indeed, SR-T100[®] is a highly safety medicine when compare the side effects with current and developing AK medicines. Moreover, SR-T100[®] gel can apply large area of AK lesions without any limitation. Combined cryotherapy, SR-T100[®] continuously and selectively cleans residual cancer cells without any injury to normal cells which may greatly reduce the recurrent rate of cryotherapy. These critical and benefit points of SR-T100[®] to AK have provided great advantages and alternative for the treatment. Besides, SR-T100[®] was also evidenced in highly potential to treat solid tumors by oral or injection dosage form which aims to treat head and neck SCC and related cancers. G&E will continuously complete AK phase III clinical trial of SR-T100[®] gel in Taiwan and file IND of SR-T100[®] for AK to US FDA.

Phase I/II Study of PHY906 Plus Capecitabine (CAP) in Patients with Gemcitabine-refractory Pancreatic Cancer (PC)

M. W. Saif, L. Lamb, J. Li, S. Bussom, R. Penney, G. Ananthanarayanan, R. Carbone, K. Elligers, S. H. Liu, Y. C. Cheng

School of Medicine, Yale University, USA

Background: Oral capecitabine (CAP) is a palliative chemotherapeutic option, however, diarrhea and hand-foot syndrome may compromise efficacy; this is evident in US patients given CAP 1250mg/m² BID D1-14. The botanical formulation, PHY906, used widely in Asia to treat diarrhea, nausea and vomiting, reduces side effects of chemotherapy and prolongs survival in colorectal cancer and hepatocellular carcinoma. A phase I/II study using the PHY906/CAP combination against advanced pancreatic and gastrointestinal malignancies showed synergistic anti-tumor activity and reduced diarrhea, thus providing strong rationale for extending PHY906/CAP to pancreatic cancer.

Methods: Pancreatic cancer patients (failed gemcitabine treatment) received CAP 1500 mg/m² BID D1-7 and PHY906 800 mg/m² BID D1-4 q 2 wks. Adverse events were assessed per NCI CTCAE v3.0 and response per RECIST q 6 wks. Objectives were: primary, overall survival (OS); secondary, effects of PHY906 on overall response rate, quality of life, and cytokine/chemokine levels.

Results: All 25 patients enrolled received study drug (evaluable for safety); 18 received ≥ 2 cycles (evaluable for efficacy). Patients' characteristics: median age = 64yr (range 45-84); 10 F, 15 M; median ECOG PS = 1 (range 0-2). Predominant AEs: Grade 3 diarrhea (n=3), Grade 3 fatigue (n=3) and Grade 3 HFS (n=1). For 18 patients receiving ≥ 2 cycles, 2 (11.1%) achieved PR and 11 (61.1%) had stable disease (disease control rate of 72.2%). Median PFS and median OS were 13 and 31 wks, respectively. Nine-month survival rate was 37%. Only serum cytokine IL-6 levels were inversely correlated with survival.

Conclusions: In this first clinical study to evaluate a botanical drug, PHY906, plus CAP in pancreatic cancer, the therapeutic index of CAP was increased by reducing diarrhea and HFS and a survival benefit was indicated. The PHY906/CAP combination appears a safe and feasible salvage therapy after gemcitabine failure and warrants randomized study against CAP alone.

Evolution of an Integrative Oncology Research and Education Program that includes Chinese Medicine within the Department of Oncology at McMaster University

Stephen M. Sagar, Raimond K. Wong, and Andrea Baumann

Department of Oncology and Faculty of Health Sciences, McMaster University, Canada

The Faculty of Health sciences, McMaster University pioneered biopsychosocial health care, evidence-based medicine, and problem-based learning. These are core components of a curriculum that is shared by all health care professions within the Faculty.

Chinese medicine (TCM) has recognizes these same concepts and has developed theories of diagnosis and intervention based on documented observations. Each patient is a subject for a problem based learning approach, in which the practitioner utilizes various resources for intervention, and modifies his technique according to outcome.

We propose that our health care system and TCM contain some common values. The divide is mainly cultural and the need is to translate observations and data into material concepts rather than just metaphysical systems. We have published extensive literature reviews in order to translate the Oriental concepts into data that would be credible in a Western medicine setting. This involved a reductionist approach to interpretation of data and the proposition of studies that would conform to the standards of an evidence-based approach. In addition, we gradually introduced techniques from TCM into our education programs as options for supportive care. This required introducing modern technology and standard Western diagnostic systems in order to interpret TCM concepts into solutions that are culturally acceptable in a Western oncology clinic.

We also propose the synergy of an holistic approach. This involves transcultural exchange of knowledge. In 2009, McMaster University announced a memorandum with the Guangzhou University of Chinese Medicine and Hong Kong Polytechnic University. The Department of Oncology and Faculty of Health Science International have arranged a student mentorship program with the East-West Hospital, Korean Institute of Oriental Medicine, and Daejeon University. We continue to work closely with the Society for Integrative Oncology to develop practice guidelines and research agendas. In 2010, at this meeting, we have applied for membership of the Consortium for the Globalization of Chinese Medicine.

Introduction to Natural Therapies for Health Preservation in Chinese Medicine

Yi Dang, Zhongzhen Zhao

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Lifestyle has been proven to be a critical element in health and longevity. In this report, we discuss four categories of traditional Chinese approaches to health preservation that can be incorporated into lifestyle as daily practices. These methods are also clinically proven effective in improving health and promoting rehabilitation of patients with chronic conditions.

1. Taiji Quan and Qigong exercise. The various forms of Taiji Quan, including those performed with sword, can relax and regulate many of the body's functions when practiced regularly over months and years. Qigong exercises can relax and smooth heart-blood circulation, regulate the functions of various organs. Qigong is practiced not only to preserve health but also to reverse the effects of various diseases, especially chronic conditions.

2. Food therapy, including tea and herbal tea; medicinal soups; medicinal porridge; flower food; herbal wines (using wine as a solvent to soak out the effective components of the herbs, or to boil together and then removing the dregs.); vinegar and vinegar food, for example, vinegar-soaked ginger drink; fresh vegetable, fruit and herbal juices; honey or honey products; Chinese royal medicinal food, and the use of functional foods, etc.

3. Cosmetology. Chinese medicine includes various methods that can help maintain a youthful appearance and well-proportioned body shape, for example, the methods of making eyes sparkling, the methods of making hair healthy and glossy, etc.

4. Miscellaneous practices. In addition to food, exercise, and cosmetology, traditional Chinese medicine (TCM) includes a wide range of additional practices to preserve health and prevent disease. These are prescribed based on identification of a person's physical constitution, according to season and living environment. They include: massage (body, foot); bath therapy; cupping; acupuncture (body, ear); music therapy; cloud therapy; sun therapy; poem therapy, etc.

Keywords: Natural therapy, Health preservation, Lifestyle, Chinese Medicine

Internationalization of Chinese Medicine ---- International Education of Chinese Medicine

Yi Dang, Carry Po Chu YU

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Since 2008, the school of Chinese Medicine (SCM) has developed a new module to cater for exchange students who are interested in Chinese medicine theories and applications. This new program "Introduction to Chinese Medicine" has been conducted for 4 semesters with supportive feedback from students. It is a short course with 30 study hours, students have to submit an assignment in the last

This is a good opportunity for us to promote Chinese Medicine thru international education. Asia TV Hong Kong has featured this course in their news recently as this is unique among all universities in Hong Kong.

This program consist of 11 topics :

- 1) Introduction to TCM and Development history of TCM in general
- 2) Yin-Yang Theory and Five Phase Theory
- 3) Visceral Manifestation Theory in TCM
- 4) Qi, Blood and Body-Fluid Theory and Cause of Diseases in TCM
- 5) Principles for Disease Prevention and Treatment
- 6) Four Examination Methods in TCM
- 7) The Principle of Pattern Identification in TCM
- 8) Introduction to Acupuncture and The Twelve Regular Meridians
- 9) Introduction to the treatment of Acupuncture, Moxibustion, Cupping and Tuina----Chinese Bodywork Massage Therapy
- 10) Introduction to Chinese Herbal Medicine and Food Therapy
- 11) Product Development & Marketing of Chinese Medicine

Craig Ryan Thiessen, a medical student from America said, "I found this course very interesting and interesting. Although the efficacy of Chinese Medicine is slower, its side-effect to patients is lesser. Chinese Medicine Practitioner focuses on the unique symptoms of each patient and tailor-made formula for them, this is a great care for the patients."

Keywords: International Education, Chinese Medicine

Flower Therapy for Health Preservation

Yi Dang, Hu Biao Chen

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Most people know that colorful flowers are enjoyable to look at and some people know that different types of flowers represent different meanings in special occasions, for example, roses represent love, lotus stand for purity. However, fewer people acknowledge other functions of flowers, for example, flowers can refine temperaments as well as alleviate sadness. In the traditional Chinese medicine point of view, the smell of violets and roses brings joy and relaxation and the fragrance of jasmine generates peacefulness. In addition, flowers can be used for cooking flower cuisine as well as for drinking as flower tea. Essential oils extracted from flowers also have a variety of effects, for example: lavender oil can prevent influenza; jasmine oil and orange oil can sedate nerves. The benefits of flower tea on health are not only from its effective ingredients, but also from its ornamental value, the aroma of the flowers, and the process of brewing and tasting. As the saying claims: flowers can bring joy; flowers can fight illnesses; flowers can build body strength; flowers can enhance beauty. Given the varied effective functions of flowers, more attention should be paid to discover what flowers can offer to improve our life quality and to enhance our health. The following 4 topics about different flowers which are blossom in 4 seasons, namely spring, summer, autumn and winter, will be discussed accordingly in this report concerning the functions and properties of 50 kinds of flowers with lively pictures and 200 medicine food or drink prepared with flowers. With the current report, the authors aim to raise more attention from multidisciplinary researchers and practitioners to explore the functions and a better usage of flowers.

Keywords: Flower Therapy, Health Preservation, Chinese culture, Chinese Medicine

New Area of Chinese Medicine Education ---- Launching the Program of Master of Science in Personal Health Management (Chinese Medicine) in China

Yi Dang, Liang Liu

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Recently, the School of Chinese Medicine (SCM) has launched a new taught Master program to cater the need of students for broader-based, interdisciplinary training in personal health management with intensive integrated training of Chinese and Western medicine theories and applications. This new program titled in "Master of Science in Personal Health Management (Chinese Medicine)" is positioned as a pioneer in the local tertiary education aiming to train new health maintenance for personals at postgraduate level students in Hong Kong, as well as Mainland China.

Intended program learning outcomes:

1. Acquire the basic concepts of traditional Chinese Medicine (TCM).
2. Relate the concept of the quality of life to the well-being of human body and affects of diseases.
3. Acquire the basic concepts of health maintenances based on the concepts of TCM and scientific approaches of the conventional health management.
4. Demonstrate knowledge of basic principle in health maintenance and food therapy in Chinese medicine and apply the knowledge in enhance health awareness.
5. Demonstrate knowledge of using traditional TCM techniques to improve various aspects of health.
6. Apply critical thinking to manage the health-related problem in everyday life.

Curriculums of the program include 10 major aspects as below:

- 1) The Rationale of the Program
- 2) Program Aims and Objectives
- 3) Program Courses
- 4) Special Features of the Program
- 5) The Learning Goals
- 6) Target Students
- 7) Social Demand for the Program
- 8) Program Structure
- 9) Teaching Methods and Strategies
- 10) Requirements of Dissertation

Keywords: Personal Health Management, Chinese Medicine, Program of Master Degree

Emotionless Holism: Factor and Rasch Analysis of the Chinese Integrative Medicine Attitude Questionnaire

Vincent CH Chung*, Lau Chun Hong, Marc Chong, Eric MC Wong, Samuel YS Wong, Eng Kiong Yeoh, Griffiths

School of Public Health and Primary Care, Chinese University of Hong Kong, Hong Kong

Background: Originating in the U.S., the 28 item Integrative Medicine Attitude Questionnaire (IMAQ) is used for assessing attitudes towards complementary and alternative medicine amongst allopathic western medical doctors (WMD). Our preliminary Chinese adaptation has shown the need in (1) transforming the IMAQ to align with the philosophy of traditional Chinese medicine (TCM), and (2) redesigning items relating to holistic doctor-patient relationship. This indicates a potential East-West difference in the interpretation of holistic healthcare. In this study, we aim to examine this difference by assessing the psychometric properties of a re-orientated IMAQ (TCM-IMAQ) in the Chinese context.

Methods: Factor and Rasch analyses were performed using data collected from a mail survey of 166 Hong Kong WMD randomly sampled from the official registry. The structural validity, unidimensionality, item fit, and differential item functioning of the HKTCM-IMAQ were evaluated.

Results: Confirmatory factor analysis demonstrated that the original IMAQ factor structure was not consistent with our data on Chinese WMD and subsequent explanatory factor analysis validated a new three factor model for TCM-IMAQ: (1) attitude towards "tonification", (2) attitude towards the effectiveness of TCM, and (3) attitude towards TCM knowledge. The original IMAQ factor on holism and doctor-patient relationship disappeared. Rasch analysis confirmed the unidimensionality of "tonification" and the effectiveness domains, but further refinement of the knowledge domain is needed.

Conclusion: Cultural adaptation of the IMAQ has demonstrated differences between Eastern and Western medical doctors trained in allopathic medicine in their interpretations of holism in healthcare. For Chinese WMD, the emphasis of holistic care is placed on "tonifying" the body rather than on nurturing the mind and spirit. Confucian and Taoist conceptualizations of mental health, as well as the persistent stigma towards mental illness within modern Chinese culture may explain why Chinese WMD do not regard mental health promotion as part of routine healthcare.

Developing Ontological Search Engine for Global Access Portal of Academic TCM Websites

Lawrence Wing-Chi Chan, Meyrick Chow, Josiah Poon, Daniel Man-Yuen Sze

Department of Health Technology and Informatics, School of Nursing, The Hong Kong Polytechnic University, Hong Kong

School of Information Technologies, University of Sydney, Australia

The strategic direction of World Health Organization (WHO) implicates the urgent need for a global hub connecting the sources of TCM information scattered throughout the world. A one-stop portal linking up these TCM information sources is of critical importance to train and provide clinical decision support to the novice TCM practitioners. Recently, a prototype of the web portal has been established by the research team. The web presentation and content of the portal are automatically driven by a schema of relational database. To search for TCM websites relevant to a new clinical case, novice practitioner can enter the keywords of the case to the search engine and a list of the relevant academic websites will be returned. The web portal is different from the existing search engines, like Google and Yahoo, as it is supported by the built-in TCM ontology. Each TCM website hyperlink in the database is indexed with a feature vector, which is obtained by an ontological inference mechanism. The retrieved hyperlinks have to be conceptually similar to the provided keywords. The development of the portal and search engine is ongoing and the user and system evaluation will be performed to assess its accuracy, usefulness, user-friendly and performance in clinical practice and training. The success of the portal and search engine will provide a global access point for TCM practitioners to search for useful reference information without being distracted by irrelevant websites and information.

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The Research on Human Rights Protection of Chinese Elderly Patients with Alzheimer's: Case Studies

Chunhuan Lao, Li-Jen Cheng

Institute of Chinese Medical Sciences, University of Macau, Macau

This paper describes the current situation of human rights violations of the elderly patients with Alzheimer's disease in China and analyses their causes through case studies. Elderly patients with Alzheimer's disease lose self-care ability, lack of care, suffered from discrimination and abuse, with very bad living environment, which led to many social problems, such as homeless elderly, elderly abuse, and so on.

There are many reasons that may contribute to these social problems: (1) People are under a lot of living pressure. (2) They know little about Alzheimer's disease. (3) The social security system is uncertain. (4) The old age support in the country leans too much on the offspring. (5) The traditional morality is being lost.

The countermeasures as follow may help to protect the human rights of the elderly patients with Alzheimer's disease: Not only should the legal and institutional human rights protection of ill elderly be improved, but also the education of the social ethics and the legal awareness of taking good care of the elderly should be strengthened. Moreover, the service facilities for Alzheimer's Disease patients and the old-age security system in rural areas should be well developed. Finally, the social assistance network should be encouraged and supported.

First Master Degree of TCM in Europe

Henry Johannes Greten, Sven Schröder and Tobias Greten

Institute of Biomedical Sciences, University of Porto, Portugal

TCM Centre at the University Clinic Hamburg-Eppendorf, Germany

Heidelberg School of Chinese Medicine, Germany

Background: True academic recognition is necessary to add impact on the process of integration of TCM. Prospective, controlled, randomized trials such as the GerAc- and ART studies have so far not been able to prove specific effects. Neither did a meta-analysis of 33 studies show a difference between the use of acupuncture points and random skin areas. These negative data are one of the reasons why academic institutions hesitate to recognize acupuncture in the West, and thereby diminish the reputation of TCM on the whole.

Solution: We have defined the theory of TCM according to old scriptures of medicine, daily practice, scientific translation and mathematical analysis more precisely, thus providing a quotable scientific basis of defined diagnosis in TCM. This Heidelberg Model of TCM is allows to translate the key termini of TCM into western measurable physiology.

We also developed a first double blinding assay of acupuncture and showed in double-blinded and also triple-blinded studies, that acupuncture shows specific effects. These effects can be increased dramatically in comparison to the metaphysical diagnostic standards common in acupuncture practice.

On this basis, we were credible enough to establish the first academic Master Degree of TCM for health care professionals in Europe at the ICBAS, University of Oporto, in cooperation with the Heidelberg School of Chinese Medicine, the International Scientific Medicine Association (ISCMA), the German Association of TCM (DGTCM), and the WFAS.

We invite everybody interested in structural, process and clinical outcome quality control in TCM to cooperation.

Comparison of Chinese Medicine Education in Different Countries

Peter Karl Mayer, Jui-Shan Lin, and Yi-Chang Su

School of Chinese Medicine, China Medical University, Taichung

As Chinese Medicine (CM) is getting more popular worldwide, many universities teaching CM developed their own curricula and educational system. However, until now there is no worldwide accepted guideline for CM education. In order to evaluate the current situation, curricula of top institutions with a regular CM degree program were compared. The degree should allow the person to work as a CM physician in the respective country. About 20 curricula of 14 different universities from ROC Taiwan, PR China, Korea, Japan, USA and Australia were analysed. The curricula were compared in relation to duration of study, courses, didactic hours and degree. The courses were classified into different categories like CM Foundation Studies, CM Classical Texts, CM Clinical Studies, Western Medicine (WM), other courses and Internship. The didactic hours for each category were calculated and compared.

Within the selected universities, the amount of CM didactic hours differed from around 1200 to 3000 hours and the amount of WM hours varied between 500 to 4800 hours. CM universities in PR China have a high amount of non medical courses (more than 700 hours) with political content. Great differences could be found within the universities regarding the internship duration. Compared with the 13-week CM internship at RMIT University, Australia and a 52-week internship at Nanjing University of TCM, China Medical University is the only university, which, beside a 48-week WM internship in the double degree program, also offers a 32-week WM clerkship in the five and seven year CM curriculum. On the other hand, the 7-year master degree curricula of universities from PR China offer a 40-week cultivation program which includes clinical practice and research besides the regular CM internship.

The differences and similarities shown in this research can provide a basic platform for the discussion of CM education, recognition and improvement worldwide.

Amount of Alkaloids in Processed Aconite Roots: Comparison of Specific Hplc Determination of Toxic Aconite Alkaloids with a Titration Method of Total Alkaloids

Csupor D¹, Borcsa B¹, Heydel B², Hohmann J¹, Zupkó I³, Ma Y⁴, Widowitz U², Bauer R²

¹ Department of Pharmacognosy, University of Szeged, Hungary

² Institute of Pharmaceutical Sciences, Pharmacognosy, Karl-Franzens University Graz, Austria

³ Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

⁴ Department of Pathophysiology, Center of Physiology, Pathophysiology and Immunology, Medical University, Austria

Unprocessed aconite roots are very toxic due to diterpene ester alkaloids [1]. Processing reduces toxicity because of hydrolysis of the ester groups [2]. Therefore, Chinese pharmacopoeia allows only application of processed aconite roots. Nevertheless, several cases of poisoning have been reported [3], and proper quality control is essential. Recently, a specific sample preparation method for HPLC analysis of toxic aconite alkaloids has been suggested [4]. Now, an improved HPLC method is presented, which has been suggested as a purity test for the European Pharmacopoeia. The content of mesaconitine, aconitine and hypaconitine has been determined in 30 commercial samples of processed aconite roots by this method. Most of the samples were void of toxic aconite alkaloids, or contained only traces. However, in four samples more than 0.04 % of hypaconitine and mesaconitine, the highest with a content of 0.16 % were detected. Acute toxicity of batches high in hypaconitine and mesaconitine has been confirmed in CFLP mice. In Homeopathic Pharmacopoeias, alkaloids in aconite roots are determined by titration. The results of HPLC analysis of toxic alkaloids (mesaconitine, aconitine and hypaconitine) were compared with the results obtained by the titration. No correlation was found. Samples which were lacking mesaconitine, aconitine and hypaconitine, still contained up to 0.2 % alkaloids determined by titration. Therefore, titration of alkaloids is not appropriate as an assay for toxic alkaloids in aconite roots.

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Phylogeny and Cultivation Origination of Medicinal Plants of Genus *Coptis*

Juan Hu; Ai-Juan Shao; Qing-Jun Yuan; Lu-Qi Huang

Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing

Coptis in China, group of three all split palmate leaves, is divided into four species and one variant by morphology according to Flora of China. The five species present dispute and confusion in taxonomy, especially in cultivation origination. The present study constructed the molecular phylogeny with 226 collected samples, using two fragments of non-coding regions of chloroplast *psbA-trnH* and *trnL-trnF*, the variable sites were 23 and 9, with a total of 17 haplotypes. An evolutionary tree was built with following conclusions: 1) Three clear branches were constructed. A branch of *C. teeta* with the farthest genetic distance; a branch contained *C. omeiensis* accompanied cultivated products; the other branch with mainly *C. chinensis*, were all cultivated products. 2) *C. omeiensis* were divided into two distinct sub-branches due to the complicated geographical structure of Mount. Emei; There presented confusing problem of cultivated germplasm from haplotype distribution; *C. deltoidea* with HapH and HapI, are only 1-2 base variation between HapA of *C. omeiensis*, and *C. deltoidea* in population DCZX are all HapA, speculated cultivation of *C. deltoidea* originated from *C. omeiensis*; *C. chinensis* in the other branch retained more abundant haplotypes, speculated that *C. chinensis* cultivated from another species (the ancestor have disappeared); *C. chinensis* var *brevisepala*, only 4-step variation between HapF of *C. chinensis*, as sub-species of *C. chinensis* were confirmed by genetic variants.

From ancient herbal literature, with *C. teeta* rarely recorded, there are two main kinds, which related to the two cultivated species, *C. chinensis* and *C. deltoidea*. Cultivation history record with description of *C. chinensis*, cultivated germplasm from Wuxi, Chongqing, multiplication with seed, suggesting that Wushan area most likely an ancient cultivated germplasm source of *C. chinensis*. From Hongya historical cultivation records and records of *C. omeiensis* distributed in Hongya, confirmed the cultivation of *C. deltoidea* originated from *C. omeiensis*.

Introducing Novel Herbal Products in Canada: Safety, Quality, and Efficacy by Controlled Environment Production and Chemical Profiling

Praveen K. Saxena

Department of Plant Agriculture, University of Guelph, Canada

The Plant Based Medicine (PBM) industry in Canada is continuously searching for novel health products for the native population as well as a rapidly growing Asian immigrant community. Medicinal products from various cultures including the Traditional Chinese Medicine offer an exceptionally broad range of preventative and curative health-promoting benefits for many serious health conditions such as dementia, arthritis, cardiovascular disease, and diabetes. A major concern with plant medicines has been the quality and consistency of the products. The medicinal metabolite production of plants is affected by plant genetics and environmental conditions of cultivation, harvesting, processing and distribution causing widespread occurrence of chemical variability, compromised product quality and consequently, inconsistent results in clinical trials.

This presentation illustrates the importance of an integrated process referred to as "Optimum Medicine Technology" for safe and effective production of plant medicines. The Optimum Medicine Technology employs in vitro cell culture, bioreactors, and controlled environment greenhouse production systems for the selection and seasonally independent propagation of elite lines with specific, consistent levels of medicinal metabolites with minimum contamination. Several unique lines of medicinal species such as Saint John's wort, Echinacea, ginseng, and Scutellaria were chemically profiled using standard and emerging metabolomics techniques and successfully tested using in vitro bioassays and animal model systems. The Optimum Medicine technology provides a model for the development of novel medicinal products with consistent chemistry and medicinal efficacy for the Canadian market.

Quality Evaluation of Radix Polygoni Multiflori and its Dregs by Determination the Contents of Stilbene Glucoside, Emodin and Physcion

Ngon Ngon Leung, Zhitao Liang, Hubiao Chen, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Radix Polygoni Multiflori is the processed dried root tuber of *Polygonum multiflorum* Thunb. (Family Polygonaceae). In the herbal markets, Radix Polygoni Multiflori is often sold for replenishing the liver and kidney with vital essence and blood, blackening the hair and strengthening the tendons and bones. However, in our investigations at local markets, we found a wide variety in the characteristics of Radix Polygoni Multiflori on sale. Some were cut into irregular thick slices or sections; others were cut length-wise into thin slices. Additionally, the diameter differed, apparently according to whether the herbs came from cultivation or from the wild, and according to age. The prices of Radix Polygoni Multiflori varied, based on these differences. In order to evaluate the commercial processed Radix Polygoni Multiflori and its dregs for ensuring quality and fully utilizing the resources of Radix Polygoni Multiflori, more than twenty batches of processed Radix Polygoni Multiflori as well as their decoction dregs were collected for determination of the contents of 2,3,5,4-tetrahydroxystilbene-2-O-β-D-glucopyranoside (THSG), emodin and physcion, which are considered to be its potent active ingredients. The results showed that the contents of THSG, emodin and physcion varied greatly and did not always correlate with price and size. The results also indicated that the content of THSG decreased after decoction while the contents of emodin and physcion varied. In some samples, the contents of emodin and physcion decreased after decoction but in others it increased. However, distinctly, the dregs of Radix Polygoni Multiflori still contained higher contents of THSG, emodin and physcion. The results demonstrated (1) that the dregs of Radix Polygoni Multiflori could be further utilized, and (2) that apparent morphological characteristics, such as size, are not necessarily accurate indicators of potency.

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Traditional Experiences and Modern Understanding of Morphological Identification of Chinese Materia Medica

Zhitao Liang, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Morphological identification is fundamental for standardization of Chinese Materia Medica (CMM). Morphological identification is an important method for authentication; it has a long history, derived from practice and experience over generations. In ancient times, morphological identification was used not only to identify the genuine or counterfeit nature of CMM, but also to evaluate quality. There are lots of traditional, picturesque descriptions used to describe the quality of certain specific CMM in ancient documents. The traditional morphological descriptions used for identification are filled with characteristics, quality and specifications of CMM that are confirmed by modern scientific analyses. Today, CMM quality is chiefly evaluated by determination of active components using modern analytical instruments. In this report we correlate results of modern chemical analyses with traditional descriptions of quality evaluation of two CMM. The CMM *Caulis sinomenii* (CS) and *Radix Polygoni Multiflori* (RPM) were selected as representatives for study. In CS, the chemical sinomenine is considered as the potent active ingredient, and quality indicator. The results of our HPLC analyses showed that the content of sinomenine in large stems (i.e., stem diameter >3 cm) of CS was much higher than in small stems (stem diameter <3 cm), and that sinomenine was mostly distributed in xylem regions. For RPM, the results indicated that its active constituent, 2,3,5,4-tetrahydroxystilbene-2-O- β -D-glucopyranoside, was distributed in all tested tissues with relative content as cork > cortex > xylem of allotype vascular bundles > xylem of central vascular bundles; distinctly the anthraquinones mostly distributed in cortex. Thus, CS with large diameter and RPM with broader cortex and fewer vascular bundles apparent in a transverse cut surface should be considered to have better pharmaceutical quality. These findings demonstrate how, in at least two cases, modern science confirms traditional experiences relating herbal quality to morphological characteristics.

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Molecular Authentication of the Chinese Herb Huajuhong and its Antimicrobial Activity against Pathogenic Microorganisms

Chang Su, Pang-Chui Shaw, Wei-Wei Su, Mamie Hui, Margaret Ip

*Department of Biochemistry & Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong
School of life science, Sun Yet-sen University, Guangdong*

Citrus grandis Osbeck 'Tomentosa' (COT) is a popular Chinese medicinal material for the relief of tussis and phlegm symptoms. Different Citrus species are often used as substitutes or adulterants of COT in herbal market, and a reliable authentication method is essential for the prevention of misuse. We employ chloroplast trnH-psbA intergenic spacer, nuclear internal transcribed spacer (ITS) and inter-simple sequence repeats (ISSR) marker to differentiate COT from other Citrus variants. Our study shows that ISSR method and DNA sequencing methods are effective in differentiating the closely related species. The essential oil from the fruit of COT was extracted by steam distillation extraction and analyzed by GC-MS and GC-FID. The extract of COT, its major component limonene and a related chemical citral were shown to inhibit three common pathogenic organisms *Candida albicans*, *Trichophyton rubrum* and *T. mentagrophytes*.

Study on Quality Specification of *Isatis Indigotica*

Yanhong Shi¹, Zhiyong Xie¹, Rui Wang^{1,2}, Yiming Li¹, Zhengtao Wang^{1,2}

¹Shanghai University of Traditional Chinese Medicines, Shanghai

²Shanghai R&D Center for Standardization of Traditional Chinese Medicines, Shanghai

Purpose: To establish a technical method for quality control of *Isatis indigotica* and identify goitrin isomers and its analogues using chiral column coupled with HPLC-MSⁿ spectrometry. **Method:** *R*, *S*-goitrin in *Isatis indigotica* was identified by TLC method. Its content was determined by HPLC method with Ultimate AQ-C₁₈ (4.6 mm × 250 mm, 5 μm) column using the mobile phase MeOH/0.02% H₃PO₄ (7/93) solvent at a flow rate of 1.0 mL · min⁻¹. The column temperature was 30°C and the detection wavelength was 245 nm. The HPLC-MSⁿ method was carried out on a SHISEIDO CD-Ph column (250 mm × 4.6 mm, 5 μm). Compounds were eluted using a mobile phase of H₂O/MeOH: 70/30 at a flow rate of 0.5 mL · min⁻¹. The detector for MS spectra was an ion trap mass spectrometry with an ESI negative ion mode. The full scan mass and MSⁿ spectra were recorded in the range of *m/z* 150-800. **Results:** Calibration curve showed good linear regression (*r*=1) within test ranges of 2.4~309.0 μg · mL⁻¹. Average recovery was 100.9 % with RSD of 2.0 %. Goitrin isomers were well separated on SHISEIDO CD-Ph column. The relative intensity of product ions showed different among the isomers by the high quality mass spectra of the isomers. At the same time, other pairs of goitrin analogues isomers such as progoitrin in *Isatis indigotica* were tentatively identified and characterized in the same method. **Conclusion:** The developed qualitative and quantitative detect methods of *R,S*-goitrin were proved to be sensitive, reliable and useful by analysis of twenty-two different *Isatis indigotica* samples. The effective method has been successfully applied to the quality evaluation of *Isatis indigotica* and adopted by Chinese Pharmacopoeia 2010 Edition as a new published compendia method.

Genetic Diversity and Relationship of *Fritillaria thunbergii* Landraces and Related Taxa

Shan Li¹, Kai Hu¹, Jing Liu¹, Xiaoling Yang¹, Yunguo Zhu¹, Zhou Cheng^{1,2*}

¹School of Life Science and Technology, Tongji University, Shanghai

²Tongji University-Lishui Institute of Chinese Traditional Medicine, Zhejiang Lishui

Bulbs *Fritillariae* refers to the dry bulb of *Liliaceae Fritillaria* varieties of plants, which is an important traditional Chinese medicine with very long history of medicinal use for phlegm-heat cough, bloody expectoration, scrofula, ulcer, and tumor. The genetic diversity of *Fritillaria thunbergii* landraces and its relationship with *Fritillaria cirrhosa* D. Don, *Fritillaria anhuiensis* were evaluated using ISSR marker, chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) sequence analysis. 9 selected ISSR primers amplified 129 loci in the three kinds of fritillaria, of which 100% were polymorphic. For *F. thunbergii*, the genetic diversity was high at the species level ($h = 0.2372$; $I = 0.3732$; $PPB = 91.47\%$) but relatively lower at the population level ($h = 0.0971-0.2364$; $I = 0.1464-0.3586$; $PPB = 28.68-74.42\%$), and a high level of genetic differentiation among populations was detected based on the gene differentiation coefficient ($G_{ST} = 0.4248$) and the hierarchical analysis of molecular variance (AMOVA) ($\Phi_{ST} = 56.37\%$), in line with the low inter-population gene flow ($N_m = 0.6769$). Both Principal Coordinates Analysis (PCoA) and UPGMA cluster analysis showed that the three kinds of fritillaria can be divided into two groups, *F. thunbergii* landraces as one, *F. cirrhosa* and *F. anhuiensis* as the other, among which there occurred a remarkable genetic differentiation ($\Phi_{ST} = 29.42\%$, $G_{ST} = 0.5774$, $N_m = 0.3660$) among three kinds of fritillaria. cpDNA, mtDNA sequence analysis revealed that *rbcl*, *trnS-trnfM*, 18S-5S rRNA gene and gene spacer sequences are completely identical in the three kinds of fritillaria, except for only two bases difference in *Rps14-cob* gene spacer in *F. cirrhosa*. These findings have profound implications for the effective conservation and sustainable utilization of those fritillaria species.

Code	Species name	Origin	Genetic diversity		
			PPB (%)	h	I
KY	<i>Fritillaria thunbergii</i> Miq.	Zhangshui, Zhejiang province	74.42	0.2364	0.3586
DZ	<i>Fritillaria thunbergii</i> Miq.	Yinjiang, Zhejiang province	29.46	0.0977	0.1476
XY	<i>Fritillaria thunbergii</i> Miq.	Longguan, Zhejiang province	28.68	0.0971	0.1464
XB	<i>Fritillaria thunbergii</i> Miq.	Hengjie, Zhejiang province	37.98	0.1286	0.1934
Species-level	-	-	91.47	0.2433	0.3811
CB	<i>Fritillaria cirrhosa</i> D. Don	Lixian, Sichuan province	31.78	0.1182	0.1741
WB	<i>Fritillaria anhuiensis</i>	Bozhou, Anhui province	23.26	0.0867	0.1275

Table 1 Geographic localities and genetic diversity of three species

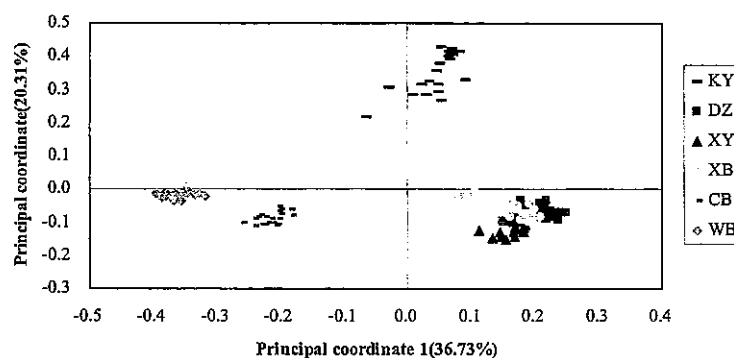


Fig.1 Principal Coordinates analysis (PCoA) using ISSR data of 120 individuals of *Fritillaria thunbergii* landraces

Cloning and Molecular Characterization of Microsomal Oleate Desaturase Gene (*FAD2*) from *Safflower* (*Carthamus Tinctorius* L.)

Ling-Liang Guan, Ying-Wen Xu, Yuan-Biao Wang, Jin-Feng Shao, Wei Wu*

Agronomy College, Sichuan Agricultural University, Sichuan

Linoleic acid is an important Polyunsaturated fatty acid (PUFAs), which plays crucial role in plant and human health. Very recently, the potential roles of linoleic acid in reducing the heart disease and improving the vision sensitivity have been verified. The enzyme responsible for the conversion of oleic to linoleic acid is delta 12- desaturase. Safflower (*Carthamus tinctorius* L.), which is the richest plant source of linoleic acid, contains 75% linoleic acid in its seed oil. But no information about the mechanism of the biosynthesis of linoleic acid in this plant has been reported so far. Based on the sequence information of the published delta 12- desaturase genes from other higher plant species, a cDNA sequence putatively encoding a delta 12- desaturase was isolated from the total RNA of immature safflower seeds using the combination of reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) method. Sequence analysis indicated that it had an open reading frame (ORF) of 1140 bp, coding for 345 amino acid residues of 43 kDa. The deduced amino acid sequence of the cloned *FAD2* showed the three histidine residues characteristic of all membrane-bound desaturase and a C-terminal endoplasmic reticulum retention signal. Phylogenetic analysis shows that *CtFAD2* is grouped with other plant *FAD2* sequences which were specifically or highly expressed in developing seeds. Functional identification was done heterologously in *E. coli* (DE3) pLysS and *Saccharomyces cerevisiae* strain INVSc1. The results confirmed that the cloned sequence encodes an approximately 43kDa membrane-bound microsomal oleate desaturase, partially convert oleic acid to linoleic fatty acid by analyzed with SDS-PAGE and GC-MS. This is the first time of cloning and expression analysis of *FAD2* genes from safflower. Further characterization of the mechanisms that regulate the safflower linoleic acid synthesis, such as the copy numbers and the tissue expression, is currently underway in our group.

Keywords: *Carthamus tinctorius* L.; Microsomal oleate desaturase (*FAD2*); *Saccharomyces cerevisiae*; Prokaryotic expression; Linoleic acid

An observation for the cultivation of TCM herbs

Chong-Ren Yang, Hui Cao and De-Shang Deng

Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan

National Engineering and Research Center for Modernization of TCM, Guangdong

It is well known that herbs are the origin of TCM. The sustainable supply of crude herbal materials with high quality is important not only for the TCM industry but also for the clinical application of TCM. As a necessary and developed measure, the cultivation of TCM herbs occurred throughout the country in recent twenty years. The large scale cultivation of a variety of herbs is becoming one of the new industries in villages and the GAP system is recommended to be put in practice. In order to build the modern herbal agriculture, there are a lot of academic researches still needs doing. Such as the problem of germplasm resources, quality controls, cultivation modes, cultivate and primary processing techniques, as well as coordinate development between the agriculture and industry of TCM herbs. In this paper, a brief observation of the cultivation of TCM herbs is carried out. Some important herbs, such as notoginseng, *Paris polyphylla*, *Erigeron breviscapus*, as well as *Lonicera japonica*, will be discussed.

AgDNA Microarray for Genotyping Danshen (*Salvia Miltiorrhiza*) and Other Economically-important *Salvia* Species

Alexandra Olarte, Nitin Mantri, Gregory Nugent, Chun Guang Li, Charlie Xue and Edwin Pang
RMIT Health Innovations Research Institute, RMIT University, Australia

This study aims to generate a microarray containing polymorphic and divergent DNA sequences for fingerprinting several economically important members of the genus *Salvia*. Suppression subtractive hybridization between a pool of ten *Salvia* species and a pool of non-angiosperm and angiosperms (excluding Lamiaceae) was used to selectively enrich the array for *Salvia*-specific sequences. A total of 285 cloned genomic fragments were amplified and arrayed. DNA fingerprints were obtained for twenty *Salvia* genotypes including three which were not part of the original subtraction pool. Cluster analysis revealed genetic relationships consistent with geographical origins. Three major clusters were obtained; the first one included Danshen provincial varieties and two Chinese species, *S. sinica* and *S. przewalskii*. The second cluster included the European *S. officinalis* (three accessions) and *S. lavandulifolia*, while the third cluster included the five American and African species. Species-specific features were also found for *S. elegans*, *S. officinalis*, *S. glarea*, *S. przewalskii*, *S. runcinata*, *S. lyrata* and *S. fruticosa*. The results show that the array was not only able to fingerprint effectively species and varieties of *Salvia* that were used to develop the array, but also was able to fingerprint *Salvia* species that were not used in its construction. This technique has the potential to be used in phylogenetic analyses, simultaneous marker-assisted selection of several loci and future plant breeding programs of non-model organisms such as Danshen.

Acknowledgments: The authors gratefully acknowledge the support from the RIRDC, RMIT University, the Australian Postgraduate Scholarship, Hans Wohlmuth from the Southern Cross University and Reg Lehmann from MediHerb.

Study on Extracting *Magnolia officinalis* by Using O/W Microemulsion

Hong Yi, Liya Sun, Jin Gao, Yanfei Ren, Hua Yang

Institute of Chinese Materia Medica, China Academy of Chinese Medical Science, Beijing

Objective: To investigate the feasibility of extracting the fat-soluble compounds from *magnolia officinalis* with O/W microemulsion. **Method:** To measure the particle size of blank microemulsion by dynamic light scattering when it is being heated and compare with its viscosity and other physical index. To choose O/W microemulsion, water and ethanol as solvents to extract *magnolia officinalis* and determine the content of magnolol and honokiol in extracts of *magnolia officinalis* by HPLC. Compared with efficiency of extracting *magnolia officinalis* by using the different media, different formulations of O/W microemulsion and using different extraction methods and the differences of *magnolia officinalis*' extracts by TLC. **Result:** The extracting yield of magnolol and honokiol are more than 90% by using microemulsion at mean time, which is similar to 60% ethanol. The formulations of microemulsion and extracting methods have remarkable effect for extracting the fat-soluble compounds from *magnolia officinalis*. There is no significant difference for blank microemulsion pre and post heated. **Conclusion:** It is feasible to extract *magnolia officinalis* by using O/W microemulsion as a solvent. It could not only ensure the extracting efficiency of fat-soluble compounds, but also avoid the usage of organic solvents, save energy and reduce the links of manufacture, which are beneficial to the sustained development and low-carbon economy of Chinese herbs.

Study on Extracting *Angelica Sinensis* by Using O/W Microemulsion

Gao Jin, Yi Hong, Ren Yanfei, Yang Hua

Institute of Chinese Materia Medica, China Academy of Chinese Medical Science, Beijing

Microemulsions are clear or opalescent, isotropic and thermodynamically stable dispersion, which are mixtures of oil, water and surfactant, frequently in combination with a cosurfactant. With its excellent solubilization ability both for water-insoluble and water soluble constituents, this study's goal is to use microemulsion as a new solvent to extract *Angelica Sinensis*. First, a HPLC method for the determination of ferulic acid from microemulsion extracts of *Angelica Sinensis* was established. Through the solubilization of ferulic acid and determining the content of ferulic acid by HPLC, we can find in various solvent systems, microemulsions there are some differences. Next, compared with different solvents (microemulsions, water) and different extracting methods (reflux, microwave, ultrasonic) to extract *Angelica Sinensis*, the extraction rate of microemulsion and factors of extraction were investigated. In order to determine the content of ferulic acid. The result showed that the extraction rate of microemulsion was up to 99% and it has the similar ability of 50% ethanol, and higher than water. The more amounts of surfactant and cosurfactant, the more extraction rate of microemulsion. Different extracting methods result in significant difference and it is the highest extraction rate for refluxed extraction. At the same time, microemulsion extraction of *Angelica Sinensis* by TLC proved microemulsion has a similar power with 50% ethanol which could extract both water-insoluble and water soluble constituents. In conclusion, it appears that microemulsion has the advantage of wide dissolution range and could not only increase solubility of water-soluble constituents, but also water-insoluble constituents. It could simplify the links of manufacture and consume less energy, which is more environmentally friendly without using ethanol, which are beneficial to the sustained development of low-carbon economy of Chinese herbs.

Screening of Bioactive Components in Cerebrospinal Fluid after Administration of Chinese Medicine

Peng Zhang, Shuo Meng, Jianxun Liu

Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing

There are two major directions in the research methods to study the active components of Chinese herbs. One is to separate the active elements one by one from the single or mixed herb, and then to screen the obtained monomer by pharmacological activity. The other is to extract the active components under the direction of biological activity. Once, some researchers combined these two directions to propose the term "seropharmacology". But the neurological drug screening presents particularity due to the blood-brain barrier. So not all components can get through blood-brain barrier, Only components which go through blood-brain barrier perhaps is bioactive components. Therefore the present study concentrated on the target, i.e., bioactive components in the cerebral spinal fluid (CSF), and reconfirm the activity by pharmacological test.

In this paper, we detect chemical components in cerebrospinal fluid by puncturing cistern after administration of Chinese medicine. Remove protein by adding methanol precipitation, detect with HPLC and HPLC-MS method.

Three components were found in the CSF of normal dogs. Total flavone in *Radix Puerariae* was detected, and we found Puerarin, Daidzins and isoflavones Aglycone. Using nerve cell culture *In vitro*, by MTT staining, three of them showed the neurological protection in isolated cells suffering from hypoxia and lack of glucose.

Use of ITS2 Region as the Universal DNA Barcode for Plants and Animals

Hui Yao¹, Jingyuan Song¹, Chang Liu¹, Yingjie Zhu², Kun Luo³, Jianping Han¹, Ying Li¹, Hongxi Xu⁴, Shilin Chen¹

¹Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing

²School of Bioscience and Engineering, Southwest Jiaotong University, Chengdu

³College of Pharmacy, Hubei University of Chinese Medicine, Wuhan

⁴Hong Kong Jockey Club Institute of Chinese Medicine, Hong Kong

The Internal Transcribed Spacer 2 (ITS2) region of nuclear ribosomal DNA is regarded as one of the candidate DNA barcodes because it possesses a number of valuable characteristics, such as the availability of conserved regions for designing universal primers, the ease of amplification, and the presence of enough variability to distinguish even closely related species. However, a general analysis of its ability to discriminate species in a comprehensive sample set is lacking. In current study, a total of 31,596 dicotyledon, 10,572 monocotyledon and 10,758 animal ITS2 sequences were evaluated according to sequence length, GC content, intra- and inter-specific divergence, and the efficiency of identification. The results show that the inter-specific divergence of congeneric species in dicotyledons, monocotyledons and animals was greater than their corresponding intra-specific variations. Furthermore, the success rates for using the ITS2 region to identify dicotyledons, monocotyledons and animals were 76.8%, 74.0% and 91.9% at the species level, respectively. Moreover, the ITS2 region unveiled a different ability to identify closely related species within different families and different genera. In addition, the secondary structure of the ITS2 region could provide useful information for species identification and could be considered as a molecular morphological characteristic. Therefore, as one of the most popular phylogenetic markers for eukaryota, we proposed that the ITS2 locus should also be used as a universal DNA barcode for identifying plant species and as a complementary locus for CO1 to identify animal species. Finally, to facilitate ITS2-based cross-kingdom species identification, we have developed a web application (<http://its2-plantidit.dnsalias.org>).

The Strategies on Medicinal Plant Genome Sequencing Project

Shi-Lin Chen*, Hong-Mei Luo, Jiang Xu, Yong-Zhen Sun, Chao Sun, Liu He

¹Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing

The next-generation sequencing technologies, represented by Roche/454 GS FLX, Illumina/Solexa and ABI/SOLiD, have achieved rapid development and wide application and provide an effective platform for whole genome sequencing of medicinal plants. The progress of plant genome sequencing was briefly introduced and a comprehensive strategy of whole genome sequencing for medicinal plants was illustrated in detail in this paper. The medicinal plants possessing physiological and genetic characteristics of model plants, obtaining mature tissue culture and genetic transformation system, having research foundation of pharmaceutical chemistry and functional genomics as well as significant pharmacological effect could be selected for whole genome sequencing. The option of using favorable platforms to sequence the whole genome of selected species will facilitate to sequence assembly and gene annotation, combined with genetic maps and physical maps to draw draft. Complete genomic information will lay a solid foundation for comprehensive analysis of the secondary metabolic pathways, growth and development, and molecular mechanisms of disease/stress resistance in medicinal plants.

Flow Cytometric Analysis of Nuclear DNA Content in *Poria cocos*

Ya-zhi WANG, Qiu-shi LI, Jing-yuan SONG, Chao SUN, Shilin CHEN*

Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences & Peking University Medical College, Beijing

Poria cocos, which belongs to Polyporaceae in basidiomycetes, is a well known Traditional Chinese medicine (TCM) for centuries. However, the genetic background of *Poria cocos* is relatively unclear. To elucidate the fungal genome size which is essential for genome sequencing projects, the present study aimed to estimate DNA content as well as the relationship between ploidy and DNA content. The fungal material was prepared according to a protocol adopted from Jaroslav Dolezel et al. (2007) and stained with propidium iodide (PI) which is a nucleic acid dye. For this study, a nuclei isolating method was optimized by adjusting centrifugal force and staining period. The DNA quantity was quantified by flow cytometry and calculated as a ratio between mean fluorescence of the G₀/G₁ peak of the *Poria cocos* and *Aspergillus niger*. The *Aspergillus niger* was used as an internal reference. The result showed *Poria cocos* was mixoploid, consisting of cells with 2C, 4C DNA content. After comparing the peak of the fluorescence intensity of *Poria cocos* and *Aspergillus niger*, the estimated genome size of *Poria cocos* is 57.50±0.74 Mb, or 2C DNA about 0.12pg (1pg=0.978×10⁹bp). The main coefficient values (CV) below 5% obtained for *Poria cocos* and *Aspergillus niger* showed that the extraction and staining resulted in the best histograms in this study. In summary, the present study described a modified method of genome size analysis by using flow cytometry in *Poria cocos*. The availability of DNA content in this study is helpful with the future genome sequencing research.

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Studies on Macroscopic and Microscopic Identification of *Cordyceps Sinensis* and its Counterfeit

Siutsau Chan, Zhongzhen Zhao, Hubiao Chen*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Composite consisting of the stroma of *Cordyceps sinensis* (Berk.) Sacc. (Clavicipitaceae) parasitizing the insects belonging to the Hepialidae family, and of the larva corpse. In the recent 20 years, the trend of Hong Xia Cao (*Cordyceps*) is rapidly developed. The counterfeit have been discovered as result of resorting. Since the fungi of *Cordyceps* and confused products mostly come from same genus, it is not easy to distinguish each other. This article is providing a rapid, simple, accurate and reproducible identification method from which *Cordyceps* can be distinguished from other species, including *Cordyceps hawkesii* Zang, *Cordyceps liangshanensis* Zang, Hu et Liu, *Cordyceps gracilis* (Grav.) Dur. et Mont and *Cordyceps crassipes* Wang, Yang et Liu. According to the literature, macroscopic identification mostly applied to distinguish different species of *Cordyceps*, or only microscopic identification of *Cordyceps sinensis* and *Cordyceps hawkesii*. The stroma's transverse section from part of literature, so the aim of this article is to optimize the identification method. The result is revealed that *Cordyceps* can be distinguished from other counterfeits by macroscopic and microscopic identifications. For macroscopic, only stroma of *Cordyceps sinensis* is mostly non-inflated, and its stipe at the tip, the caterpillar annulations of *Cordyceps sinensis* and the *Cordyceps gracilis* is distinct. The number of annulations is 1-30, and feet of above two are 8 pairs, 4 of 8 pairs in the middle are relatively distinct. There are 8 pairs of gills on the back of *Cordyceps sinensis* is clear, the above appearance shows its unique characteristics. For microscopic identification, only *Cordyceps sinensis* exists microtrichia, the tip is pointed. The arrangement of setae is irregular, the tip is blunt while the basal is gradually broader; the top of some setae is slightly like a hook. The above characteristics can provide basis for powder microscopic identification of *Cordyceps sinensis*.

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The Study of Hong Kong Chinese Materia Medica's Concocted Processing Characteristics

Tung-Kin Cheung, Hu-Biao Chen*, Zhong-Zhen Zhao

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Along, the people are few to research Hong Kong Chinese Materia Medica's concocted processing, and therefore the study of Hong Kong Chinese Materia Medica's concocted processing characteristics, to establish experience identifying method of the Hong Kong decoction pieces and guiding clinical use of Chinese Medicine is important. This article combines the survey which in Hong Kong the Chinese Medicine developed in the past, through sample collection, select a large difference between Hong Kong and Mainland decoction pieces, for comparison, and investigate commodity specifications of the decoction pieces on the Hong Kong market, after analysis induction: That the processing characteristics, the Hong Kong Chinese Materia Medica's processing mainly in stress authentic, quality, usual sell high-grade high-standard medicine, the pursuit of decoction pieces handsome good looks, combined with characteristics of Lingnan culture in Chinese Medicine, antique processing. The decoction pieces contour majority were tied up neatly, large and color pretty famous, mainly longitudinal slices, mostly artificial opening cut, handsome rules, some of them, after cutting through the special treatment, dispenses the medicament is extremely neat, no ash dregs doping; The decoction pieces concocted with steam over mainly to meet Hong Kong's hot and humid climate characteristics of drug use, its clinical treatment highlighted through XinTouQingQi, reconciliation Qushi, invigorating Qi, promoting the production of the body fluid, and the effect of clearing heat and nourishing yin; The decoction pieces of goods effect the business specifications are more than the pursuit of high quality features.

Microscopic Study on the Bark of *Schefflera Octophylla* and *Bombax Malabaricum*

LEE Lai-mei, ZHAO Zhong-zhen, Liu Bao-ling, CHEN Hu-biao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

The bark of *Schefflera octophylla* and *Bombax malabaricum* are commonly used in China for a long time. However, the research about these two Chinese medicines is not very comprehensive. To establish a quality control method for the bark of *Schefflera octophylla* and *Bombax malabaricum*. A comparison study was carried out on both of them with microscopy and on their source. The micro-morphological characteristics of two Chinese medicines have been documented and compared. Their corresponding features were described and documented with color digital micrographs, so as to authenticate the presence of genuine crude constituents in two Chinese medicines. To summarize the results, the bark of *Schefflera octophylla* is covered with patches of lichen in the outer surface and has fine longitudinal striations in the inner surface. It is granular in fracture and it odors slightly with astringent taste. Under microcopy, several layers of cells containing yellowish-brown substances can be found in the cork. Meanwhile, groups of stone cells and prisms of calcium oxalate scattered in the cortex. Phloem rays are 3~5 cells wide, and containing numerous of fiber bundles. As for the bark of *Bombax malabaricum*, it has a brown outer surface covered with stout prickles with woody conical bases. The fracture is fibrous and it odors slightly with astringent taste and viscous on chewing. Under the microcopy, several layers of cells containing brownish-red substances can be found in the cork. Clusters of calcium oxalates and secretary cells can be found also in the cortex. The phloem rays are 5~12 cells wide, and containing numerous of fiber bundles.

Differentiation of the Sweet and Bitter Variants of *Gynostemma pentaphyllum* Based on their Diterpenoid Saponin Profiles and Ribosomal DNA Sequences

Pui Kei Wu,^a William C.S. Tai,^a Roy C.Y. Choi,^b Karl W.K. Tsim,^b Hua Zhou,^a Xin Liu,^a Zhi-Hong Jiang,^a W.L. Wendy Hsiao^{*a}

^aSchool of Chinese Medicine, Hong Kong Baptist University, Hong Kong

^bDepartment of Biology, Hong Kong University of Science and Technology, Hong Kong

Gynostemma pentaphyllum (Gp) Makino, a medicinal herb called Jiaogulan, is used in traditional medicine for lowering cholesterol levels, regulating blood pressure, alleviating gastritis, and reducing inflammation. In addition to the traditional medical applications, *in vitro* and animal studies showed that Gp possess anti-oxidant, anti-apoptotic, and anti-carcinogenic activities. The major active components of Gp are a series of dammarane-type saponins, which are also known as gypenosides. More than 100 gypenosides have been isolated and identified from Gp. Bitter and sweet variants of Gp have been documented, but cannot be differentiated morphologically and there is little information on their chemical and phylogenetic properties. Because the variants also differ pharmacologically, the inability to distinguish the two poses a serious problem in quality control of herbal products containing Gp. In the present study, the chemical fingerprinting of Gp saponins in the sweet and bitter variants that were collected from different regions of China were determined by HPLC-DAD and HPLC-ESI-MS analysis. Data shows that the Gp saponins in the sweet and bitter variants have distinct and non-overlapping profiles. Sequencing data of the ribosomal ITS region shows that the two variants share only 69.01% homology in the ITS-1 region, suggesting a phylogenetic distinction between these two variants. The combinations of chemical profiling and phylogenetic analysis further confirm the distinction between these two taste variants. This information has direct application in the authentication and quality assessment of medicinal and health products of Jiaogulan.

Acknowledgements: We are grateful to Mr. Alan H. M. Ho from HKBU for his technical assistance in HPLC-DAD and HPLC-ESI-MS analysis. We also appreciate Dr. Martha Dahlen's contribution in editing the manuscript. This study was financially supported by Research Grants Council of Hong Kong under HKBU2/07C and HKRGC 260307 Grants to WLW HSIAO.

A Statistic Analysis for the Development of TCM Products

Yang, Zhijun; Ivan Wong

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

There are over 1420 TCM formula products listed in the China pharmacopeia, national health insurance & work injury insurance drug list, and national essential drug list of China. However, only 556 TCM raw materials were listed in the China Pharmacopeia 2005-edition, and about 150 TCM raw materials were added in 2010-edition. Therefore, the efficacy differences between TCM products for same disease treatment are attention-getting. Here 210 TCM formulas which are generally used in China and Japan were chosen to statistical analysis in order to search the efficacy differences between the TCM products which are used to treatment the same symptom. These formulas may be manufactured as market products in several dosage forms for oral administration or transdermal drug delivery only, on the other hand, most of them may also be directly dispensed to the patients by TCM practitioners or pharmacists. These formulas are classified to treatment 21 disorders, diseases and symptoms in internal medicine, gynecology, and dermatology. The crude drugs in the formulas were divided into 3 groups as the mostly-use, normal-use and few-use TCM raw materials. The small molecular chemical ingredients of the crude drugs were investigated and compared. As known to all, there are about 25 percent western medicines are gotten from herbs. Therefore, from the standpoints of pharmacy and cure of diseases, drugs should not to be labeled into Chinese, or Indian, or western medicine, etc. The statistical analysis may establish an order of priority for re-evaluation the TCM formulas, and help pharmaceutical researchers to evaluate the potential of the development new drugs from TCM, and may also help the TCM practitioners to make a compendious and effective prescription to a special patient with special disorders. Meanwhile, the herbal resources and bio-environments might be well protected and higher efficiency herbal products may achieve the goals of public health services.

Authentication of Chinese Materia Medica Decoction Dregs by the Comparison of Morphological and Microscopic Characteristics before and after Decoction

Queenie Lailai Wong, Zhitao Liang, Hubiao Chen, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Decoction dregs are the residues of medicinal materials after decoction. Accurate identification of CMM in decoction dregs will be helpful for exploring the causes of poisoning or other medical incidents arising after the decoction of CMM decoctions. In order to determine how decoction affects the characteristics used to authenticate specific CMM, a systematic study was carried out. In this study, four pairs of Materia Medica that are commonly confused – namely, Baizhu (*Rhizoma Atractylodis Macrocephalae*) and Cangzhu (*Rhizoma Atractylodis*), Baishao (*Radix Paeoniae Alba*) and Chishao (*Radix Paeoniae Rubra*), Fenge (*Radix Puerariae Lobatae*) and Shanyao (*Rhizoma Dioscoreae*), Shu Dihuang (*Radix Rehmanniae*) and Huangjing (*Rhizoma Polygoni*) – were chosen for investigation. Each pair of Materia Medica has similar morphology in appearance but they have different functions in Chinese clinic. After decoction, with regard to gross morphological characters, the results showed that bark and wood could be easily distinguished. The striation of vessels and fibres became more prominent due to the contraction of parenchymatous cells but the lignified vessels did not. As for the microscopic characteristics, the cells with thickened walls, such as stone cells and sclerenchyma, were basically stable. Most of the parenchymatous cells were broken. Crystals of calcium oxalate showed no changes as they were insoluble in water. Starch granules were gelatinized and aggregated in parenchymatous cells. Inulins were substantially reduced in number as they dissolved in water during decoction. According to these changes in morphological and microscopic characteristics after decoction, the dregs of four pairs of Materia Medica could be distinguished. Further researches on toxic and potent CMM decoction dregs are in progress. The authentication of CMM decoction dregs will be very helpful to ensure the therapeutic effects and clarify the causes of poisoning or medical incidents after taking decoction.

The Application of Fluorescence Microscopy in the Authentication of Dregs and Processed Products of Chinese Materia Medica

Zhitao Liang, Queenie Lailai Wong, Hubiao Chen, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

The microscopic characteristics of crude herbal materials, dregs and processed products of CMMs are very similar. It is difficult to differentiate them by normal light microscope. The fluorescence microscope can precisely determine which plant tissues emit fluorescence when illuminated. After decoction or processing, the chemical profiling of herbal dregs or processed products can change. The changed chemical profiles may produce different fluorescent characteristics. In order to explore a new method for the authentication of dregs and processed products of Chinese Materia Medica, the fluorescence microscope was applied to differentiate the raw and processed *Radix Polygoni Multiflori* (RPM) as well as the raw and processed *Radix Rehmanniae* (RR) corresponding with their decoction dregs. The RPM and RR are commonly used CMMs in clinic. In the herbal markets, some merchandisers will paint black color on the surface of raw RPM as its processed products for sale. For RR, it is difficult to distinguish the raw and processed products as they are very similar in appearance after decoction. When observed under fluorescence microscope, the results showed that the fluorescent characteristics and intensity of raw and processed samples as well as their dregs were different. In general, the fluorescent color changed and the intensity becomes weakened. For example, investigated under the excitation filter BP 450-490 nm and LP 515 nm emission filter, the cortex emitted yellowish-green fluorescence and normal phloem parenchyma as well as xylem parenchyma emitted bright yellow fluorescence in the transverse section of the raw RPM; while the cortex, normal phloem parenchyma and xylem parenchyma emitted brownish-yellow fluorescence yellow fluorescence in the transverse section of the processed products. The fluorescence microscope can be helpful for the authentication of raw materials, processed products and the decoction dregs from the same CMM.

Identification of Powdered Chinese Materia Medica by Fluorescence Microscopy

Yaqiong Wang, Zhitao Liang, Qin Li, Hua Yang, Hubiao Chen, Ping Li, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

The light microscope has been successfully used in identification of Chinese Materia Medica (CMM) for more than a century. However, positive identification is not always possible. Given the popularity of fluorescence microscopy in bioanalysis, researchers dedicated to finding new ways to identify CMM more effectively are now turning to fluorescence microscopy for authentication purposes. Some studies on distinguishing confused species from the same genus and on exploring distributions of chemicals in tissues of CMM by fluorescence microscopy have been reported; however, no systematic investigations on fluorescent characteristics of powdered CMM have been reported. Our recent investigations showed that many microscopic features of powdered CMM emit fluorescence rendering them easily observed, even against complex backgrounds. Under the fluorescence microscope, different microscopic features from the same powdered CMM or, in some cases, the same features from different powdered CMMs fluoresced differently. This information is very helpful for the authentication of CMM in powder form. Hence, fluorescence microscopy could be a useful additional method for the authentication of powdered CMM if the fluorescent characteristics of specific CMM are known.

Microscopic Identification of Commonly Used Chinese Materia Medica in Hong Kong

Hua Yang, Yaqiong Wang, Li Liang, Ping Guo, Hubiao Chen, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Chinese Materia Medica (CMM) has been well accepted by the community of Hong Kong. However, questions about its safety and efficacy have been raised internationally after toxic effects and even poisonings have been reported, caused by misusing or confusion of CMM. Most of the CMMs distributed worldwide are sourced from Hong Kong; thus, establish CMM standard in Hong Kong is increasingly critical to the safe and effective use of CMM throughout the world. Toward this end, the Hong Kong government has recently established the Hong Kong Chinese Materia Medica Standards (HKCMMS). Microscopic identification is an important component of the HKCMMS. In this work, the macro- and micro-scope identification standards of 6 commonly used CMMs, namely Herba Gynostemmatis, Rhizoma et Radix Baphicacanthis Cusiae, Radix Isatidis, Spica Schizonepetae, Fructus Corni and Herba Houttuyniae, which will be recorded in HKCMMS are systematically described and illustrated. In addition, the characteristics by which confused species of some CMMs can be distinguished are also described. For example, Herba Gynostemmatis, a commonly used drug and health product, is usually confused with the dried herb of *Cayratia japonica* (Thunb.) Gagnep. due to their similar macroscopic characteristics. Under the microscope, differences of transverse sections and the presence or absence of raphides of calcium oxalate in power readily distinguish the genuine Herba Gynostemmatis. More of this kind of work, with results recorded and disseminated worldwide, will reduce the risk of misuse and mistakes arising from misidentification, and will ensure continued safe and effective use of CMMs.

Market Investigation and History of Spicy and Aromatic Chinese Medicinal Materials

Menghua Wu, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Spicy and aromatic Chinese medicinal materials (SACMM) is one category of Chinese medicinal materials with its own history and pattern of usage. Most SACMM contain significant amounts of essential oils that give them strong aromas and/or tastes. In traditional Chinese medical theory, SACMM are warm in nature; their functions include resolving hard masses, moistening dryness, dispersing qi, inducing perspiration, and tonifying and invigorating the body. SACMM are used not only medically, but also in Chinese cuisine. In this study, we examined three aspects of SACMM. First, we investigated the current usage of SACMM in medicine: the species used; the amounts and extent of their usage; their inclusion in patent medicines; their availability in commercial markets. Second, we examined the use of SACMM in cuisine. Third, we considered the history of usage of SACMM. In the past, a relatively few species were used, whereas today several hundred species are used. This increase reflects acculturation throughout Chinese society, as China became exposed to and absorbed many elements of foreign cultures—including vegetables, spices and herbs.

Macroscopic Identification of Chinese Medicinal Materials in Commerce

Zhongzhen Zhao*, Hubiao Chen, Ping Guo, Menghua Wu, Zhitao Liang
School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

According to the statistics, over 11,000 botanical species, nearly 1600 zoological species, and 80 mineral substances are used medicinally in China. However, the lack of precise information on diagnostic features of specific medicinal materials remains one of the constant concerns for the safety and effectiveness of Chinese medicinal materials (CMMs). Macroscopic identification has proven to be a practical and fundamental method for the authentication of CMM. By observing, touching, smelling, tasting, and testing with fire and/or water, characteristics of CMM such as shape, size, color, surface, texture, cross-section, odor and taste are used to identify the genuineness and quality. In this study, macroscopic identifications on 429 commonly used CMMs in commerce and their decocting pieces, including 383 botanical, 32 zoological, and 14 mineral substances, have been conducted. Samples were collected from herbal markets and pharmaceutical corporations all over China. Voucher specimens are deposited at Bank of China (Hong Kong) Chinese Medicines Center. The research results are presented as 429 richly illustrated monographs. The origin(s), production areas, harvesting and preliminary processing, functions and indications based on traditional Chinese medicine concepts, macroscopic characters, and quality requirements of entire or cut CMMs are included in each monograph. Core identification features are highlighted and marked in over 1000 high resolution digital pictures of medicinal materials. This study is a useful tool to enable the supply of fully authenticated CMMs, and has played an important role in the safe use of CMMs both home and abroad.

Ethnobotanical Study of 24-Herb Liangcha Used by Cantonese in Lingnan

Wing Ping Yeung, Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Background: In Southern China, people have the habit of drinking Liangcha (herbal teas which has a cooling function) to treat subclinical symptoms or maintain health. Acknowledging the value of this popular custom, the State Council of China included Liangcha on China's first Intangible Heritage list in 2006. Liangcha formulae are indeed precious and worthy of conservation. Dr. Hu Shiu Ying listed 65 species as the Liangcha ingredients in her paper in 1997; this is the only paper studying Liangcha to date. Here, 24-Herb Liangcha was studied. What is its composition? Is the formula fixed?

Method: 185 Chinese medicine shops in Hong Kong, Shenzhen and Guangzhou were visited and 26 brands of 24-Herb Liangcha samples were collected. Totally 64 different decoction pieces were summarized and were authenticated. 14 with highest frequencies were further studied microscopically.

Result: The most common ingredients were: *Herba Lophatheri*, *Herba Polygoni Chinensis*, *Radix Ilicis Asprellae* (30%); *Caulis et Folium Cratoxylis cochinchinensis* (28%); *Folium Microcotis* (25%); *Folium Eriobotryae* (22%); *Caulis Melicope* (20%); *Cortex Schefflerae Octophyllae*, *Herba Lygodii japonici* (16%); *Herba Elephantopi*, *Folium Vitis Negundo*, *Ramulus Mori*, *Folium Mori*, *Herba Desmodii Styrcifolii* (14%). The number of decoction pieces in one packet varied from 7 to 27.

Discussion: In this study, we found 28 species were not on Dr. Hu's Liangcha ingredient list. 24-Herb Liangcha is believed to contain 24 decoction pieces initially. We think the reason that the formula varies greatly is that it lacks a fixed formula and such preparation is also very difficult. In the future, the formula should be standardized to have its quality controlled.

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Application of Forensically Informative Nucleotide Sequencing (FINS) in the Identification of Traditional Chinese Medicine

LI Ming, WONG Ka-Lok, BUT Pui-Hay, SHAW Pang-Chui*

Department of Biochemistry, Department of Biology and Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong

Modernization and economic importance of traditional Chinese medicine (TCM) has increased over the decade and are likely to continue. However, counterfeit, poor quality, and adulterated TCM in the market are serious patient safety threats and the quality assurance is a bottleneck for the modernization and globalization of TCM. Molecular techniques have been applied to identify TCM for two decades and forensically informative nucleotide sequencing (FINS) is recently recognized as one of the most powerful techniques. The objective of this presentation is to introduce the application of FINS approach in the identification of TCM. Baihuasheshecao and Madouling as examples. FINS is comprised of four basic steps: (1) DNA extraction from biological samples; (2) amplification of a specific nucleotide segment; (3) determination of nucleotide sequence of the amplified nucleotide segment by DNA sequencing, and (4) phylogenetic analysis of the closest related species with reference to a database. By choosing appropriate nucleotide segments and reference database, this approach can be used to differentiate closely related species, to distinguish genuine TCM from adulterated material as well as to identify any unknown herbal material. We have recently applied FINS based on internal transcribed spacer to distinguish Baihuasheshecao (*Hedyotis diffusa*) from its adulterant derived from *H. corymbosa*. FINS identified seven samples from mainland China, Hong Kong and Boston. FINS based on trnL-trnF and psbA regions have been applied to differentiate Madouling (*Aristolochia contorta* and *A. debilis*) from its adulterant substitute derived from *Cardiocrinum giganteum* var. *yunnanense* and four samples from mainland China and Taiwan have been identified. FINS is an objective and powerful tool for identification and quality assessment of TCM which provides fundamental bases for the modernization and globalization of TCM.

Research and Development on the Taiwanese Traditional Chinese Medicinal Crop-Salvia miltiorrhiza (Tanshen): Chemical Profiling

Tung-Ying Wu, Chih-Yuan Lin, Wan-Yu Lin, Ying-Chi Du, Yang-Chang Wu, Fang-Rong Chang
College of Pharmacy, Kaohsiung Medical University, Kaohsiung

Tanshen, the rhizome of *Salvia miltiorrhiza*, is one of the most commonly used traditional Chinese Medicines. It is widely accepted as a health product in the western countries in recent years owing to its remarkable and reliable biological activities, especially in the treatment of cardiovascular disorders. There are two classes of major active constituents reported in Tanshen, lipophilic tanshiones and hydrophilic polyphenols. Among them, tanshione IIA and salvianolic acid B are the two major bioactive markers highly regarded for their anti-inflammatory and anti-oxidant activities, individually.

Besides the medicinal use, Tanshen is well-known for its aroma used as a source of perfume ingredients in Taiwan. However, the price of Tanshen is rapidly increasing due to the limitations in import from China, the main producer of *S. miltiorrhiza* rhizomes. The problems of food safety, including the residues of pesticides and heavy metals, and also the misuse of the original herbal medicines, were still concerned for the consumers. In establishment of the industry of traditional herbal medicine, cultivated *S. miltiorrhiza* was selected and developed as the treasure crop by Hualien Prefectural Agricultural Experiment Station (HPAES) on the basis of adaptation to local conditions. Nowadays, the extensive cultivation of Tanshen is successfully taking place in Hualien based after six years of research and development. Tanshen products are used in cooking as fresh food material, and can be used for traditional Chinese medicine and biotechnology products.

In this study, qualitative and quantitative analysis of various Tanshen products from different sources were obtained. The identification of several bioactive markers (tanshione IIA and salvianolic acid B) was carried out and compared by high-performance liquid chromatography with a photodiode-array detector (HPLC-PDA). The results showed that the HPLC fingerprints of Tanshen products from different sources were investigated. The content of tanshione IIA and salvianolic acid B in Taiwanese *S. miltiorrhiza* was competitive to that of different origins.

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Effect of *Polygala tenuifolia* on Penile Erection and Isolation of Two Phenolic Glycosides by HSCCC

Ji Young Kim, Yoonjung Kim, Sunghyun Lee, Mikyung Song, Dongwook Lim, Mi-Yeon Kim, Hocheol Kim

College of Oriental Medicine, Kyung Hee University, Korea
NeuMed Inc, Korea

This study was conducted to investigate the effect of the root of *Polygala tenuifolia* on electric stimulation-induced penile erection in rats and isolated the active constituents by HSCCC. The *P. tenuifolia* was extracted in 70% EtOH by using reflux apparatus and fractionated successively with *n*-hexane, EtOAc, *n*-BuOH, and EtOH fraction. Intracavernous pressure (ICP) and mean arterial blood pressure (MAP) were simultaneously monitored during electrical stimulation of the cavernous nerve, before and after oral administration of the *P. tenuifolia* extracts (10, 30, 100 and 300 mg/kg) and its fractions (100 mg/kg) in rats. The 70% EtOH extract was fractionated by column chromatography over silica gel with CHCl₃/EtOAc/MeOH (89:7:4). From fractionation, subfr. 100-31-49 and 100-296-676 showed activity. Two compounds, PTE_1 and PTE-2 were isolated by HSCCC with a two-phase solvent system composed of EtOAc-EtOH-water (15:1:10, v/v/v), the lower phase was used as the mobile phase at a flow-rate 1.0ml/min in the head to tail elution mode and a revolution of 850 rpm. Administration of 30, 100 and 300 mg/kg of *P. tenuifolia* extracts significantly increased ICP/MAP ratio and AUC values in a dose-independent manner and 30 mg/kg of *n*-BuOH fraction significantly increased ICP/MAP ratio and AUC values. The root of *P. tenuifolia* extracts and the *n*-BuOH fraction improved the penile erection and prolonged the decay period. 47mg of PTE-1 was obtained from subfr. 100-31-49 and 20mg of PTE-2 was obtained from subfr. 100-296-676. Purity was as determined by HPLC. The structures of PTE-1 and 2 are under investigation and identifying as cinnamoyl glycosides by ¹H-NMR and ¹³C-NMR. In conclusion, From this study, two cinnamoyl glycosides were purified from the root of *P. tenuifolia* extracts showing the penile erection improvement and prolonging the decay period.

Discrimination of *Polygonum multiflorum*, *Cynanchum wilfordii* and *Cynanchum auriculatum* by Genetic and Chemical Analysis

Young Hun Shim, Jong Hwa Lee, Rack Seon Seong, Ju Young Park, Chang Hee Cho, Ji Yeon Kim, Seong Ye Hyeon, Ji Sang Whang, Sun Ho kim, Dong Sup Kim¹, Wan Kyun Whang²

Herbal Medicine Research Division, National Institute of Food and Drug Safety Evaluation, Korea¹ College of Pharmacy, Chung Ang University, Korea²

Polygonum multiflorum Radix is the root of *Polygonum multiflorum* Thunberg (Leguminosae) and this is listed in the Korean Pharmacopoeia 9th edition as 'Hasuo' in Korean pronunciation. *Cynanchum wilfordii* Radix is the root of *Cynanchum wilfordii* Hemsley (Asclepiadaceae) in the Korean Herbal Pharmacopoeia as 'Baek-Su-O'. However, *Cynanchum auriculatum* (Asclepiadaceae) called 'I-Yeup-U-Pi-So' in Korean, has very similar appearances comparing with 'Ha-Su-O' and 'Baek-Su-O'. So, *Cynanchum auriculatum*, *Polygonum multiflorum* and *Cynanchum wilfordii* are often misidentified in the Korean herbal market due to their morphological similarities and similar names such as 'Ha-Su-O' and 'Baek-Su-O' as Korean pronunciation. Therefore, we have studied to discriminate *Polygonum multiflorum*, *Cynanchum wilfordii* from *Cynanchum auriculatum*.

In this study, we have discriminated by genetic study (PCR) between botanical origins and have studied the identification method to analyze using TLC and HPLC. And we have analyzed using PCR, TLC, SPLC HPLC System (UV254nm, C₁₈ column). According to the genetic and identificational methods, we have identified and discriminated botanical origins of *Cynanchum wilfordii* and *Cynanchum auriculatum* with 30 samples.

As the result, we have established the discrimination method between *Polygonum multiflorum*, *Cynanchum wilfordii* from *Cynanchum auriculatum*.

Keywords: *Polygonum multiflorum*, *Cynanchum wilfordii*, TLC, HPLC, PCR

The Preparation and Application of Continuous Two-dimensional Open-tubular Ion Exchange Reversed Phase Monolithic Column Capillary Electrochromatography System

Zhu Yaxian, Lei Wen, Qin Sasa, Wan Li, Zhang Lingyi, Zhang Weibing*

Analysis and Test Center, East China University of Science & Technology, Shanghai

Multi-dimensional separation becomes the research hotspot in recent years due to the high selectivity and peak capacity^[1-2]. A continuous two-dimensional open-tubular ion exchange / reversed monolithic column capillary electrochromatography system was developed in this paper. Reversed monolithic column was firstly synthesized in the middle of the capillary, then N-(3-(trimethoxysilyl)propyl)ethylenediamine (PEDA) was surface coated to the other of the capillary. The migration of weak monoacids in continuous two-dimensional open-tubular ion exchange / reversed phase monolithic column of electrochromatography was proved in theory, and the separation of seven organic acids verified the effect of the separation of ion exchange in the open-tubular capillary. *Rhizoma gastrodiae* was separated to evaluate the system, and 31 peaks were recognized. The gastrodin which is the main constituent in *rhizoma gastrodiae* was identified by the retention time. This new electrochromatography system has the characteristics of gradient elution in HPLC, electrophoresis flow, electroosmotic flow and interaction between solute and stationary phase which all devote to adjusting the selectivity of the separation. This system is designed for complex samples containing highly hydrophobicity components and highly hydrophilicity components simultaneously. This new electrochromatography system combining advantages of multidimensional separation and adjusted stationary phase selectivity is suitable for the analysis method development of complicated samples.

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Characterization of the Components from Fuzheng Huayu Decoction in Rat Serum by High-Performance Liquid Chromatography – mass Spectrometry

Danping Shen^{a,b}, Li Yang^{c,d}, and Chenghai Liu^{a,b}

^aEast China University of Science and Technology, Shanghai

^bInstitute of Liver Diseases, Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai

^cMOE Key Laboratory for Standardization of Chinese Medicines and The SATCM Key Laboratory for New Resources and Quality Evaluation of Chinese Medicines, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai

^dShanghai R&D Center for Standardization of Chinese Medicines, Shanghai

Fuzheng Huayu decoction (FZHY) is a SFDA approved anti-fibrotic medicine in China. But the effective substance of FZHY is still not fully understood. The liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI/MSⁿ) was used to identify the chemical components in serum from FZHY. The chromatographic separation was achieved on a reversed-phase C₁₈ column by gradient elution using methanol and water containing 5mM Ammonium acetate as mobile phase, at a flow rate of 0.2 mL/min. The ion trap mass spectrometer equipped with electro-spray ionization was operated in both negative and positive ion modes. By LC-ESI/MSⁿ method, the components of migrating to blood was affirmed by comparing the extracted ion chromatography (EIC) of black serum, the serum containing drug, FZHY and reference substance, correlated ion peak in mass-spectrogram were analyzed at the same time. The results showed that the phenolic acids and four lignans components in rat serum were identified. The method was successfully applied to the study of the serum effective constituents of rat after oral administration of FZHY. 13 components above-mentioned are likely the effective substances of FZHY in human body. More study by means of combining with active ingredient verification will illuminate the therapeutic basis of this preparation.

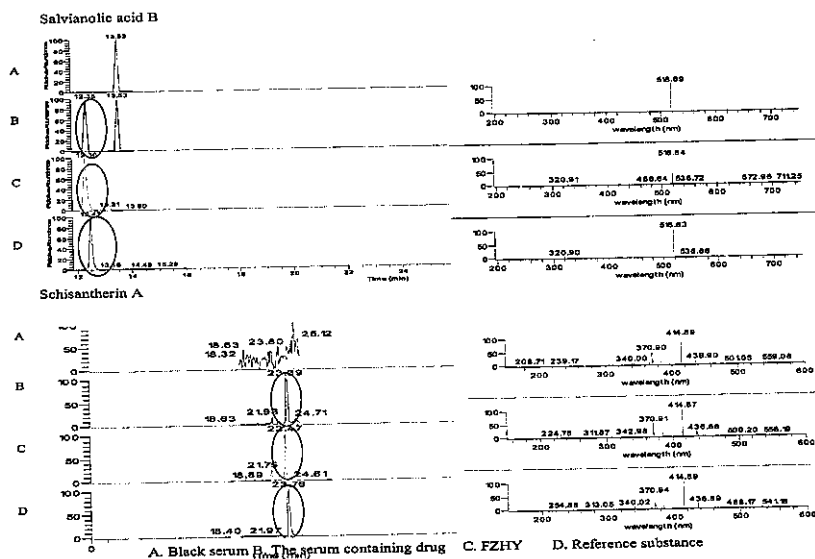


Figure 1 MS² spectrum of Salviaanolic acid B and Schisantherin A in rat serum

Genetic Diversity and Effective Components of *Cordyceps Sinensis* Populations in China

Shan Li¹, Xiao-Ling Yang¹, Zhu Yun-guo¹, Hong-Hui Liang¹, Yang Geng¹, Zhou Cheng^{1,2*}

¹ School of Life Science and Technology, Tongji University, Shanghai

² Tongji University-Lishui Institute of Chinese Traditional Medicine, Zhejiang Lishui

Cordyceps sinensis is one of the most valuable medicinal caterpillar fungi native to China. However, its productivity is extremely limited and the species is becoming endangered. The genetic diversity of eight *C. sinensis* populations across its major distributing regions in China was evaluated by morphological characteristics, ISSR markers, mitochondrial *Cytb* sequences (Fig.1). Meanwhile, the potential factors related to the effective components of *C. sinensis* were also discussed.

1) Based on the morphological analysis, the 18 populations are approximately divided into four groups correlated with the producing areas around Qinghai Lake (I), Mid-eastern (II), Southern (III) of Qinghai Province, and the Tibet-Sichuan-Yunnan (IV&V), respectively.

2) Based on the ISSR analysis, the 18 populations are divided into five groups and the grouping matches with the geographic distribution along latitudinal gradient. 3) Based on the mitochondrial *Cytb* sequences analysis, the host insects of *C. sinensis* and those of the 18 cordyceps populations could be divided into 3 distinct clades with high bootstrap values in the NJ tree. 4) Based on the effective components analysis, the contents of D-mannitol, polysaccharide and adenosine were very different among the different populations and groups (Table 1). The quality differences among the groups might be due to the species diversity and larger ecosystem while the effect of micro-ecological environment on effective components might exceed that of district and climate differences among populations from the same group. The information illustrated by this study is useful for making the strategic plan for effective conservation and sustainable exploitation of this precious Chinese traditional medicine resource.

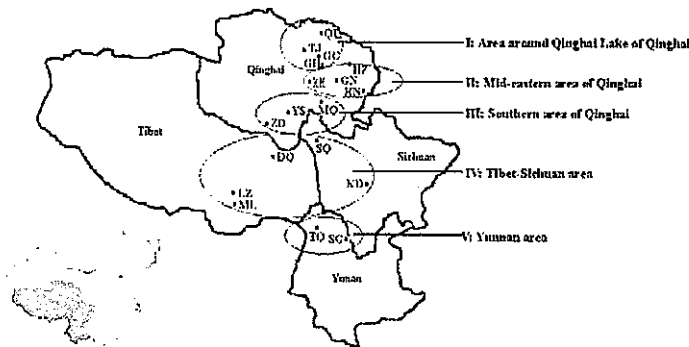


Fig.1 Sketch maps of 18 *C. sinensis* populations from 4 provinces of Qinghai, Tibet, Sichuan and Yunnan, China.

Content (%) Group	Group I 环青海湖	Group II 青海中部	Group III 青海南部	Group IV 四川、西藏	Group V 云南
Mannitol	16.19±0.55	18.43±1.20	18.10±0.69	17.92±0.84	17.16±0.74
Polysaccharide	1.10±0.22	1.88±0.78	1.26±0.07	0.80±0.5	1.19±0.5
Adenosine	0.016±0.011	0.017±0.005	0.020±0.009	0.023±0.011	0.024±0.018

Table 1 Mannitol, polysaccharide and adenosine content of the 5 groups of *C. sinensis* divided by their genetic diversity analysis

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Rapid Identification of the Medicinal Plant *Taraxacum mongolicum* from its Adulterants by Ribosomal DNA Internal Transcribed Spacer (ITS)-Primed Polymerase Chain Reaction

Ying-Chen Chiang¹, Mann-Duan Chen², Guan-Hua Lai³, Hsi-Jien Chen⁴, Jung Chao⁵, Wen-Te Chang², Ming-Kuem Lin², Yuan-Shiun Chang¹, Yu-Mei Chou², Meng-Shiunn Lee⁶, Meng-Shiou Lee²

¹ Graduate Institute of Chinese Pharmaceutical Sciences, College of Pharmacy, China Medical University, Taichung

² School of Chinese Medicine Resources, China Medical University, Taichung

³ Institute of Biochemistry, College of Life Science, National Chung Hsing University, Taichung

⁴ Department of Safety, Health and Environmental Engineering, Mingchi University of Technology, Taipei

⁵ Graduate Institute of Life Sciences, National Defense Medical Center, Taipei

⁶ Department of Medical Research, Tung's Taichung MetroHarbor Hospital, Taichung

Taraxacum mongolicum is a traditional Chinese medicine (TCM) that used as an important component in healthy drink at Taiwan. Due to its similarity on morphological features between *Taraxacum mongolicum* and *Taraxacum officinale*, *Ixeridium laevigatum*, *Youngia japonica*, *Ixeris chinensis*, *Emilia sonchifolia* var. *javanica*, is very common misused and becomes its adulterant of *Taraxacum mongolicum*. In this study, the internal transcribed spacer 2 (ITS2) nuclear ribosomal DNA (nrDNA) served as DNA barcode and allele-specific sequence-primed polymerase chain reaction were exploited for their application in the differentiation of *Taraxacum mongolicum* from its related adulterants. Using extracted genomic DNA from *Taraxacum mongolicum* and others *Taraxacum* plants leaves as template, the PCR reaction was performed with a set of specific designed primers. The results showed that highly specific 250 bp PCR product of *Taraxacum mongolicum* was successfully amplified; however, no any fragment was amplified from other *Taraxacum* plants and species. This indicated our allele specific primers have highly specificity to discriminate *Taraxacum mongolicum* from other *Taraxacum* plants. The ribosomal DNA internal transcribed spacer (ITS)- primed polymerase chain reaction for authentication of *Taraxacum mongolicum* was first developed herein that is a very sensitive and specific method to rapid and precisely discriminate *Taraxacum mongolicum* and its adulterant. Applying this allele-specific primers to detect DNA barcode, it might be an alternative way to rapid authenticate the TCM or to develop a TCM identification kit in the future.

Keywords: polymerase chain reaction (PCR), Authentication, *Taraxacum mongolicum*, traditional Chinese medicinal, internal transcript spacers 2 (ITS2).

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The Effects of Qianlie-Bao Capsule on Animal Model of Prostatic Hyperplasia and Prostatitis

Xiao-hua Hong, Jian-Xun Liu, Wei-Lin Yu

Department of Basic Theory, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing

Objective: To observe the effects of Qianlie-Bao (QLB) capsule on animal model of prostatic hyperplasia and prostatitis. **Methods:** The weight and index of prostate, leukocyte count and colony growth in prostatic hyperplasia by fetal urogenital sinus implants and rats prostatic hyperplasia by ovariectomization and testosterone propionate subcutaneously, as well as abacterial prostatitis and non-abacterial prostatitis of rats by 1.4×10^7 /mL *E. coli* and 1% carrageenin injection in ventral prostates respectively. **Results:** QLB capsule in dosage of 5, 10 and 20 g /kg could decrease prostatic weight and index ($P < 0.01$), but only the dosage of 20 g /kg reduce acinar luminal area of prostate in mouse ($P < 0.01$). QLB capsule in dosage of 7, 14 g /kg could not only decrease prostatic weight and index in prostatic hyperplasia of rats, as well as acinar luminal area of prostate ($P < 0.01$), but also inhibit colony growth, reduce leukocyte count and elevate lecithin corpuscular density ($P < 0.01$) in succus prostaticus after administration at 10 days in rat abacterial prostatitis and non-abacterial prostatitis. **Conclusion:** QLB capsule can improve prostatic hyperplasia and prostatitis in animal model.

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Up-regulation of Licochalcone A Biosynthesis and Secretion by Tween 80 in Hairy Root Cultures of *Glycyrrhiza Uralensis* Fisch

Haichao Zhang, Jingmei Liu, Chunchun Gao, Hua Zhou

National Engineering Research Center for Crop Molecular Design, Beijing

We evaluated the effect of Tween 80 as elicitor on licochalcone A from hairy root cultures of *Glycyrrhiza uralensis* Fisch. After a 15-d treatment with 2% Tween 80, hairy roots still grew well and produced higher levels of licochalcone A and total flavonoids than the control (without treatment). Licochalcone A content and total flavonoid content were 3.103 and 127.095 mg per flask (9- and 11-fold higher), respectively, compared with controls. Secretion of licochalcone A and total flavonoids into the culture medium was remarkably high, up to 98% and 94% of the total production, respectively. The enhanced flavonoid production was associated with elevated mRNA levels and enzyme activities of phenylalanine ammonia-lyase (PAL), 4-coumarate:coenzyme A ligase (4CL), and cinnamate-4-hydroxylase (C4H). These results clearly demonstrated that Tween 80 treatment permeabilized the roots to enhance secretion, but also acted as an efficient elicitor of licochalcone A and total flavonoid production in hairy roots of *G. uralensis* Fisch.

Systems Chemistry: Systemic Properties and Energy Management in Bioactive Molecules

Gregory A. Chass

Global Institute of Computational Molecular and Materials Science, Canada

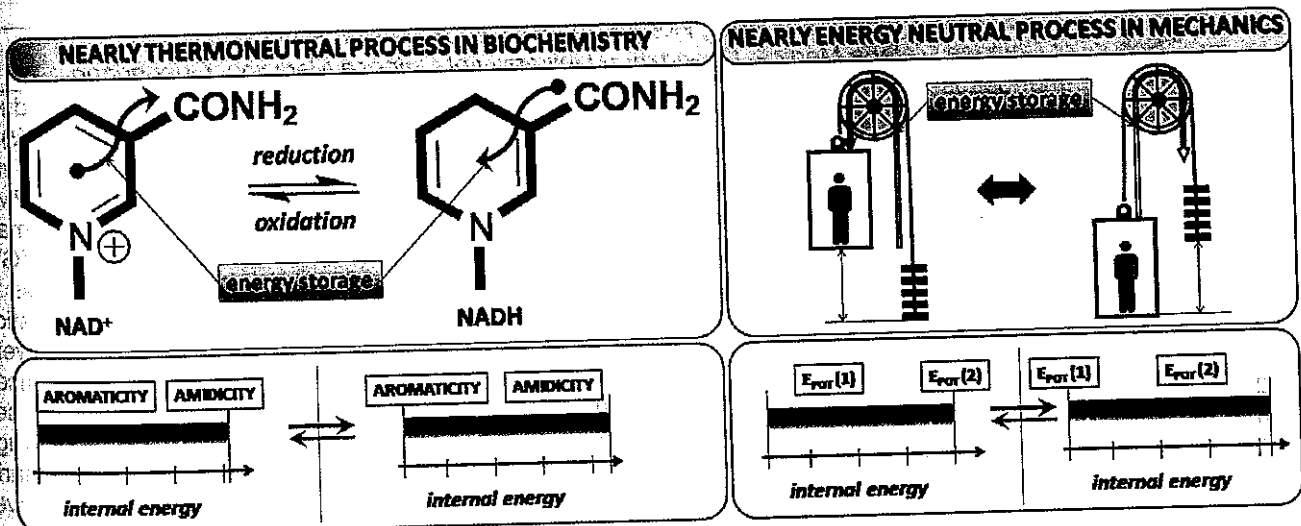
In view of the development of Systems Biology, focus has been on interactions and the cooperativity between separate molecules in larger mixed systems, such as host-guest complexes, protein-protein interfaces, chaperon molecules and other complex processes. Systems Chemistry represents a continued effort by the author to develop a novel discipline, extending these concepts towards quantitatively characterising the systemic effect of functional group cooperativity within a single molecule; particularly in the context of (natural) bioactive molecules and closely related molecular analogues.

This novel discipline is defines biomolecular systems as strategically located functional components within molecular frameworks, "valued" at more than their components' sum, acting in unison to effect efficient energy management. Systems chemistry seeks to define and quantify the efficiency of Nature's molecules, which nearly exclusively outperform compounds "assembled" (synthesised) by man.

Natural metabolic pathways and associated processes do not circumvent well-ordered, high-energy structures (e.g., antiaromatic rings, strained geometries, charged species). Energy management and recovery are near-quantitative, mediated through efficiently designed, systematic molecular frameworks that very effectively store energetic potential in the highly-ordered molecular structures. Such systemic chemical principles may be universally applied in all life-related processes.

Evidently, Nature is able to store reactive potential within the molecular system in carefully designed chemical structures which act as reversible energy carriers; effectively molecular free-energy capacitors. This modus operandi of Nature relies on near thermoneutrality, neither extra heating nor cooling is required in metabolic processes by living organisms. When these systems are damaged or less than optimal, energy management also suffers – hence the emergence of latent heat among other bases of illness.

The talk covers the development of Systems Chemistry in the context of energy management in bioactive molecules including those found in green tea and Traditional Chinese Medicines.



Hyperglycemic Effects of Mangiferin Hydrastis Granulein in GK Rats with Diabetes Mellitu

Na Li, Xiao-Bo Qu, Zhe Lin

Department of Scientific Technology, Development Center of Traditional Chinese Medicine and Bioengineering,
Changchun University of Chinese Medicine, Changchun

To investigate the effects of Mangiferin hydrastis granule (MHG) on blood glucose, blood fat and insulin in Goto-Kakizaki (GK) model rat and its mechanism.

Methods Thirty -six male GK rats were randomly divided into control group, rosiglitazone group and MHG (20.0, 40.0mg·kg⁻¹) groups (n=9). The rats were administered by intragastric administration for 4 weeks. The change of body weight was observed and the FBG, blood fat, insulin in serum were detected.

Results: The body weight rats in control group was significantly decreased, and continually dropped during administration, the body weights of rats in rosiglitazone group and MHG 20.0,40.0mg·kg⁻¹ groups were increased. Compared with control group, the contents of blood glucose in rosiglitazone group and MHG (40.0mg·kg⁻¹) group were decreased (P<0.05). There were no difference of the content of insulin between various groups (p>0.05). The content of triglyceride (TG) and total cholesterol (Tc) in rosiglitazone group were decreased (P<0.05). The content of total cholesterol (Tc) in MHG (40.0mg·kg⁻¹) group was decreased (P<0.05).

Conclusion: MHG can reduce the risk of diabetes by up-regulating intervent the contents of FBG and blood fat in GK rats.

A Traditional Chinese Patent Prescription, You-Gui-Wan, can Maintain the Balance of Sex Hormones and Alleviate the Atrophy of Sex Organs in Female Natural Aging Rats

Liang-Feng Liu^{a,b}, Ping Lai^b, Wei-Jin Liu^b, Ji-Yan Su^b, Da-Ke Cai^b, Jia-Hong Lu^a, Siva-Sundara Kumar^a, Min Li^a and Mu-Rong Ye^{b,*}

^a School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

^b School of Chinese Materia Medica, Guangzhou University of Chinese Medicine, Guangdong

Introduction: Perimenopausal syndrome (PMS) is a disorder characterized by hormonal changes that induce physical, psychological, and emotional symptoms found in a huge amount of women. You-Gui-Wan (YGW) is one of the famous traditional Chinese patent prescriptions which has been used for healing PMS. However, not much PMS animal study on YGW was reported. In this study, female natural aging rats were raised as a PMS model to verify the efficacy of You-Gui-Wan on healing PMS. **Methods:** Eleven and a half months old female Sprague-Dawley rats with menstrual disorder confirmed by vagina smear were randomly separated into YGW group, Premarin group and model group. All the rats were housed in standard cages and had free access to food and water. YGW was grinded and resuspended in ddH₂O to make it 450mg/mL. Premarin, a preparation of conjugated estrogens, was used as positive control and the concentration was 10.42µg/mL. Drugs were respectively administered 1mL per 100g body wt via P.O. for 4 months. After the treatment, rats were sacrificed and the serum E₂, FSH, LH was examined by radio-immuno-assay to evaluate the effect of drugs on sex hormones. Accordingly, organ indices of ovarian, womb, vagina were tested. **Results:** After 4 months of drug administration, the serum E₂ of YGW group is 29.4% higher than that of the model group whereas Premarin group is 135% higher than the model group. The FSH/LH ratio of YGW group and Premarin group are lower than that of the model group with 0.68, 0.74 and 0.81 respectively. The ovarian indices of YGW group and Premarin group are higher than model group with 16%, 22% respectively. The womb indices of YGW group and Premarin group are higher than that of the model group with 7%, 45% respectively. The vagina indices of YGW group and Premarin group are higher than that of the model group with 19%, 11% respectively. **Conclusions:** Our findings verified that You-Gui-Wan can alleviate the atrophy of ovarian, womb and vagina by increasing E₂ and maintaining the balance of FSH and LH in female natural aging rats.

Keywords: You-Gui-Wan, perimenopausal syndrome, natural aging, estrogen, organ index

Xanthones from the Stems of *Cratoxylum Formosum* ssp. *Pruniflorum* and their RXR α Transcriptional Inhibitive Activities

Ying-hui Duan¹⁾, Yi Dai^{2,3)}, Guang-hui Wang⁴⁾, Xue Zhang¹⁾, Hai-feng Chen⁴⁾, San-lin Jin¹⁾, Jie-bo Chen⁵⁾, Xiaokun Zhang^{4,5)}, Xin-sheng Yao^{1,2,3)}*

¹College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang

²Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangdong

³Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangdong

⁴Institute for Biomedical Research, Xiamen University, Xiamen

⁵Burnham Institute for Medical Research, Cancer Center, USA

Retinoid X receptors (RXRs) are members of the nuclear receptor superfamily and play an important role in many diverse physiologic processes, including embryogenesis, calcium homeostasis, and lipid and glucose metabolism. There has been tremendous interest in identifying agents that regulate RXR activities. In our previous work in screening for natural RXR α transcriptional regulators from 100 traditional Chinese medicine (TCM) extracts, we found three bioactive TCM extracts, including *Cratoxylum cochinchinense*. Further bioactivity guided isolation on this plant afforded several xanthones, some of which could regulate RXR α transcriptional activities in various degrees. *Cratoxylum formosum* ssp. *Pruniflorum* also belongs to the genus *Cratoxylum* family Clusiaceae, which is widely distributed in several Southeast Asian countries. This plant is locally known as "Kuding Tea" in southwest mainland China and has been used as a folk medicine for the treatment of fever, coughs, ulcers, and diarrhea. Previous chemical investigations on this species have revealed a series of xanthones and anthraquinones. Some of these compounds possessed various bioactivities, such as antimalarial, antibacterial, and cytotoxic effects. As a continuation study in searching for natural RXR α transcriptional regulators from the stems of *C. formosum* ssp. *Pruniflorum*, we reported the isolation of six new xanthones (1-6), and nineteen known xanthones (7-25). Compounds 5-6, 7, 11, 15, 16, 17, 19, and 24 showed potent transcriptional inhibitive activities of RXR α . Among them, compounds 6, 7 and 11 exhibited concentration-dependent activities.

This work was supported by grants from the National Natural Science Foundation of China (NSFC-30873146).

In Vitro Study on Kang Bin Du Granule against Swine-origin Influenza A Virus (A/H1N1)

Hui Cao^a, Desheng Tao^b, Yongqing Zeng^c, Yi Guan^d

^a National Engineering Research Center for Modernization of Traditional Chinese Medicine, Guang

^b Livzon Pharmaceutical Group Inc, Guangdong

^c Sichuan Guangda Pharmacy Co., Ltd, Chengdu

^d Department of Microbiology, The University of Hong Kong, Hong Kong

Kang Bin Du granule (KBD), produced by Sichuan Guangda Pharmacy Co., Ltd under Livzon Pharma Group Inc, has been shown to be inhibitory effect against highly pathogenic avian influenza A virus (H5N1) *in vitro* & *in vivo*. This paper objectives have the specific aims i.e. to provide a comprehensive of the anti-virus profiles in swine-origin influenza A virus (H1N1) *in vitro* after the treatment using KBD confirm the efficacy of the KBD in swine-origin A/H1N1 strain.

Material and Method:

KBD-GB formula (certificate no. Z20010127): 4.7g /ml of extract equal to 4.28g crude drug per granule; KBD-SC formula (certificate no.Z51020072): 3.7g/ml of extract equal to 4.28g crude drug per granule.

Positive control drug: Tamiflu (Oseltamivir phosphate) from Roche Inc.

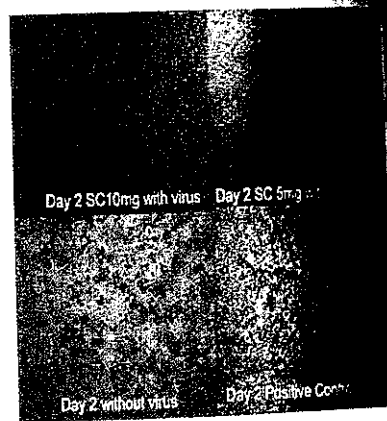
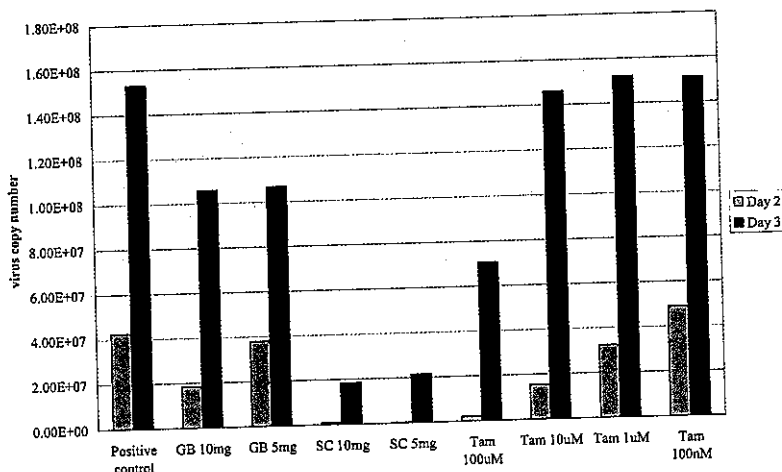
Cell line: MDCK from HKU.

Virus strain: H1N1 (A/California/4/2009) from HKU.

1. Cytotoxicity assay: MTT assay.
2. Antiviral assay: cytopathogenic effect (CPE) inhibition assay.
3. M detection: real time PCR.

Result and Discussion:

Realtime PCR for drug test with 50 TCID₅₀ H1N1 influenza virus



	Day 2		Day3	
KBD (mg/ml)	10	5	10	5
GB formula	2/2	2/2	2/2	2/2
SC formula	0/2	0/2	1/2	2/2
Positive control(μmol/ml)	100	10	100	10
Tamiflu	0/2	1/2	2/2	2/2

Conclusion: KBD has been shown to be inhibitory effect against swine-origin A/H1N1 *in vitro* at 5 mg/ml-10mg/ml dose.

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2. Hui Cao, Desheng Tao, Yongqing Zeng, Yi Guan. *In vivo* study on a TCM product (Kang Bin Du Granule) against highly pathogenic H5N1 avian influenza A virus (genotype E). The 6th Meeting of Consortium for Globalization of Chinese Medicine Program & Abstract, 2007: 69-70

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Simultaneous Characterization of Hydrolysable Tannins, Naphthalene Derivatives and Flavonoids in *Juglans Mandshurica* by UPLC Coupled to ESI-Q-TOF-MS

Tian-Min Wang^{1,2}, Hu-Biao Chen^{1*}, Tao Yi¹, Jing Liu¹, Zhong-Zhen Zhao¹, Ting-Guo Kang²

¹School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

²School of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian

The roots, branches and leaves of *Juglans mandshurica*, a folk medicine used for treating cancer, have been reported to exhibit inhibitory effects to cancer cells. Several kinds of compounds, including tannins, naphthalene derivatives, diarylheptanoids and flavonoids, have been isolated from this plant. Herein, comparative analysis of the multiple constituents in the roots, branches and leaves of *Juglans mandshurica* was conducted by on line ultra-performance liquid chromatography with photodiode array detector (UPLC-PDA) and electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS). UPLC and TOF-MS was applied to obtain high resolution and accurate molecular weight, respectively. All of the constituents were characterized by matching their accurate molecular masses, characteristic fragments (obtained by operating MS under high collision energy) and UV absorptions with those of reference compounds or reported in the literature. Consequently, gallotannins, ellagitannins, naphthaquinones, naphthalenyl glucosides and flavonoids were identified in the methanol extracts of the roots, branches and leaves of *Juglans mandshurica*. The roots and branches of *Juglans mandshurica* showed similar UPLC-UV and ESI-MS chromatogram, which was different from those of the leaves. Naphthalene derivatives were found in all of the three parts studied. Besides naphthalene derivatives, the main constituents found in the roots and branches were hydrolysable tannins including gallotannins and ellagitannins, while the major compounds in the leaves were flavonoids. The research was a good application of UPLC-Q-TOF-MS method to the rapid analysis of multiple constituents in herbal medicine and provided useful information on the chemical constituents of *Juglans mandshurica*, which could contribute to further research on the anti-cancer constituents of this plant.

New Acylated Protopanaxadiol-type Ginsenosides from the Root of *Panax Ginseng*

Guo-Yuan Zhu, Ying-Wei Li, Desmond Kwok-Po Hau, Zhi-Ling Yu, and Wang-Fun Fong*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Panax ginseng C. A. Meyer (Araliaceae) is a well-known oriental medicinal plant that has been used as a general tonic for thousands of years in China. Previous phytochemical work on *P. ginseng* led to isolation of ginsenosides (triterpenoid saponin glycosides), polyacetylenes, sesquiterpenoids, flavonoids, and polysaccharides. Due to the continued interests in the structure-bioactive relationship of ginsenosides, we reinvestigated the root of *P. ginseng* and obtained 6 new acylated protopanaxadiol-type ginsenosides (1-6) and 14 known protopanaxadiol-type ginsenosides (7-20). Their structures were elucidated by spectroscopic methods including HRMS, 1D and 2D NMR as 3-O-[β -D-6-O-E-2-butenoyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-20-O-[β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-arabinopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol (1), 3-O-[β -D-6-O-acetyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-20-O-[β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-arabinopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol (2), 3-O-[β -D-6-O-E-2-butenoyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-20-O-[β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol (3), 3-O-[β -D-6-O-E-2-butenoyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-20-O-[α -L-arabinopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol (4), 3-O-[β -D-4-O-E-2-butenoyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-20-O-[α -L-arabinofuranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol (5), 3-O-[β -D-6-O-E-2-butenoyl-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl]-20-O-[α -L-arabinofuranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-20(S)-protopanaxadiol (6) ginsenosides Ra₁ (7), Ra₂ (8), Rb₁ (9), Rb₂ (10), Rb₃ (11), Rc (12), Rd (13), Rs₁ (14), Rs₂ (15), malonyl-ginsenoside Rb₁ (16), gypenoside XVII (17), pseudoginsenoside RC₁ (18), quinquenoside R₁ (19), vinaginsenoside R₁₆ (20).

Synthesis and DNA G-quadruplex Binding Activity of Glucosaminoside Derivatives of Epigallocatechin in Green Tea

Li-Ping Bai, Zhi-Hong Jiang

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

G-quadruplexes in nucleic acids have been postulated to play important roles in a lot of biological processes including gene regulation and the inhibition of enzyme function. Various G-quadruplex interactive ligands may be necessary for the development of anticancer drugs. Two 5,7,3',4',5'-penta-O-methylepigallocatechin glucosaminosides **2** and **3** have been designed and synthesized starting from epigallocatechin. Their telomeric G-quadruplex binding activities were examined by ESI-TOF-MS technique. The ESI-TOF-MS results show that compounds **2** and **3** can stabilize intermolecular G-quadruplex DNA formed by two molecules of telomeric sequence dTAGGGTTAGGGT with both 1:1 and 1:2 binding stoichiometry (Figure 1). In contrast, compound **1** does not bind to G-quadruplex DNA by the evidence that no any G-quadruplex-ligand complex was detected in the ESI-TOF-MS chromatograph. This finding indicated that introduction of nitrogen atom into **1** resulted in G-quadruplex stabilizing activity. Aminoglycosidation of natural product is an effective way to design and synthesize small molecules targeting G-quadruplex DNA.

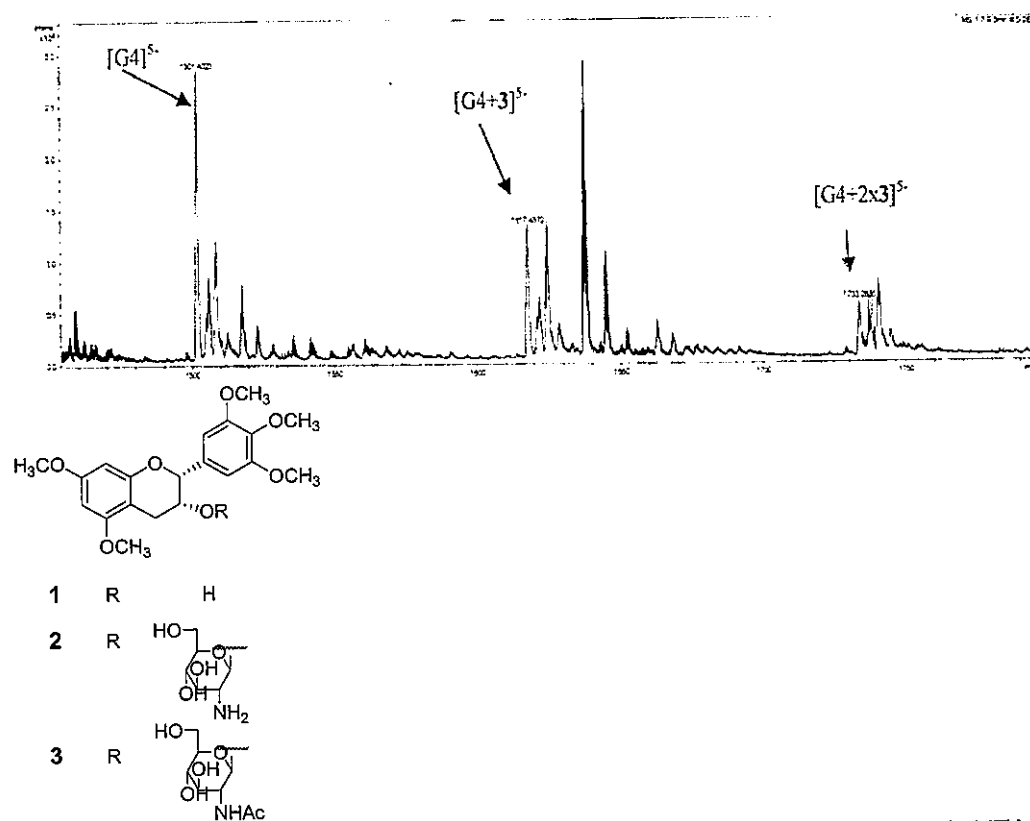


Figure 1. Negative ESI-TOF-MS spectra of intermolecular G-quadruplex DNA (dTAGGGTTAGGGT, 100 μM) with compound **3** (100 μM) in 25% methanol containing 100 mM ammonium acetate

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Potential Application of Traditional Medicine (TCM) for the Treatment of Methicillin-resistant *Staphylococcus Aureus* (MRSA) Infections – Bioassay-guided Fractionation of *Sophora Flavescens* Ait

Quan-Bin Han, ¹Ben Chung-Lap Chan, ¹Eric Wong, ²Margaret Ip, ^{1,2}Sau-Lai Lui, ¹Ching-Po Lau, ¹Clara Bik-San Lau, ^{1,3}Kwok-Pui Fung & ¹Ping-Chung Leung

¹Institute of Chinese Medicine, ²Department of Microbiology and ³School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong

Objective: Bacterial resistance to antibiotics has become a serious problem of public health that concerns almost all antibacterial agents and that manifests in all fields of their application. The main objective of the present research is to identify herbs and their active components exhibiting inhibitory effect either on the growth of *Staphylococcus aureus* (SA) or on the bacterial resistance mechanisms.

Methods: The antibacterial effects of the herbal extracts of 33 Traditional Chinese Medicine (TCM) which are conventionally use in antibacterial treatments were tested against: 1- *Staphylococcus aureus* (ATCC 25923), 2- Methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC BAA-43) and 3- *Escherichia coli* (ATCC 25922). Suspension of each organism was prepared from fresh colonies on blood agar plates after overnight incubation in Mueller-Hinton (MH) broth (Bio Rad) at 37 °C. The determination of the antibacterial activity of extracts was carried out in liquid medium using a Biomek model 2000 liquid-handling robot (Beckman-Coulter) and 96-well microtiter plates. The most active herbal extracts against MRSA were further fractionated by high-speed counter-current chromatography (HSCCC), and the antibacterial activities against MRSA were identified.

Results: Among 33 tested herbal extracts, the ethanolic extracts of *Sophora flavescens* Ait (SF) were the most active against both SA and MRSA, but ineffective against the growth of *E. coli*. Finally, two active compounds were found out from SF using bioassay guided fractionation.

Conclusion: Two active compounds, with strong activities against the growth of MRSA, were isolated from SF.

Effect of the Seed of *Nelumbo Nucifera*, on Electric Stimulation-induced Penile Erection in Rats

Dong Wook Lim, Donghun Lee, Juyeon Park, Jae-Goo Kim, Mikyung Song, Mi-Yeon Kim, Ho-young Choi, Hocheol Kim

College of Oriental Medicine, Kyung Hee University, Korea
NeuMed Inc, Korea

The seed of *Nelumbo nucifera* was reported to have anti-depression, antioxidant and enhancing neurogenesis in the hippocampus, however, the erectile effect of the seed of *N. nucifera* yet to be reported. The enhancing effect of penile erection of the seed of *N. nucifera* was evaluated in vivo rat model, in which erection is induced by electric stimulation of the cavernous nerve. The seed of *N. nucifera* was extracted with 70% EtOH by using reflux apparatus and fractionated successively with *n*-hexane, EtOAc, *n*-BuOH, and H₂O fraction. Intracavernous pressure (ICP) and mean arterial blood pressure (MAP) were simultaneously monitored during electrical stimulation of the cavernous nerve, before and after oral administration of the seed of *N. nucifera* crude extracts (NNC) (100, 300, and 1000 mg/kg) and its fractions (100 mg/kg) in rats. The oral administration of the NNC at 300 mg/kg and the *n*-BuOH fraction at 100 mg/kg resulted in a significant increase in ICP, ICP/MAP ratio, and AUC of the ICP/MAP ratio compared with the control group at 2 Hz, 6 Hz, and 10 Hz. The ICP/MAP before and after administration of NNC showed no significant change in the NNC, while it showed a significant drop in case of sildenafil. In these experimental results, the seed of *N. nucifera* enhances penile erection and prolongs the decay period without lowering blood pressure in rats.

Inhibitory Compounds on LPS-induced NO Production in BV-2 microglial Cells from *Abies*

Sa Wang Baek, Jinwoong Kim

College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University

Backgrounds: The plant genus *Abies* consisted of 51 species widely distributed in temperate and regions of northern hemisphere. *Abies* species have been used against cold, stomachache, indigestion, and vascular diseases. The seeds of *A. koreana* Wilson (Pinaceae), mainly ranged in mountainous areas in southern Korea, have been used for hypertension and uterine bleeding as Korean traditional medicine. Although several monoterpenes and lignans isolated from this plant, detail investigations on their components or biological activities have not yet been performed.

Methods: The fresh leaves of *A. koreana* (10 kg) were collected and extracted with MeOH and evaporated under reduced pressure. The methanolic extract was suspended in H₂O and partitioned successively with hexane, CHCl₃, EtOAc and n-BuOH. Eleven compounds were isolated from EtOAc fraction by various chromatographic methods including MPLC and preparative RP-HPLC. Anti-inflammatory activities *in vivo* were evaluated based on the inhibition of NO production in BV-2 microglial cells. BV-2 microglia cells were treated with test samples for 1 hr and then exposed to 100 ng/ml of LPS. After 24 hrs, nitrite in culture media was measured to assess NO production in BV-2 cells using Griess reagent. Cell viability was determined by MTT assay.

Results and Discussion: The compounds were identified as, maltol-3-O-β-D-Glucoside (1), 3-hydroxy-4-methyl-4-pyrone (2), 4-(3-hydroxybutyl)phenol (3), ferulic acid (4), 4-(4-hydroxyphenyl)butan-2-one (5), isolariciresinol (6), Secoisolariciresinol (7), naringenin-7-rhamnosidoglucoside (8), (-)-epicatechin (9), naringenin 7-O-β-D-glucopyranoside (10) and kaempferol 3-O-β-D-glucopyranoside (11). Among the isolated compounds, compound 11 significantly reduced LPS-induced NO production in BV-2 microglia cells. Compounds 4, 6 and 7 showed mild S-nitroso-N-acetylpenicillamine (SNAP)-derived NO radical scavenger activity.

Anti-obesity Effects of *Dioscorea opposita* on High-fat Diet-induced Obesity in Mice and in Vitro Assays

Min Hye Yang and Jinwoong Kim

College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Korea

Backgrounds: According to recent pharmacological studies, *Dioscorea* species possess significant antidiabetic, cholesterol-lowering, and hypolipidemic effects which were closely associated with obesity-related metabolic diseases. In this study, tests were carried out to evaluate the hypolipidemic effects of n-BuOH soluble extract from *D. opposita* on high-fat diet-induced obese mice. Furthermore, the anti-obesity effects of the phenolic compounds isolated from a *D. opposita* n-BuOH fraction were measured based on the induction of apoptosis in 3T3-L1 preadipocyte and inhibition of adipogenesis.

Methods: Female ICR mice were fed a high-fat diet with the 100 mg/kg of *D. opposita* n-BuOH extract for 8 weeks. The high-fat diet mice received either 15 mg/kg orlistat or 0.5% CMC orally as a positive or negative control. For *in vitro* experiments, cell proliferation and apoptosis induction were determined by MTT methods and caspase 3/7 assay kits. Effects of compounds isolated from *D. opposita* on PPAR γ , C/EBP α , adiponectin, AMPK and CPT-1 expression in differentiated 3T3-L1 cells were measured by Western blot analysis.

Results and Discussion: The body weights, parametrial adipose tissue weight, and the levels of TG, TC and LDL-cholesterol in blood serum of female ICR mice were significantly decreased by feeding a high-fat diet with n-BuOH extract for 8 weeks. The treatment with the compounds batatasin I, (4E,6E)-1,7-bis(4-hydroxyphenyl)-4,6-heptadien-3-one and 3-methoxychrysin isolated from *D. opposita* induced apoptosis in 3T3-L1 preadipocytes, mediated through the activation of caspase-3. Batatasin I and (4E,6E)-1,7-bis(4-hydroxyphenyl)-4,6-heptadien-3-one were also suppressed adipogenesis by inhibitions of C/EBP α and PPAR γ expressions. The inhibitory effect of batatasin I was related to the activation of AMPK-CPT-1 signaling pathway in 3T3-L1 adipocytes. These results suggested that the anti-obesity effects of n-BuOH extract from *D. opposita* on high-fat diet-induced obese mice may be partly mediated through inducing the apoptosis and inhibiting adipogenesis in 3T3-L1 preadipocytes and adipocytes.

Enzymatic Transformation of Platycosides and One-step Separation of Platycodin D by High-speed Counter-current Chromatography

Minseok Kang, In Jin Ha, Young Wan Ha, Jongsung Lee, Deokhoon Park, Yeong Shik Kim*

College of Pharmacy, Seoul National University, Korea

Platycosides, the saponins found in the roots of *Platycodon grandiflorum* (Platycodi Radix), are typically composed of oleanene backbones with two side chains. Of the platycosides, platycodin D (glucose unit at 3) is a major component and it has several pharmacological activities. Because of the high demand for this compound, we attempted to enzymatically convert platycodin D₃ and platycoside E, having two and three glucose units at C-3, respectively, into platycodin D. In this study, we tested the ability of several glycosidases to transform platycosides, or more specifically, the ability to transform platycoside E and platycodin D₃ into platycodin D. To get pure platycodin D on a preparative scale, high-speed counter-current chromatography (HSCCC) with a solvent system ethyl acetate-*n*-butanol-water (1.2:1:2, v/v/v) was used for separation of the enzymatically-transformed product from the reaction mixture. Total 40.5 mg of platycodin D (99.8 % purity) was obtained from 200 mg of the product in one-step separation.

Platelet Anti-aggregation and Blood Anti-coagulant Effects of Compounds Isolated from *Evodiae Fructus*

Yean Kyoung Koo^a, Ja Yong Koo^a, Jung Jun Lee^c, JinHo Chung^b, Yeong Shik Kim^a, and Hye Sook Yun-Choi^{a*}

Natural Products Research Institute, Seoul National University, Korea

Platelets play a key role in normal hemostatic process caused by injury of blood vessels, as well as in the pathogenesis of thrombosis occurred by the improperly regulated hemostatic stimulus and blood flow with platelet plug or hemostatic plug. Thus, anti-platelet compounds have wide therapeutic potential for various circulatory diseases. *Evodia officinalis* Dode (or *Evodia rutaecarpa* Benth) have long been used as oriental traditional medicines in Korea, China and Japan; the dried fruit of *E. officinalis* under the traditional names of *Evodiae Fructus* (WuChu-Yu). *E. Fructus* were known to be used for the treatment of headache, thoracoabdominal pain, vomiting, cold and reduced blood circulation. The seven compounds of isolated from *E. Fructus* were secured and evaluated for their effects on platelet aggregation and blood coagulation to identify the common active constituents.

Evodiamine, goshuyunamide II, rutaecarpine acid and rutaevine were inhibited 15~30 % shear stress-induced aggregation. Evodiamine, goshuyunamide II and rutaecarpine were observed 6~30 times stronger inhibitory effects on collagen induced platelet aggregation than ASA (IC₅₀: 59.5, 31.3 and 10.5 μM vs. 340.0 μM, respectively). 1-methyl-2-[(6Z, 9Z)-6, 9-pentadecadienyl]-4(1H)-quinolone (KR 11) and rutaecarpine showed approximately 2~20 times more inhibitory than ASA on AA induced platelet aggregation (IC₅₀: 26.7 and 3.0 μM vs. 63.0 μM, respectively). Evodiamine exhibited stronger inhibitory effect than ASA on U46619 induced platelet aggregation (IC₅₀: 49.0 μM vs. 501.0 μM). Also, rutaevine showed mild anti-platelet aggregatory and blood anti coagulant activity.

Above compounds isolated from *E. Fructus* were suggested to be the major active constituents which contributes to improve blood circulation with their inhibitory effects on both platelet aggregation and blood coagulation.

Keywords: *Evodia officinalis*; platelet aggregation; anticoagulation

Different Flavonoids from *Hylocereus Undatus* Flower

Yi Yan, Zhang Qinwen

Institute of Chinese Medical Sciences, University of Macau, Macau

The flower of *Hylocereus undatus*, Ba Wang Hua, has been used as traditional herbal medicine and vegetable in South of China for hundreds years. A chemical investigation led to the isolation of 10 flavonoids from the ethanol extracts of dry flower of *H. undatus* (collected from Guangzhou, China). The structures were identified as tamarixetin, isorhamnetin, quercetin, kaempferol, Gelidolin, Herbacetin-3-glucoside, Tamarixin, Kaempferol-3-glucoside, Kaempferol-4'-glucoside and Isoscutellarein-4'-glucoside. Structural elucidation was accomplished by spectroscopic methods including NMR and MS. All of them were isolated from the flower of *hylocereus undatus* for the first time. The research was supported by a grant from Macao Science and Technology Development Fund (013/2008/A1).

Preparative Separation of Patchouli Alcohol from Essential Oil of *Pogostemon Cablin* Using High Performance Centrifugal Partition Chromatography

Xiao-cen Li, Qing-wen Zhang*

Institute of Chinese Medical Sciences, University of Macau, Macau

Pogostemon cablin, like many plants within the Lamiaceae, accumulates large amounts of essential oil. Patchouli alcohol, the main volatile constituent, was successfully isolated from the essential oil of *Pogostemon cablin* by high-performance centrifugal partition chromatography (HPCPC). The separation was performed with a nonaqueous two-phase solvent system consisting of petroleum ether (bp.30-60°C)-acetonitrile (1:1, v/v) in combination of descending separation modes. A total of more than 2 g of patchouli alcohol was isolated from the essential oil (12.5 g) in 300 min. This component was separated and identified by comparing its retention time and MS spectra with its standard substance analyzed by GC-MS. The purity was more than 98%. About 450 mL petroleum ether and 550 mL acetonitrile used for each independent run, 60% and 80% of petroleum ether and acetonitrile could be recovered for next run respectively. The research was supported by grants from Macao Science and Technology Development Fund (013/2008/A1) and Research Committee of University of Macau (RG087/07-08S/ZQW/ICMS).

The Use of *Caenorhabditis Elegans* as the Model for Anti-Infective Screening Assay

Tengku Sifzizul Tengku Muhammad^{1,2}, Komalavali Dharmalingam², Mohamed Isa Abdul Majid², Saiedatu Akmal Mohamed Sedek², Alexander Chong Shu Chien², Lokman Md Din¹, Noraznawati Ismail¹ and Tan M. Wah³

¹*Department of Biological Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu, Malaysia*

²*Malaysian Institute of Pharmaceuticals and Nutraceuticals, Ministry of Science, Technology and Innovation, Universiti Sains Malaysia, Malaysia*

³*Departments of Genetics, and, Microbiology and Immunology, Stanford University School of Medicine, USA*

Antibiotic resistance is a major complication faced by health practitioners in the treatment of or prophylaxis against infections. In view of only a few new classes of anti-microbial agents becoming available for clinical use in the near future, the development of novel anti-infective, which can circumvent the mechanisms of resistance of the microorganisms, would be the key to halt the emergence of further resistance. In our study, *Caenorhabditis elegans* was used as the host model to rapidly screen natural products with anti-infective activity. There is a plethora of evidence suggesting the advantages of using *C. elegans* as the host model for substitution to human. The genes in this nematode were found to be very similar to those in humans and a number of genes that are associated with human disease have homolog in the *C. elegans* genome. It was demonstrated that human pathogens such as *Pseudomonas aeruginosa* were able to infect and kill *C. elegans*. In addition, the mechanisms and pathways of the immune system in *C. elegans* have been found to conserved in human. Therefore, with these unique properties, *C. elegans* is a suitable model to be used as a screening model for anti-infective agents. We found that the methanol extract prepared from a marine gastropod, *Faunula*, increased the survival rate of *C. elegans* when the worm was co-cultured with *P. aeruginosa*. Interestingly, the extract did not kill the pathogen. Therefore, it is tempting to speculate that the methanol extract produced potential anti-infective activity not by exerting the anti-microbial activity but by increasing the immune system of *C. elegans*, preventing the infection of *P. aeruginosa* to the host or/and reducing the virulence of the pathogen.

Analysis of Catalpol Derivatives by Characteristic Neutral Losses Using Liquid Chromatography Combined with Electrospray Ionization Multistage and Time-of-flight Mass Spectrometry

Jun-Li Hong, Xiao-Ying Qin, Pan Shu, Gang Wu, Qiang Wang and Min-Jian Qin

Department of Resources Science of Traditional Chinese Medicines, China Pharmaceutical University, Nanjing

Utilizing the combination of LC-TOF-MS and LC-ESI-MSⁿ to gather accurate mass measurements and complementary structural information, we apply the powerful approach for the identification and structural elucidation of the iridoid glycosides with an oxygen bridge from *Veronica linariifolia*, especially the catalpol derivatives. 2 compounds were tentatively identified as new iridoid compounds with other 10 catalpol derivatives identified. The 3, 4-dihydroxy-cinnamoyl substituent is more characteristic of this plant while the cinnamoyl substituent is more common in other plants of the genus *Veronica*, which can be studied for the further chemotaxonomy research. Characteristic neutral losses and fragment ions of the catalpol derivatives were identified, there are some characteristic product ions and a series of neutral losses such as 182 Da (aglycone), 162 Da (glucose), 114 Da (^{6,9}X⁻ ion), 68 Da (C₄H₄O), 44 Da (carboxyl unit) and 28 Da (carbonyl unit). The product ions formed by subsequent or simultaneous fragmentation of deprotonated molecules, can be used for identifying constituents with the same mother nucleus or similar structures. The appropriate fragmentation pathways were proposed based on the definite composition of the fragment ions.

Investigation on Material Foundation of Antioxidant and Anti-lung Cancer of *Prunella Vulgaris* L.

Liang Feng^{1,2}, Xiao-bin Jia², Yan Chen², Guangmin Liu², Feng Shi²

¹Key Laboratory of Delivery Systems of Chinese Materia Medica, Jiangsu Provincial Academy of Chinese Medicine, Nanjing

²Biotechnology Laboratory of Chinese medicine, Macau university of science and technology, Macau

Prunella vulgaris L. (PV) has anti-cancer and antioxidant activity, but its material foundation is still not clear. In this paper, XKC-95, XKC-60, XKC-30 and XKC-W(extracted sequentially by 95% ethanol, 60% ethanol, 30% ethanol and water extraction) four extracts were obtained from PV. Anti-lung adenocarcinoma activity of mixture of four extracts in C57BL/6 mice were developed and antioxidant activity (SOD activity and MDA content) were evaluated in serum of tumor-bearing mice. The results showed that PV enhanced SOD activity (48.28±1.85 U/ml in 5mg crude drug/ml, p ≤ 0.01; 40.44±2.26 U/ml in 10mg crude drug/ml, p ≤ 0.01; 32.38±8.54 U/ml in saline group) and reduced MDA content (3.20±1.25 nmol/ml in 10mg crude drug/ml, p ≤ 0.01; 7.25±1.85 in saline group). In addition, PV had strong antitumor activity (63.56±6.79%; 33.45±10.98%). MTT assay of four extracts on SPC-A-1 (Human lung adenocarcinoma cell) were developed for anti-tumor activity. IC₅₀ of four extracts were 687.79, 650.36, 1627.73, 4842.80 μg/ml, respectively. The result showed that XKC-60 had the strongest anti-tumor activity. The ABTS⁺, DPPH, FRAP radical scavenging methods were utilized to compare antioxidation activity of different extracts. In ABTS⁺ test, TEAC value of four extracts were 8.926, 89.307, 37.335 and 9.779 μmol Trolox/g crude drug, respectively (R² = 0.9988); in DPPH test, 13.710, 19.662, 17.645 and 17.536 μmol Vc/g crude drug, respectively (R² = 0.6284); in FRAP test, 0.0252, 0.1567, 0.0802 and 0.0558 μmol Fe(II)/g crude drug, respectively (R² = 0.9673). These results showed that XKC-60 had the strongest antioxidant activity. There were a significant correlation of total phenolic and antioxidant activity. HPLC-DAD method was used for the content of phenolic acid in PV. Caffeic acid, rosmarinic acid, rutin, quercetin were 0.09, 1.32, 0.35 and 0.09 mg/g, respectively. These above results indicated that phenolic acids was the material foundation of antioxidative and antitumor activity.

Keywords: *Prunella*; material foundation; Phenolic acids; Antioxidant activity; Anti-lung cancer; HPLC-DA

Current Research Status of Targeting Interference of Leukemia Stem Cell with Components from Natural Products

Yang Bin-feng¹, Liu Zhi-hui², Sun Xue-mei²

¹Nanjing University of TCM, Nanjing

²Jiangsu Provincial Hospital of TCM, Nanjing

Objective: Leukemia stem cell (LSC) is a group of heterogeneous cells. There are some similarities between LSC and HSC, such as proliferation extensively, the self-renewal capacity, in G₀ phase and CD34⁺ immunophenotype. LSCs account for the disease progression and therapeutic failure. It has been a new way to cure leukemia via targeting LSCs in recent years, but little study based on research on natural products. This article reviewed literatures trying to present current research status in this field and provide clues for further studies.

Methods: Literatures of LSCs in the recent years are reviewed and the status of targeting therapies with natural products on LSCs is included.

Results: According to reports in the literature, natural products can kill LSCs by interfering certain signaling pathways. Firstly, LSCs apoptosis was induced via inhibition of NF- κ B. Secondly, signal paths maintaining self-renewal capacity, such as Wnt pathway, could be interfered. Thirdly, multi-drug resistance can be reversed by down-regulating the transporter ABC (ABC-binding cassette) highly expressing on the LSCs surface. Finally, interacting factors between LSCs and niches could be regulated to weaken the homing and migration ability.

Conclusion: Use of extracting effective components from natural products for targeting LSCs could be a strategy for the exploitation and development as molecular-targeted agents. They might play an important role in curing acute myelogenous leukemia.

Keywords: Leukemia Stem Cell; Natural products; Target therapy

Lead Screening and Optimization Based on Traditional Chinese Medicine

Ke Liu

School of Pharmacy, Yantai University, Shandong

Great pharmaceutical companies in west lands would be in the face of difficult situation on drug innovation, though the many new technologies have been innovated, some things like high throughput screening, combination chemistry; and many new drug targets have been discovered. But the cost of drug R&D is getting higher and higher and the time would be taken longer and longer, the successful ratio was lower and lower. On the other hand the chemical drugs show still the clinical limitation effects in the chronic degenerative diseases such as cancer; Alzheimer disease; diabetic and autoimmune disease.....What is reason?

(1) Target based drug discover has its fatal disadvantage. In general speaking, disease is not only one target (target) disorder but a protein net disorder. One target based drug discover must be substituted by multi-target drug discover.

(2) Modern drug screening has a fatal disadvantage, by Hit screening stage *In vitro* only the compound which has a high affinity to the target has been taken in counter, it means the IC₅₀ must be < 0.1 μ M. Minuteneous to one target must cause the scathing change of the relative protein net. It results in serious side effects and even toxic effects.

(3) The generation of chronic disease takes long time. The recover take also long time. But the chemical drugs hanker for always short-term clinical effects. They don't pay attention to the spring of disease. Punchy adjusting to the relative protein net of chronic disease can also cause serious side effects and even cause other organic disease.

Based on these openings, the auto has raised a point of drug R&D based. It means: "Lead Screening and Optimization Based on Traditional Chinese Medicine" and take some examples: like Salvianolic acid from *Salvia miltiorrhiza*; Celastrol from *Tripterygium wilfordii* and Hydroxynaphthoquinone from *Arnebia euchina*.

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Chemical Constituents of *Psidium guajava* L. and *Verbena officinalis* L., and Quality Evaluation of Herba Verbenae

Jicheng Shu, Guixin Chou, Zhengtao Wang

Shanghai R&D Center for standardization of Chinese Medicines, Shanghai

Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai

This dissertation consisted of three parts: part one was studies on chemical constituents of leaves and fruits of *Psidium guajava* L.; part two was studies on chemical constituents of *Verbena officinalis* L.; part three was studies on quality evaluation of *V. officinalis*.

Part one: studies on chemical constituents of leaves and fruits of *P. guajava*.

Psidium guajava L. (Myrtaceae) is commonly known as Guava, distributes mainly in Lingnan of China, an important food crop and medicinal plant in tropical and subtropical countries. Different parts of the plant are reported for the treatment of different ailments such as anti-diarrhoeic, stomach ache, wounds, cold, cough and fever. Previous investigations of *P. guajava* have reported a variety of triterpenoids, flavonoids, and other phenolic compounds. For the purpose to find biologically active compounds, elucidate the pathways of active components, we carried out systematic studies on chemical constituents of this plant.

Systematic chemical studies were carried out on the fruits and leaves of *P. guajava*, 46 compounds were isolated and 43 compounds were elucidated from the fruits, and 44 compounds were isolated and 41 compounds were identified from the leaves, mainly by chemical and spectroscopic methods. Among them, three new triterpenoids, one new phenolic acid, four new bezophenones, and 34 known compounds were isolated from the plant for the first time.

Part two: studies on chemical constituents of *V. officinalis*.

V. officinalis L. a commonly used Chinese herb, is the verbenaceae family. Its action is considered as removing heat and counteracting toxicity, blood-quicken and stasis-dissipate, disinhibit water and disperse swelling. Previous investigations of *V. officinalis* have reported a variety of flavonoids, triterpenoids, phenylethanoid glycosides and iridoids. For the purpose to find biologically active compounds, identify the active constituents and establish the quality standard of *V. officinalis* we carried out systematic studies on chemical constituents.

Systematic chemical studies were carried out on *V. officinalis*, and 39 compounds were isolated. 36 compounds were elucidated by chemical and spectroscopic methods. Four of them were new compounds and 12 compounds were isolated from the plant for the first time.

Part three: studies on quality evaluation of *V. officinalis* L.

In the Chinese Pharmacopoeia (2005 volume I) of *Verbena officinalis* L., the reference standards of TLC identification and quantitative determination were ursolic acid, the quality control methods could not fully represent the quality of the herb. Therefore we established the qualitative and quantitative analysis methods for *V. officinalis* by TLC and HPLC using hastatoside and verbenalin, which are more specific constituents of the herb. 11 samples were analyzed by the established methods, and the methods showed good suitability and reliability.

Hyperglycemic Effects of Mangiferin Hydrastis Granulein in GK Rats with Diabetes Mellitu

Na Li, Xiao-bo Qx, Zhe Lin

Department of Scientific Technology, Development Center of Traditional Chinese Medicine and Bioengineering, Changchun University of Chinese Medicine, Changchun

To investigate the antidiabetic effects of Mangiferin hydrastis granule (MHG) in GK rat. MHG can increase the body weights and decrease the contents of FBG and total cholesterol. There were no difference of the content of insulin between various groups. MHG can reduce the risk of diabetes in GK rats.

Five Kinds of Alkaloids from *Rhizoma Coptis* Improve Glucose Uptake of 3T3-L1 Cells without Adipogenesis Activity

Jiachuan Li, Xianli Meng*, Yi Zhang

Department of Pharmacology, Traditional Minority Medicine College, Chengdu University of Traditional Chinese Medicine, Sichuan

Insulin resistance is the key link of metabolic syndrome, and *Rhizoma Coptidis* has definite clinical effect on improving insulin resistance. We separated five alkaloids from *Rhizoma Coptis* (berberine, palmatine, coptisine, jatrorrhizine and epiberberine), and observed the effect of five alkaloids on 3T3-L1 cell proliferation and induce differentiation and insulin resistance, then explored the mechanism. The results showed that these five alkaloids had obviously promoted cell proliferation, inhibited differentiation, decreased the consumption of glucose in culture medium, improved insulin resistance. These effects are similar to rosiglitazone maleate, its possible mechanism might be attributable to high expression of PPAR α mRNA. The coptisine showed the most significant activity on regulating lipid metabolism and improving insulin resistance at the concentration of 16.5 $\mu\text{mol}\cdot\text{L}^{-1}$. Moreover, the activity was obviously beyond berberine which has been studied more in the world at present. Based on these results, *Rhizoma Coptidis* can obviously improve insulin resistance, the effect attributed to the synergism of each component; and suggested that coptisine has potential advantage on the new drugs development of improving insulin resistance, which is worth for further study.

Inhibition of NADPH Oxidase-produced Oxidative Stress-stimulated JNK/NF κ B Signaling by Diallyl Trisulfide (DATS) Suppresses High Glucose-induced Cardiomyocyte Apoptosis

Wei-Wen Kuo¹, Wei-Jan Wang¹, Chia-Li Way¹, Chih-Yang Huang²

¹Department of Biological Science and Technology, China Medical University, Taichung

²Graduate Institute of Basic Medical Science, China Medical University, Taichung

Hyperglycemia is an important risk factor for cardiovascular diseases. High glucose can induce reactive oxygen species (ROS), leading to cardiomyocyte apoptosis and cardiac dysfunction. In our previous study, we show that NADPH oxidase-derived ROS induces JNK signaling to enhance nuclear factor- κ B (NF- κ B) nuclear activation, contributing to high glucose-triggered cardiomyocyte apoptosis. In this study, we investigate the mechanism of antiapoptosis by diallyl trisulfide (DATS) on high-glucose treated H9c2 cells. H9c2 cells were treated with media containing 5.5 or 33 mM of glucose for 36hr with presence or absence of DATS. Our data demonstrated that ROS measured by flow cytometry, p22, phosphorylated I κ B and c-Jun detected by western blot were dose-dependently decreased after the treatment of DATS in H9c2 cells exposed to high glucose. The results of activated caspase 3 levels and TUNEL assay showed a significant decrease of cell death by DATS. The results of nuclear translocation detected by immunofluorescence assay, promoter sequence binding ability estimated by EMSA, transcription activity evaluated by luciferase assay and downstream gene, COX, expressions examined by RT-PCR indicated that increased NF- κ B nuclear activation by high glucose exposure were decreased by the treatment of DATS in H9c2 cells. Furthermore, all of the increased protein levels, TUNEL-positive cell number and NF- κ B activation were decreased following the treatment of inhibitor of NADPH oxidase. Therefore, we elucidated the roles of the DATS on inhibiting high glucose induced cardiomyocyte apoptosis is mediated through inhibiting NADPH oxidase-derived ROS and its downstream JNK/NF κ B signalings.

Deoxyelephantopin Protects Mice from Lipopolysaccharide/D-Galactosamine - Induced Fulminant Hepatitis through Attenuating LPS, TNF- α , or IL-6 Mediated Signaling Pathways

Ya-Wen Cheng¹, Chi-Chang Huang,¹ Kun-Ju Lin,² Chih-An Hsu,¹ Sien-Sing Yang,³ and Lie-Fen Shyr¹

¹Agricultural Biotechnology Research Center, Academia Sinica, Taipei

²Department of Nuclear Medicine and Molecular Image Center, Chang Gung Memorial Hospital Linkou Medical Center and Chang Gung University, Taipei

³Liver Unit, Cathay General Hospital, Taipei

We aimed to provide scientific evidence for the *in vivo* efficacy of an abundant phytochemical, deoxyelephantopin (DET), isolated from an anecdotally used hepatoprotective phytomedicine *Elephantopus scaber*, in fulminant hepatitis induced by LPS/D-GalN in mice. Protective (preventive) and therapeutic effects and the underlying molecular mechanisms of DET were elucidated and compared with those of a hepatoprotective drug, silymarin. Post-treatment with 10 mg/kg DET or silymarin and pre-treatment with DET (designated Post-DET10, Post-SM10 and Pre-DET10) all effectively attenuated LPS/D-GalN-induced increase in serum aminotransferase activities, and TNF- α and IL-6 levels, as well as NF- κ B(p65) nuclear translocation and I κ B α phosphorylation in mice livers. Pre-DET10 prevented LPS/D-GalN-induced infiltration of F4/80 monocytes/macrophages, and increase of nitrotyrosine and COX-2 protein in liver tissues, and Post-DET10 and Post-SM10 protected against liver cell apoptosis; the 3 treatments all suppressed serum and hepatic MMP-9 activity. Induction of HO-1 expression protected mice from LPS/D-GalN-induced mortality in part with Pre- or Post-DET10 and Post-SM10 treatment, with improved survival from 50% to 75% and 62.5%, respectively. We used serial liver scintigraphy with ^{99m}Tc-diisopropyl iminodiacetic acid (DISIDA) on single-photon emission computed tomography (SPECT) to monitor the LPS/D-GalN-induced acute liver failure with or without compound treatment. Both liver uptake and excretion times of DISIDA were significantly delayed in LPS/D-GalN-treated animals and were effectively recovered by DET and silymarin treatment, reaching the efficiency of the vehicle control. This report demonstrates that DET possesses a novel hepatoprotective function superior to that of silymarin in fulminant hepatitis induced by LPS/D-GalN by downregulating proinflammatory mediators.

Butanol extract of *Gentiana Scabra* bge. has immunomodulatory Effects and Attenuates Pathological Signs in MRL/lpr Mice

Yo-Jui Kuo, Hen-Hong Chang, Tzung-Yan Lee

Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taipei

Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Taipei

Background/aim: *Gentiana scabra* Bge. is a Chinese herb that is prescribed as an anti-inflammatory aid, a hepato-protectant, and an immunostimulant. In this study, we examined the biological effects of butanol extract of *Gentiana scabra* Bge (BG) administration in MRL/lpr mice. MRL/lpr mice develop immunological disturbances and deregulated production of Th1 and Th2 cytokines and are a good model of systemic lupus erythematosus (SLE). **Methods and Results:** Female MRL/lpr mice were randomly separated into two groups. The experimental group received BG (250 mg/kg/day, po) from 19 to 21 weeks of age. At 21 weeks of age, the animals were euthanized and kidneys and spleens were removed for evaluation. Splenic CD3⁺CD4⁺, CD3⁺CD8⁺, and CD4⁺CD25⁺ T cells were increased in the BG-administered mice compared to MRL/lpr controls, and this was associated with splenomegaly. There was a marked reduction in IFN- γ , TNF- α , anti-dsDNA, and there were reduced IgG immune complex deposits in the glomeruli. BG also restored kidney glutathione levels, thereby limiting the toxic effects of the inflammatory mediator iNOS protein and, TNF-alpha mRNA which are overproduced in MRL/lpr mice. Two-dimensional gel electrophoresis was used to analyze proteome changes. BG protected MRL/lpr mice against developing the lupus syndrome through up-regulation of alpha-enolase, which were identified by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry. **Conclusion:** This study indicates that BG at this dose and time course of administration was effective in reducing oxidative stress associated with disease progression in MRL/lpr mice. BG could be useful as adjunctive therapy for reducing distress in SLE. Further research will be necessary to determine the clinical relevance of these findings.

Andrographolide Inhibits iNOS Expression by Down-regulating LPS-induced Activity of NF- κ B and Enhances Suppressor of Cytokine Signaling 3 in Raw 264.7 Cells

Ko-Chen Lee, Hen-Hong Chang, Tzung-Yan Lee

Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taipei
Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Taipei

Background/aim: Andrographolide has been reported to possess a variety of pharmacological activities. In activated macrophage, large amounts of nitric oxide (NO) are generated by inducible nitric oxide synthase (iNOS), resulting in acute or chronic inflammatory disorders. To investigate the mechanism by which andrographolide inhibits iNOS gene expression, we examined the activation of NF- κ B in Raw 264.7 cells. **Methods and Results:** Andrographolide inhibited the lipopolysaccharide (LPS)-induced expression of both iNOS protein and mRNA in a parallel dose-dependent manner. Andrographolide-treated Raw 264.7 cells showed significantly higher gene expression of SOCS1 and SOCS3 in LPS treatment Raw 264.7 cells, while that of TNF- α was reduced. These findings were associated with increased STAT3 phosphorylation. Furthermore, andrographolide significantly improved the oxidative stress and apoptosis pathways in a concentration-dependent manner. Moreover, andrographolide significantly inhibited the DNA-binding activity of nuclear factor-kappaB (NF- κ B) and CCAAT/enhancer-binding protein beta (C/EBP β) as well as activator protein-1 binding protein. Taken together, these results suggest that andrographolide acts to inhibit inflammation through inhibition of NO production and iNOS expression through blockade of C/EBP β DNA-binding activity and NF- κ B activation. **Conclusions:** These results have an important implication for using andrographolide toward the development of an effective anti-inflammatory agent.

Benzoxazinoid Glycosides from *Scoparia Dulcis*

Chia-Chuan Chang*, Tzu-Yu Chen, Rui-Wen Lu

Department of Medicinal Chemistry, National Research Institute of Chinese Medicine, Taipei

Scoparia dulcis L. is a perennial herb widely distributed in tropical and subtropical regions of Asia, Africa and America. In Taiwan and China, it has traditionally been used for disorders such as bronchitis, diabetes, hypertension, hepatitis and gastric ulcer. From the high polar extract of *S. dulcis*, 17 compounds were identified, including four 2-O-glycosyl-7-methoxy-1,4-benzoxazine-3(2H)-ones (GMBOAs) (1-4), three 6-methoxy-benzoxazolin-2(3H)-one (MBOAs) (5-7), one phenylpropanoid sugar (acteoside, 8), four flavonoids glycosides including vicenin-2 (9), scutellarin (10), tricetin 8-O- α -D-glucoside (11), apigenin 8-O-pentopyranoside (12), together with coumaric acid (13) and 4 monosaccharides (myoinositol 14, D-mannitol 15, D-glucose 16 and L-rhamnose 17). Among them, five of them (1 and 3-6) were new. These compounds were isolated by repeated Sephadex LH-20 and C₁₈ column chromatographic methods. The structures of compounds 1-17 were identified by comparison with reported physicochemical data or rigorously elucidated by detailed spectral analyses (UV, MS, ¹H and ¹³C NMR). The proton and carbon signals were assigned according to the DEPT, COSY, HSQC and HMBC NMR spectra.

New Cytotoxic Prostanoids from Taiwanese Soft Coral *Clavularia Viridis*

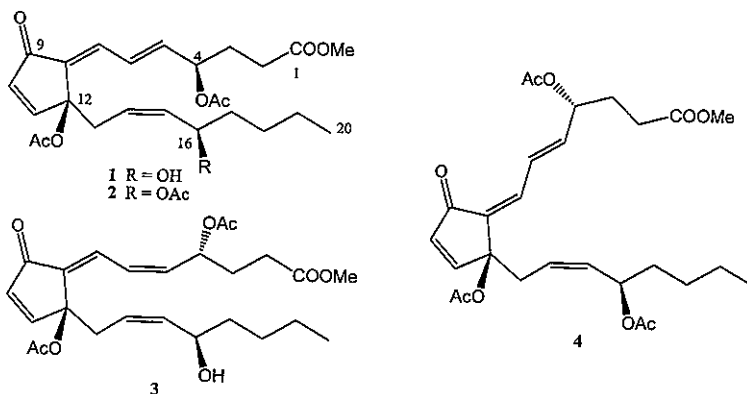
Ya-Ching Shen^a, Kuang-Liung Lo^b, Jiun-Yang Chang^b, Yun-Sheng Lin^a, Khongorzul Mendbayar^a, Yao-Hsiung Kuo^c and Yu-Chi Lin^a

^aSchool of Pharmacy, College of Medicine, National Taiwan University, Taipei

^bInstitute of Marine Resources, National Sun Yat-sen University, Kaohsiung

^cNational Research Institute of Chinese Medicine, Taipei

Chemical investigation of the EtOAc-MeOH extract of *Clavularia viridis* collected in Taiwan has afforded four new prostanoids, designated claviridins A-D (1-4). The structures of compounds 1-4 were determined on the basis of 1D and 2D-NMR techniques including COSY, HMQC, HMBC and NOESY experiments. Pharmacological study revealed that compounds 1-4 exhibited potent cytotoxicity against human cancer cells.



Nortriterpene Lactones from the Fruits of *Schisandra Arisanensis*

Yuan-Bin Cheng,[†] Tzu-Ching Liao,[†] Yi-Wen Lo,[†] Yu-Chen Chen,[†] Yuh-Chi Kuo,[‡] Shun-Ying Chen,[§] Ching-To Chien,[§] Tsong-Long Hwang[⊥] and Ya-Ching Shen^{*,†}

[†]School of Pharmacy, College of Medicine, National Taiwan University, Taipei

[‡]Department of Life Science, Fu-Jen University, Taipei Hsie

[§]Division of Silviculture, Taiwan Forestry Research Institute, Taipei

[⊥]Graduate Institute of Natural Products, Chang Gung University, Taipei

Fractionation of acetone extract from the fruits of *Schisandra arisanensis* afforded five new nortriterpene lactones, together with four known compounds, schindilactones D and E and pre-schisanartanins A and B. Compound 1, a wuweiziartane-type nortriterpenoid, contains a new class of fused ring system with a γ -lactone ring between C-15 and C-17. Compounds 2, 6, and 7 may be categorized as schisanartane-type and compounds 3-5, 8, and 9 as pre-schisanartane-type nortriterpenoids. All the structures of the above compounds were elucidated by 1D, 2D-NMR and single crystal X-ray diffraction analyses. The antiviral activity against HSV-1 and inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB of compounds 1-9 were evaluated.

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Anticancer and Anti-AIDS Clinical Trials Candidates from Traditional Chinese Medicine (TCM)

Kuo-Hsiung Lee, Yizhou Dong, Keduo Qian, Tian-Shung Wu, and Yung-Chi Cheng

Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, USA

Chinese Medicine Research and Development Center, China Medical University, Taichung

Department of Pharmacology, School of Medicine, Yale University, USA

New therapeutic agents with diverse structures and mechanism of action have been and will continuously be discovered from TCM-derived natural products. Accordingly, Dr. Lee's Natural Products Research Laboratories (NPRL) have discovered more than 3,000 novel cytotoxic antitumor and anti-HIV natural products and synthetic analogs in near four decades of support mostly from NIH grants. These discoveries are being used as new leads to develop future anticancer and anti-AIDS agents. Research in the NPRL previously led to the discovery of GL-331, a synthetic etoposide analog, which was in Phase II clinical trials as an anticancer drug. More recently, JC-9, a novel curcumin analog developed in the NPRL, was found to be potent androgen receptor degradation enhancer. Planning is in progress to pursue clinical trials with JC-9 for prostate cancer in the near future, and it currently has succeeded in a Phase II clinical trial for treating acne. Moreover, synthetic analogs of tylophorine and neotanshinlactone have shown efficacy in both cell and animal models and are in preclinical studies for clinical trials for treating lung and breast cancers, respectively. In addition, MPC-4326 (formerly known as bevirimat) has succeeded in Phase IIb Anti-AIDS clinical trials recently and is on planning for phase III clinical trials this year.

Hepatoprotective and Antioxidant Effects of the Methanolic Extract from *Halenia Elliptica*

Youwei Wang*, Bo Huang, Xiaoquan Ban, Jingsheng He, Hong Zeng, Peng Zhang

College of Pharmacy, Wuhan University, Wuhan

Halenia elliptica is a medicinal herb of Tibetan origin, and in China, was commonly used for clearing the "fire and heat of the liver and gall bladder" in the treatment of hepatitis. Research shows that hepatoprotective effects have been associated with plant extracts that are rich in phenolic compounds. The aim of the present study is to evaluate the hepatoprotective and antioxidant activity of *H. elliptica* against experimentally induced liver injury. The antioxidant property of methanolic extract (ME) of *H. elliptica* was investigated by employing various established *in vitro* systems. The ME of *H. elliptica* was studied here for its hepatoprotective effects against CCl₄-induced liver toxicity in rats. Activity was measured by monitoring the levels of alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total bilirubin. The ME possessed strong antioxidant activity *in vitro*. The results of CCl₄-induced liver toxicity experiment showed rats treated with the ME of *H. elliptica* (100 mg/kg and 200 mg/kg), and also the standard treatment, silymarin (50 mg/kg), showed a significant decrease in ALT, AST, ALP, and total bilirubin levels, which were all elevated in the CCl₄ group ($p < 0.01$). The results observed after administration of 100 mg/kg ME were comparable to those of silymarin at 50 mg/kg ($p > 0.05$). The ME did not show any mortality at doses up to 2000 g/kg body weight. Histopathological observations of ME group liver sections showed a relatively normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration that was more similar to the Control group. These results seem to support the traditional use of *H. elliptica* in pathologies involving hepatotoxicity, and the possible mechanism of this activity may be due to strong free radical-scavenging and antioxidant activities of ME.

Extracts of *Halenia Elliptica* Exhibit Antioxidant Properties *In Vitro* and *In Vivo*

Bo Huang, Haibin Ke, Xiaoquan Ban, Jingsheng He, Peng Zhang, Youwei Wang*

College of Pharmacy, Wuhan University, Wuhan

The genus *Halenia* (family Gentianaceae) has approximately 100 species primarily distributed in America, with a few species in Asia. *Halenia elliptica* is one of two species distributed in the Qinghai-Tibet Plateau. It is a medicinal Tibetan herb in China, and is used to treat hepatitis. Research shows hepatoprotective effects are associated with plant extracts that are rich in antioxidants, and the antioxidant activity of *H. elliptica* largely remains an area to be studied. The aim of this work was to test the *in vitro* and *in vivo* antioxidant properties of different fractions of *H. elliptica*: n-hexane extract (HE), chloroform extract (CE), ethyl acetate extract (EE), n-butanol extract (BE) and 70% methanol extract (ME). The results showed that various extracts possessed strong antioxidant activity *in vitro* systems: α, α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging assay, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay, hydroxyl radical scavenging assay, reducing power assay, ferrothiocyanate (FTC) assay and thiobarbituric acid (TBA) assay, and ME had the strongest antioxidant activity. Based on our *in vitro* results, ME was used for investigating the antioxidant properties of *H. elliptica in vivo*. Compared with the control group, the carbon tetrachloride (CCl₄)-intoxicated animals exhibited a significant decrease in superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels, together with a significant increase in the level of malondialdehyde (MDA) in both liver and kidney ($p < 0.001$). Free radical scavenging provides important means to protect against CCl₄-induced oxidative injury. It is believed that ME may act as reductones, by donating electrons and reacting with free radicals to convert them to more stable products, thereby terminating the free radical chain reactions. Thus, it may be concluded that the ME possesses potent antioxidant properties, and might be valuable natural source of antioxidants that could be applicable to both the medical and food industries.

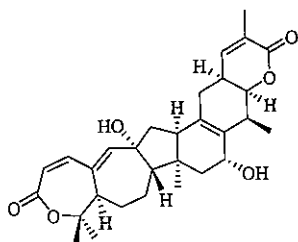
Chemical Constituents from *Kadsura* Species and their Bioactivities

Jian-Xin Pu, Jian-Hong Yang, and Han-Dong Sun*

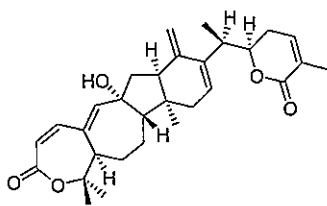
Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan

The genus *Kadsura* belongs to the family Schisandraceae, 8 species of this genus chiefly distribute in the southwest and southeast of China. The stems, roots and fruits of some species have been used as important folk medicine in Taiwan, Japan, and mainland China, and some species of this genus have been reported to mainly contain dibenzocyclooctadienlignans, lanostane, and cycloartane triterpenoids with pharmacological properties, including anti-HBeAg, antitumor, anti-HIV, and anti-lipid peroxidative activities.

In the past five years, more than 300 compounds (including over 120 new ones) have been isolated from six species of the genus *Kadsura* by our research group. Classes of these compounds include triterpenoids, sesquiterpenoids, lignans, and flavanones. Some interesting results have been discovered in this genus. Firstly, two novel series of triterpene dilactone with unprecedented rearranged skeletons, named as kadlongilactones A-F and longipedlactones A-I, were isolated from *K. longipedunculata*, and the cytotoxicity assay disclosed that some of these novel triterpenoids showed significant activity against several kinds of the human tumor cell lines. Secondly, some nortriterpenoids, which were the characteristic chemical constituents from the genus *Schisandra* belong to the same family Schisandraceae, have been isolated from *Kadsura* species firstly. In addition, due to different geographical distribution and climatic condition, it comes true that more novel and biologically potent active compounds have been found from these plants.



Kadlongilactone A



Longipedlactone A

Identification of Tylophorine Compounds as Novel Potent Anti-Coronaviral Agents for Porcine Enteropathogenic Coronavirus Transmissible Gastroenteritis Virus and Human Severe Acute Respiratory Syndrome Coronavirus

Cheng-Wei Yang, Yue-Zhi Lee, Iou-Jiun Kang, Dale L. Barnard, Jia-Tsong Jan, Du Lin, Chun-Wei Huang, Teng-Kuang Yeh, Yu-Sheng Chao, Shio-Ju Lee*

Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan
Institute for Antiviral Research, Utah State University, USA
Genomics Research Center, Academia Sinica, Taipei

For development and optimization of phenanthroindolizidines into therapeutic drugs, the variety of tylophorine derivatives must be expanded to provide fundamental data and to better understand the relationship between structure and activity. We identified tylophorine compounds, including naturally occurring and synthetic phenanthroindolizidines and phenanthroquinolizidines, as potent *in vitro* inhibitors of enteropathogenic coronavirus transmissible gastroenteritis virus (TGEV). The compounds showed low nanomolar 50% median effective concentration (EC₅₀) values as determined by immunofluorescent assay of the expression of TGEV N and S proteins and by real time-quantitative PCR analysis of viral yields. Furthermore, the tylophorine compounds exerted profound anti-TGEV replication activity and thereby blocked the TGEV-induced apoptosis and subsequent cytopathic effect in ST cells. Analysis of the structure-activity relations indicated that the most active tylophorine analogues were compounds with a hydroxyl group at the C14 position of the indolizidine moiety or at the C3 position of the phenanthrene moiety and that the quinolizidine counterparts were more potent than indolizidines. As well, tylophorine compounds strongly reduced cytopathic effect, with low nanomolar EC₅₀ values, in Vero 76 cells induced by human severe acute respiratory syndrome coronavirus. Moreover, pharmacokinetic study demonstrated high and comparable oral availabilities of 7-methoxycryptoleurine (53.5%) and the naturally occurring tylophorine (65.5%) in rats. Thus, our results suggest that tylophorine compounds are novel and potent anti-coronavirus agents that may be developed into therapeutic agents for treating TGEV or SARS CoV infection.

Isolation and Evaluation of Biological Activities In Vitro and In Vivo of Phenanthroindolizidine and Septicine Alkaloids from Formosa *Tylophora Ovata*

Yue-Zhi Lee¹, Cheng-Wei Yang^{1,2}, Chun-Wei Huang¹, Hsing-Yu Hsu¹, Iou-Jiun Kang¹, Yu-Sheng Chao¹, I-Sheng Chen³, Hwan-You Chang², Shio-Ju Lee^{1*}

Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Zhunan
College of Pharmacy, Kaohsiung Medical University, Kaohsiung
National Tsing Hua University, Hsinchu

From leaves and stems of *Tylophora ovata* (Lindl.) Hook. Ex Steud, we isolated 7 phenanthroindolizidine and 4 septicine analogues, including 2 new compounds, 13a(S)-(+)-2-demethoxyl-6-demethyl-septicine (1) and 13a(S)-(+)-6-demethyl-septicine (2). The isolation of 13a(R), 14(R)-(-)-tylophorincine (3) was the first as an enantiomer compound and that of 13a(S)-14(S)-(+)-3, 14-dihydroxyl-6,7-dimethoxyphenanthroindolizidine (4) was the first from a plant. After compounds were isolated, their structures were established by detailed physical data analyses, and all were found to be isolated from *T. ovata* for the first time. *In vitro*, these compounds showed anti-inflammatory activity in suppressing nitric oxide production in RAW264.7 cells stimulated by lipopolysaccharide and interferon- γ , with half maximal inhibitory concentration values ranging from 84 nM to 20.6 μ M. Moreover, growth inhibition testing in human cancer cell lines (HONE-1 nasopharyngeal, NUGC-3 gastric carcinoma, HepG2 hepato-carcinoma, SF-268 glioblastoma, MCF-7 breast carcinoma, and NCI-H460 lung carcinoma) gave half maximal growth inhibitory concentration values from 4 nM to 24.2 μ M. In addition, compounds 5 (13a(S)-(+)-tylophorine) and 3 exhibited potent *in vivo* anti-inflammation efficacy in a rat paw edema model. The isolated phenanthroindolizidine and septicine compounds were analyzed for structure-activity relations.

A Case Study of the Key Factors of Globalization of TCM Enterprises

Shi Yin, Yiran Rong, Hao Hu*

Institute of Chinese Medicine Science, University of Macau, Macau

Aim: TCM enterprises have been facing excessive obstruction in the attempt to globalize their business. Since a long time ago, it is considered that TCM with unclear sophisticated components is the main obstacle of its globalization. However, clarifying the definite components of TCM is not legally required in Euro and US. The obstacle seems not to be the above. This study is to investigate globalization strategies of two TCM enterprises and try to find the real obstacles of TCM globalization; we also attempt to show some effective ways of achieving TCM globalization.

Method: Case study: TongRenTang, EuYanSang

1. Is TCM with unclear sophisticated components a real issue to demonstrate its quality?
 - Veregen approved by FDA
 - TongRenTang & EuYanSang's behaviors
2. Some difficulties and failures the companies meet
 - TongRenTang vs EuYanSang
3. How the companies attempt to globalize their business? How about the efficacy?
 - TongRenTang & EuYanSang's strategies
 - TongRenTang & EuYanSang's progress in TCM globalization.

Result:

1. Both companies adopt modern enterprise system and new TCM technology, and manufacture management with strict GMP to fit the overseas standard (e.g. Australia & Malaysia) is carried out to assure products quality.
2. Cultural gap and brand controversy are big obstacles especially in European and American markets.
3. Both companies first market in overseas Chinese regions, and they seek partners in the target markets. Also, some TCM clinics with western medicine are opened by them. Their networks have extended to non-Chinese society during these several years.

Conclusion:

- Modern management mode is required to make the products quality reliable.
- Culture needs to be delivered and integrated when marketing products, TCM clinic is an effective way.
- Brand protection should be engaged to achieve the value.

Location-quotient Based Analysis of Industry Structure of Traditional Chinese Medicine in China

Chen Cong, Hu Yuanjia, Wang Yitao*

Institute of Chinese Medical Sciences, University of Macau, Macau

Objective: To identify the TCM industry structure on a regional level and give a comprehensive ranking; to find out which areas have specialized or regional advantages and make suggestions for optimizing the industry structure of TCM in China.

Methods and Results: Collect serial data (2004-2008) on gross industrial output value (GIOV) of entire pharmaceutical industry (PI), TCM herbs, and TCM patent prescription drugs (sum in TCM) in province level from National Bureau of Statistics of China and database ChinaInfobank;

Figure out the TCM location quotient (LQ) and national market share (MS) of 31 provinces to indicate the structure and specialization of TCM industry; then broke them down into types of matrix followed and mapped in bubble chart.

<p>B (High LQ, low MS.) Comparative advantage but small scale without nationwide competitiveness. Chongqing, Anhui, Yunnan, Gansu, Qinghai, Xizang.</p>	<p>A (High LQ, high MS.) Most comparative advantage and competitiveness. Guangdong, Guangxi, Sichuan, Guizhou, Jilin, Jiangxi, Beijing (A->C).</p>
<p>D (Low LQ, low MS.) No nationwide competitiveness, small industrial scale and no comparative advantage. Shanxi, Heilongjiang, Shanghai, Fujian, Ningxia, Hainan, Liaoning, Tianjin.</p>	<p>C (Low LQ, high MS.) Nationwide competitive but no comparative advantage. Jiangsu, Shandong, Henan.</p>

$$LQ = (GIOV_{TCM} : GIOV_{PI})_{regional} / (GIOV_{TCM} : GIOV_{PI})_{national}$$

$$MS = (GIOV_{TCM})_{regional} / (GIOV_{TCM})_{national}$$

Conclusion: Type A TCM sectors have most comparative advantage and competitiveness inside countrywide, which mostly is steady. Type B has certain advantages relative to other sectors in regional pharmaceutical industry while national competitiveness is below normal. TCM sectors in type C always have a strong overall pharmaceutical industry base and would transform into type A if they were taken into account seriously. However, nearly 40% in the 31 provinces have not shown any advantage in scale or competitiveness.

The Potential Use of Traditional Chinese Medicines in Finding Telomerase Activators

William H. Andrews

Sierra Sciences, LLC, USA

In the last three decades, there has been a tremendous upsurge of scientific knowledge of how and why we age. We now know that there is a clock that ticks inside every dividing cell of our bodies, and that our cells contain a gene for an enzyme called telomerase that can stop and even reverse this clock. Unfortunately, the gene for telomerase is turned off in our cells.

Scientists have begun a search for a substance that turns the telomerase gene back on. In 2001, the first telomerase-activating compound known to mankind was discovered in an extract of the root of the traditional Chinese medicine Astragalus root.

The telomerase-activating compound in Astragalus root, known as TA-65, turns on telomerase, but only at weak levels. However, it's likely that an extract from another traditional Chinese medicine contains an even more powerful telomerase activator. Screening traditional Chinese medicines has many advantages over screening manufactured chemicals. In particular, they are generally recognized as safe for human use and therefore do not require FDA approval.

Screening a library of extracts from traditional Chinese medicines could be the first step in allowing humans to exceed the theoretical 125-year limit on our lifespan.

International Collaborations Session

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Interaction of Traditional Chinese Medicine Components with Phospholipid Bilayers

Lijing Ke^{1,2}, Pingfan Rao¹, Thomas Haus³, and Jeremy P Bradshaw²

¹Institute of Biotechnology, Fuzhou University, Fujian

²Royal (Dick) School of Veterinary Studies, The University of Edinburgh, UK

³Hahn-Meitner-Institut, Germany

Radix Isatidis, the root of the plant *Isatis indigotica* Fort. or *Isatis tinctoria* L., and bitter melon (*Momordica Charantia* L.) are widely used in China as antiviral and antidiabetic agents, respectively. Arginine and Glucose products (MRPs) of these two plants and Arginine-Glucose were prepared by heating process to obtain RIE, MCE and Arg-Glc MRPs. The interaction of these MRPs and the stack of membrane phospholipids DOPC bilayers was investigated by neutron scattering.

The three products have significantly different distribution across the DOPC bilayer. Since the principal site for interaction of RIE is 18.2 Å from the centre of the bilayer, RIE is the one which inserts deepest into the bilayer. The MRPs from MCE display some similarity to the RIE profiles, but lies in the interface region. In the profiles of Arg-Glc MRPs, there is a major peak at 15.1 Å from bilayer centre. This lies much further in that the MCE peak, indicating that the Arg-Glc MRPs is able to penetrate deep into the core of the bilayer, similar to the RIE.

The introduction of the different types of MRPs slightly decreased the *d*-repeat of lipid bilayers. The most significant decrease in *d*-repeat occurs in Arg-Glc MRPs, reflecting the fact that these samples have the highest level of bilayer-incorporated MRPs.

The three types of MRPs all bound to the DOPC multilayer stacks, though on different manners of distribution. As a result of this binding, they had slightly different impacts on the way membrane lipid packed. This is the first evidence of the attachment of MRPs to cell membrane lipids bilayers. This attachment is the types of MRPs and lipids dependant, which might indicate a cell binding specificity of MRPs from different herbal Chinese medicine.

The new HanseMerkur Centre for TCM at the University Medical Centre (UKE) in Hamburg

Roland Salchow, Sven Schroeder

*HanseMerkur Centre for Traditional Chinese Medicine at the University Medical Centre,
Hamburg Eppendorf (UKE), Germany*

In Hamburg a comprehensive TCM institute at university level has been established. The title indicates its special character:

- the centre is running on a donation by the HanseMerkur Health Insurance Company
- TCM is the basic medical instrument, but cooperation with western medicine is demanded (TCM, not just acupuncture)
- the UKE clinicum is hosting the centre.

The idea goes back to political incentives, Hamburg and Shanghai are sister cities. Western medicine physicians at the UKE have agreed to accept the center as a separate self-organised institute.

The center will have two financially and legally different units: for research and education a limited liability company, and for treatment of patients a private company medical practice. 50 % of the activities regard treatment of patients, 50 % research. The medical doctors are working half-time in *both* units. There is a board of supervisors and a scientific council.

One field of research is using system science and complex analysis, incorporating non-linear and system thinking - thus trying to understand the holistic (multi-component) basis of TCM. Western medicine relies on a *detailed* classification of diseases and treatments - isolating and studying single molecules and cells, taken as responsible for health or disease.

But single proteins/moleculars are often part of a complex system. Most diseases are multi-factorial, it has to be accounted for the synergy of the constituents - as is the basis of TCM's holistic approach. We make use of ideas already used in Utrecht, Yale, and Mainz. The system approach is to classify diseases not by symptoms but through comprehensive molecular descriptions of the disease *and* the response to treatment.

An Investigation of Contemporary Medicinal Plants Based on International Collaborations

Zhongzhen Zhao*, Peigen Xiao

*School of Chinese Medicine, Hong Kong Baptist University, Hong Kong
Medicine and Health Engineering Division, Chinese Academy of Engineering, Beijing*

Supported by the Hong Kong Jockey Club Institute of Chinese Medicine (HKJCICM), a systematic investigation of contemporary medicinal plants throughout the world has been conducted during the past 6 years. More than 100 experts in the field of pharmaceutical sciences from mainland China, Hong Kong, Taiwan, Japan, South Korea, the United States and Europe participated in related field investigation, laboratory work, and literature research. The research results have been published by HKJCICM (traditional Chinese version in 2006) and by World Publishing Corporation (simplified Chinese version in 2008 and English version in 2010) as the 4-volume *Encyclopedia of Medicinal Plants*. This Encyclopedia comprises 4 volumes. Volumes 1-2 cover commonly used medicinal plants of traditional Oriental medical systems, such as those from China, Japan, the Korean Peninsula, and India. Volume 3 covers commonly used American and European medicinal plants, such as those from Europe, Russia, and the United States. Volume 4 covers medicinal plants commonly used, produced, and/or commercially available in the Lingnan area of southern China. A total of 500 commonly used medicinal plants (involving more than 800 species) are recorded with the latest botanical, phytochemical, pharmacological and clinical data, expert opinions on characteristics and perspectives, and supporting references. 1358 high resolution digital pictures of the original plants, medicinal materials and their plantation sites are included. The related voucher specimens collected from all over the world are deposited at Bank of China (Hong Kong) Chinese Medicines Center. The traditional Chinese version is circulating in Hong Kong, Taiwan and Macau. In 2007, the simplified Chinese version was named as one of the most outstanding imported science books in China. The publication of this Encyclopedia is a prime example of successful in-depth international collaboration.

Heritage, Medicinal Material Resources and Our Efforts

Zhongzhen Zhao*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

China has one of the longest histories of using ethnomedicine and some of the most abundant medicinal material resources of any country on the planet. Its time-honored medical system has been continuously making great contributions to the health of people at home and abroad.

In the past decade, our research team at Hong Kong Baptist University (HKBU) has been conducting field investigations and laboratory work related to Chinese medicinal resources. As a result, we have achieved the following:

- (1) Academic books and papers regarding Chinese medicinal material (CMM) resources, macro-/microscopic identification and quality evaluation of CMMs, microscopic identification of proprietary TCM products, Chinese herbology and formula, medicated diet, and medicinal material processing have been published. Macro-/microscopic identification of some Western herbs have been included in 2009-2010 USP Dietary Supplements Compendium.
- (2) The Hong Kong Chinese Materia Medica Standards project is going smoothly with international collaboration. The standards of 61 CMMs covered in phases I to III have been issued. A further 36 CMM standards for phase IV research work will be finished at the end of 2010. Building upon the proven value of this work, the Department of Health has planned to further develop the standards of another 104 CMMs in mid 2010 (Phase V).
- (3) The Bank of China (HK) Chinese Medicines Center has been established. The Centre displays over 3000 voucher specimens of ethnomedicines (mainly Chinese medicinal materials) from all corners of the world.
- (4) An authentication center for CMMs has been established at HKBU. This authentication center has been playing an important role in guaranteeing the effectiveness and safety of ethnomedicines including, but not restricted to, TCM.

In-depth international collaboration is needed to further explore ethnomedicine-related cultural heritage and medicinal material resources throughout the world.

Cochrane Reviews for Clinical Trials of Traditional Chinese Medicine

Lu Li *, Li Xia Dou, Ping-Chung Leung, Chi Chiu Wang

Department of Obstetrics and Gynaecology, Institute of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong

Division of Perinatal and Reproductive Medicine, The University of Liverpool, UK

Introduction: The Cochrane Collaboration is famous for providing up-to-date, accurate information about systematic assessments of healthcare interventions, known as Cochrane Reviews, which are published online in "The Cochrane Library"[1]. It consists of 50 different topic-based review groups, which review the best clinical trials available on various subjects and oversee the preparation and maintenance of the reviews, which are widely accepted as "Gold Standards" and used to help providers, practitioners and patients as the most comprehensive and reliable source of evidence.

Workflow: Following the workflow is the best and only way to get involved and write a Cochrane review with high efficiency. It could be concluded with the following steps: contact the co-ordinator to set up collaboration, check the vacant list and register the title, get accounts to download study materials and access working website, attend related workshops, write the Protocol, go through editorial process, publish, write the Review, go through editorial process, publish.

TCM Reviews: With the wide spread of Traditional Chinese Medicine (TCM) as treatments to various diseases and daily health care, more and more Chinese doctors seek collaboration opportunities with Cochrane groups and contribute to work on TCM application trials with Cochrane Reviews. However, there are some differences and difficulties for TCM reviews. First, TCM has unique theories and diagnosis systems, which should be paid with more attentions to be clearly explained. Second, many clinical trials are with low qualities and not using real randomization methods, thus authors should be more strict on the criteria for trials inclusion and exclusion. Third, the medicines, therapies, durations and courses of TCM treatments mostly depend on the experience of practitioners, and vary a lot sometimes. Therefore, authors should correctly set up the comparisons between interventions and controls, and make clear definitions on the outcomes to get appropriate conclusions for the review.

[1] <http://www.cochrane.org/> Available on May, 2010.

Establishment of Sasang Constitutional Medicine Clinical Research Collaboration in Korea

Jonghyang Yoo, Eunsu Jang, Siwoo Lee

Division of Constitutional Medicine Research, Korea Institute of Oriental Medicine, Korea

Sasang constitutional medicine (SCM) is one of the unique traditional Korean medicine, which was originated from the book named [Longevity and Life Preservation in Oriental Medicine] by Dr. Lee, Jema of Chosun dynasty (old name of Korea) in the late 19th. It focused on the customized medicine, preventive medicine and mind-body medicine which is consistent with the recent medical trends.

Lee Jema project is the Korean government supported project for scientification and objectification of SCM diagnosis and development of constitution-based treatment system. Korea institute of oriental medicine (KIOM) has collaborated with more than 20 nationwide oriental medical hospitals and clinics to collect various data – clinical data, measurement data and biological data - from the participants whose constitutions were established from clinical practices including SCM herbal prescription responses. KIOM made these data as web based database named CIB (Constitution Information Bank, <http://crf.kiom.re.kr>). There are more than 5,000 cases in CIB from 2006 to 2010.

Recently KIOM started to collaborate with two Cohort study teams – Ajou Univ. and Korea Univ. - in Korea and make international collaboration with Tohoku Univ. in Japan.

Global Development and Influential Countries in Acupuncture Research: A Statistical Analysis

Shikai Zhang, Yiran Zhang, Yuanjia Hu, Yitao Wang*

Institute of Chinese Medical Sciences, University of Macau, Macau

Objectives: This survey of clinical research articles aims to (1) characterize the global development of acupuncture research and (2) identify the key countries promoting globalization of acupuncture.

Methods: Acupuncture research articles including clinical studies and their meta-analysis published between 1990 and 2009 were retrieved from PubMed. Extracted data include: first author's address, location of publication and the year of the journal's launch. An influence indicator of each country was calculated using a weighted formula derived from the impact factors (SCI Citation Report 2008) of the journals published in the country. Statistical and social network methods were employed to analyze various characteristics of acupuncture research articles and to explore the key countries promoting globalization of acupuncture.

Results: The international publication of acupuncture research articles ($n=2153$) was increasing in the past 20 years at a mean annual growth rate of 63%. The acupuncture publications in China ($n=835$) grew rapidly between 2005 and 2009 at a mean annual rate of 102%. Among English journals, top five countries with acupuncture authors were: USA, Germany, UK, Taiwan, and Korea. Correlation analysis showed that the number of English journals was significantly correlated with the number of published articles with Chinese authors ($P < 0.01$). The top five most influential countries were: USA, UK, Germany, Denmark and Norway. In the social network of acupuncture research, USA and UK were the countries with top centrality.

Conclusions: USA and UK are the strongest influencers in terms of publication impact. China is catching up in publication of English research articles. This effort would help globalize the Chinese acupuncture research.

Open Access Journals and Globalization of Chinese Medicine

Hin Wing Yeung

*International Society for Chinese Medicine, Macau
University of Macau, Macau*

The journal, CHINESE MEDICINE (CM, www.cmjournal.org), is an open access, peer-reviewed, interdisciplinary and scientific journal in Chinese medicine. CM is devoted to bridging the gap between traditional Chinese medicine and modern medicine, and endeavours to unlock the scientific value of Chinese medicine. CM is an exciting medium for fostering evidence-led force for the advancement of Chinese medicine by committing to professional excellence and open access publishing.

The advancement, including globalization and modernization, of Chinese medicine should be led by evidence. Chinese medicine research should gather and verify evidence which can be used to break new grounds. Evidence-led Chinese medicine (ELCM), uses appropriate research methodologies, irrespective of whether they are traditional or modern, conventional or alternative, macroscopic (systems or subsystems) or microscopic (cellular and/or molecular) as long as scientific rigour is upheld. Chinese medicine research adopting the approaches and methodologies of systems biology and omics as well as those from other relevant fields, such as evidence-based medicine (EBM) and clinical and molecular epidemiology, will generate macroscopic, microscopic and integrative macroscopic-microscopic evidence for ELCM.

Qualitative Research Methods as Useful Tools to Explore Chinese Medicine in Beijing and London

¹Xing Liao, ¹Jianping Liu, ²Nicola Robinson

¹Center for Evidence-Based Chinese Medicine, Beijing University of Chinese Medicine, Beijing

²Center for Complementary Healthcare and Integrated Medicine (CCHIM), Faculty of Health & Human Sciences Thames Valley University, UK

In 2006 the National Basic Research Programme of China provided funding to support qualitative research on Chinese medicine (CM) practice. This was the first time funding had been provided for this type of research methodology. In China, CM is a specialty within the broader organization of Chinese health care and CM practitioners are able to make both Western medicine and Chinese medicine diagnosis and treatment. These differences stimulated collaborative research between Beijing and London to compare CM practitioner experiences between the two countries.

A qualitative approach was used to explore CM practitioners' practice by understanding their experience, knowledge, diagnosis and treatment principles for chronic disease; examining how such practice impacts on patients and identifying difficulties in conducting qualitative research in CM.

Semi-structured interviews were conducted with a purposive sample of 14 doctors and 18 patients with chronic health problems from endocrinology departments at five hospitals in Beijing together with 41 observations of the same doctors in routine clinical practice. Verbatim transcripts were typed and analyzed, using a framework approach. 14 CM practitioners are being interviewed in London, to compare experiences and knowledge.

Preliminary analysis of the Beijing sample identified key themes: (1) For CM practitioners: 'personal attitudes' foundation' of CM practitioners (knowledge about WM and CM; social and culture factors;), experience (learning and practice), practice (various treatment techniques and thinking style), outcome evaluation (quality and effects), and outlook (the future of CM development); (2) from patients : attitudes to CM hospital treatment, reasons for choosing CM treatment, and access to and experience of CM treatment; (3) from the observations: communication between CM practitioners and patients (practice information and daily life information), relationship between practitioners and patients (trust and dependence), and health related behaviors. These findings will be contrasted with the experience and perspective of CM practitioners in London.

Qualitative research methods are important in describing the complexity of CM doctors' practice but this may vary between countries.

Supported in part by the National Basic Research Programme of China ('973' Programme, grant number 2006CB504602)

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Objectification of TCM Four Diagnostic Methods in Sub-Health State Research

Li-min Wang, Jia-xu Chen, Xin Zhao

School of Pre-clinical Medicine, Beijing University of Chinese Medicine, Beijing

Sub-health state is a low-quality status between health and disease. Before the researchers have focused on the syndromes and symptoms of sub-health state while there have lacked research on objectification of TCM four diagnostic methods in sub-health state. As we all know, it is important for researchers to use the four diagnostic methods to collect clinical materials. Consequently, the four diagnostic materials should be complete and specific as much as possible and stage treated or quantized. Meanwhile it is necessary to study on four diagnostic combining objective index based on large sample clinical epidemiology. We will carry out a clinical epidemiology survey in five national clinical centers and obtained 2,500 samples' clinical materials including four diagnostic materials and objective index, such as IgA, IgG, β -EP, Cor, T, C, ACTH, CD3+, CD4+, etc. Clinical data are acquired using the questionnaire for basic syndrome of TCM in sub-health population. The questionnaire had good reliability and validity which had been verified in preliminary studies. Diagnostic criteria inclusion criteria and exclusion criteria of sub-health should be strictly executed in the clinical epidemiology. Since there is an increasing interest in using data-mining methods to identify or predict sub-health state, we have hereby proposed and applied the SVM method of Recursive Feature Elimination (RFE) and Random Forest method to feature selection. A fixed number of features may be selected for further analysis or to design a classifier, by which sub-health state should be predicted. Objectification of TCM four diagnostic methods in sub-health state should be realized step by step through finding the relationship between the complete and specific four diagnostic materials and objective index based on the large scale clinical epidemiology in China.

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A New Platform for Information Extraction and Analysis of Pulse-diagnosis in TCM and it's Applications in Clinic

Nanyue Wang, Youhua Yu, Dawei Huang, Zengyu Shan

Experimental Research Center, China Academy of Chinese Medical Science, Beijing

We have established a new platform for information extraction and analysis of pulse-diagnosis in TCM based on the subject supported by National Science and Technology Ministry "the study on the method of information acquisition and analysis of Pulse-diagnosis in TCM". We can extract the information and then pretreat them by DCT, build mathematical models by harmonious wave fitting, do pattern recognition by EFBS (Extended Forward Backward Leastsquare Regression) and classification judgement on the platform.

Through the platform, we have studied the pulse signals of 4 kinds of people: the normal, the patients of hypertension, cirrhosis and the pregnancy. The result is that we can classify them from the normal by 3-4 special features with the accuracy rate about 90% - 96%. For example: the features of the patients of hypertension is the phase position 1 of zuoguan and the phase position 2 of youchi, etc; And the energy of 2nd harmonics in zuoguan and the depth of youchi is the most important features to divide the pregnancy from the normal; and the phase position 1,2,3 of zuoguan are very important in the diagnosis of cirrhosis.

In conclusion, the objective and standard assessments of pulse diagnosis is always the most difficult problem in the modern research in TCM. The platform built by us can help to solve some of them and even can help the diagnosis of clinic.

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Canonical Correlation Analysis of TCM Syndrome and Related Laboratory Parameters in 350 Patients with Diabetic Kidney Disease

Liping Yang, Ping Li, Jinhang Du, Jing Li, Deqiang Deng, Yongli Zhan

Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing

Objective To investigate the distribution pattern of TCM syndrome in patients with diabetic nephropathy, and their relationship with main risk factors of Diabetic kidney disease (DKD) **Methods** Multi-centric epidemiological field survey was adopted to collect the main clinical data of 350 DKD patients conforming to the inclusive/exclusive criteria. The demographic, TCM syndrome and laboratory parameters were compared based on different DKD stages. The canonical correlation relationship between TCM syndrome score and fifteen commonly used laboratory parameters were analyzed. **Results** (1) Distribution pattern of TCM syndrome: the main deficiency syndromes of Microalbuminuric stage were Yin and Qi deficiency; the main deficiency syndrome of Macroalbuminuric stage were Yin and Yang deficiency, and renal failure stage were Yin, Yang and blood deficiency, while blood stasis and damp-heat were the main excess syndromes of every stage of DKD. (2) The results of canonical correlation analysis revealed that there were canonical correlation between serum creatinine and Yang deficiency syndrome ($CanR=0.753362$, $P<0.0001$), postprandial blood glucose and Yin deficiency syndrome ($CanR=0.477996$, $P<0.0001$), low density lipoprotein and damp-heat ($CanR=0.373819$, $P=0.0163$). **Conclusion** The developmental process of TCM syndrome of diabetic kidney disease was that Qi and Yin deficiency progress to Yin and Yang deficiency and finally progress to universal deficiency of Qi, Blood, Yin and Yang. Blood stasis syndrome is the most important excess syndrome which runs through the course of DKD. The canonical correlation between TCM syndrome score and laboratory parameters may provide evidence for treating and preventing diabetes and its main complications by TCM means.

A Study on the Characteristic and Regularity of Distribution of TCM Syndromes in Patients with Ischemic Stroke

Yan Huang, Min Zhao, Yefeng Cai, Jianwen Guo, Xiaobo Yang, Aihua Ou

Encephalopathy center, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangdong

Objective: Investigate the characteristic and regularity of distribution of Traditional Chinese medicine syndromes during 1-3 days, 4-10 days and 11-30 days after onset.

Method: There are 4077 cases from east, south, middle, north and southwest of china. Collect the Chinese medicine symptoms of all the patients at 1-3d, 4-10d and 11-30d after onset by using "Stroke syndrome questionnaire". Each symptom including the tongue and pulse is given a corresponding score according to the "The diagnostic criteria of stroke differentiation". As long as the total score is greater than or equal to 7, each syndrome can be diagnosed. Then analyze the characteristic and regularity of the distribution of Chinese medicine syndromes at three time points.

Results: The distribution of the Chinese medicine syndromes are: the percentage of wind at three time points are 79.4%, 77.2%, 75.5%, the percentage of phlegm at three time points are 62.8%, 62.5%, 52.8%, the percentage of blood stasis at three time points are 81.0%, 80.2%, 77.3%, the percentage of pathogenic fire at three time points are 30.9%, 29.5%, 24.9%, the percentage of qi deficiency at three time points are 85.1%, 83.7%, 78.5%, the percentage of yin deficiency and yang hyperactivity at three time points are 23.90%, 22.50%, 21.60%. The statistical difference of distribution of each syndrome between 3 time points is significant except for yin deficiency and yang hyperactivity. The Chinese medicine syndromes of ischemic stroke mainly manifest as the combination of several syndromes such as at 1-3d after onset the combination of 3 syndromes including wind, blood stasis and qi deficiency is most common and at 4-10d and 11-30d after onset, the combination of 2 syndromes including blood stasis and qi deficiency is most common while the secondary common combination is the phlegm and qi deficiency.

Conclusion: The Chinese medicine syndrome combination of ischemic stroke is complex because it mainly demonstrates as the combination of several syndromes while the appearance of single syndrome is rare. Wind, phlegm, blood stasis and qi deficiency are the principle syndromes of ischemic stroke and in the combination of syndromes, the combination of blood stasis and the combination of wind, blood stasis and qi deficiency are the most common ones.

Patient-Reported Outcome Scales may be Developed for Assessment of TCM Syndromes

CHEN Run Qiu, WONG Chit Ming, LAM Tai Hing

School of Chinese Medicine, School of Public Health, The University of Hong Kong, Hong Kong

Evidence shows that study designs guided by TCM theory are necessary to validate and improve future randomized controlled clinical trials (RCTs) in TCM. Such study designs require objective and standard assessment of TCM syndromes. Patient-reported outcome (PRO) approach is shown to have significantly improved reliability of clinical information and PRO scales have been widely used in RCTs. We studied whether TCM syndrome-specific PRO scales can be developed for assessment of TCM syndromes.

TCM syndromes are defined by expert opinions. Despite their description is vague and varies in textbooks and references, each syndrome is described with a definite number of symptoms and signs, and definable domains of TCM pathogenesis. The discrepancies of expert opinions may be resolved by a four-step approach we proposed for evidence-based validation of TCM syndromes, because scientific evidence is recognized to be a higher form of knowledge compared to expert opinion. The four-step approach involves thorough review of expert opinions, exploratory validation test on patients, construction of data-supported and theory-justifiable model of syndrome, and confirmatory validation testing the model on patients. As reported elsewhere, we have demonstrated that Kidney-deficiency syndromes (KDS) in menopausal women can be validated with the four-step approach and shown that the domain changes of KDS in menopausal women can be measured by patient-reported symptoms. A PRO KDS Questionnaire (KDSQ) consisting of the symptoms has been further tested for internal consistency, test-retest reliability, construct validity and responsiveness. The results have shown that KDSQ is a valid and reliable PRO instrument and may be used for outcome measurement in RCT.

Provided that TCM syndromes are described in plain language, TCM syndromes may be validated and standardized with the four-step approach based on scientific evidence, and TCM syndrome-specific PRO scales may be developed for the assessment of TCM syndromes.

Parametrizing the TCM Diagnosis by Decoding the Physiology of Yin, Yang and the Phases

Henry Johannes Greten, Mario Goncalves and Andrew B.-H. Remppis

Institute of Biomedical Sciences, University of Porto, Portugal

Heidelberg School of Chinese Medicine, Germany

Clinic for Cardiology in the Cardiovascular Centre Bad Bevensen, Germany

One of the key issues for the acceptance of TCM as a science in the West is to make Western scientists understand Chinese Medicine and its diagnostic terms. This understanding begins with the core termini of TCM, which are yin, yang and the phases (wu xing). The German philosopher and mathematician Leibniz (1646 – 1716) already decoded yin and yang lines within the I Ging as a binary numbering system. Circles can be described by these numbers which can be mathematically transformed to sinus waves. These sinus waves are a rough description of periodicity in regulated (cybernetic) systems. This can be used to describe the periodicity of regulation of the vegetative nervous system, which results in the clinical pictures of the phases (wu xing).

Yin, yang and the phases are therefore – according to this analysis – terms of regulation, and numerous physiological functions could be scientifically allocated to the curve and the phases. Mathematically analysing the I Ging links Chinese medical terms and the clinical pictures of TCM with the physiological mechanisms of the West, thus allowing to measure the vegetative effect of reflex treatments like acupuncture. As a consequence, we have defined the theory of TCM according to old scriptures of medicine, daily practice, scientific translation and mathematical analysis more precisely, thus providing a quotable scientific basis of defined diagnosis in TCM. This Heidelberg Model of TCM allows to translate the key termini of TCM into western measurable physiology. We also developed a first double blinding assay of acupuncture and showed objective and specific effects of acupuncture, the effect of which depends on the precision of the diagnostic model.

Pulse Waveform Recognition Using Fisher Linear Discriminant in Traditional Chinese Medicine based on Hemodynamics Principle

Hai Xia Yan¹, Yi Qin Wang¹, Rui Guo¹, Fu Feng Li¹, Zhao Xia Xu¹, Feng Ying Run¹, Yu Jian Hong¹ and Jian Jun Yan²

¹Department of Basic Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai
²Center for Mechatronics Engineering, East China University of Science and Technology, Shanghai

Pulse diagnosis is one of important diagnosis methods in Traditional Chinese Medicine (TCM). A number of studies on quantitative description and analysis of TCM pulse have been reported in recent decades. Recognition of TCM pulse has received more and more attention in recent years. Extracting proper features is crucial for satisfactory classification. A hemodynamics method is used to calculate the pulse waveform velocity (PWV) and pulse reflection factor (R_f), which reflects the principle of TCM pulse diagnosis. Fisher linear discriminant (FLD) has recently emerged as an efficient approach for extracting features for many pattern classification problems. FLD is employed to classify the pulse data, including normal pulse, slippery pulse, wiry pulse and wiry-slippery pulse. Leave-one-out strategy is used for accuracy assessment. The result shows that corresponding changes in the waveform shape of different TCM pulses appear as PWV and R_f increase. An average accuracy rate of 82.5 % is achieved. It is concluded that PWV and R_f may be used as the valid features of TCM pulses and FLD is an efficient approach for the classification of TCM pulses.

Acknowledgments: The research reported here was supported by the National Science and Technology Pillar Program in the Eleventh Five year Plan Period (No.2006BAI08B01-04) and the Shanghai 3rd Leading Academic Discipline Project (No. S30302). The authors are grateful to Professor Liu Zhaorong, Dr. Xiang Cheng, Dr. Qin Kairong and Dr. Huang Dong for their assistance.

Research on TCM Syndromes of CHD Inquiry Based on Latent Structure Model

XU Zhao-xia¹, WANG Yi-qin¹, YAN Jian-jun², XIA Chun-ming², LIU Guo-ping¹, XU Jin¹, LI Fu-feng¹, YAN Hai-xia¹, GUO Rui¹, HAO Yi-ming¹

¹Shanghai University of Traditional Chinese Medicine, Shanghai
²East China University of Science and Technology, Shanghai

Objective: The study observes the Traditional Chinese Medicine (TCM) inquiry clinical features of Coronary Heart Disease (CHD), and researchs the establishment of effective and objective diagnosis about TCM inquiry diagnosis of CHD based on latent structure model.

Method: This issue makes CHD as starting point, ensures possibly the consistency and normative about inquiry information through manufacturing TCM heart inquiry scale. This article collects 1391 cases of CHD using the inquiry scale of heart system in TCM. It assigns "1 and 0" separately according to "yes and no" of the inquiry information. Using Epidate3.1 to input and check data, and establishes inquiry database of CHD. We filter 50 inquiry information to analysis, based on frequency analysis and expert rating. We use computer to carry out hierarchical latent class analysis of the obtained data to find the laws and builds the latent structure model to explain these laws.

Result: (1)The clinical features of CHD: The gender differences is not significant between men and women; Most patients is older, the average age is 64.85 ± 19.60 years old; (2) The results show the probability of the relationship between the CHD inquiry information (significant variables) and TCM syndrome (hidden variables).

Conclusion: The study can provide the basis for CHD syndromes standards and promot the research on the disease and syndrome. It can prove objectivity of differentiation of syndrome in TCM to a certain degree, and also conducts methodological exploration for researching integrated analysis of information of four diagnostic methods in TCM.

Keywords: Latent Structure Model; Traditional Chinese Medicine (TCM) Inquiry; Coronary Heart Disease (CHD).

Acknowledgements: I sincerely appreciated opening source of the latent structure the Dr. Zhang in Hong Kong University of Science and Technology provided for us. This project was supported by the Shanghai 3th Leading Academic Discipline Project (No.S30302); National Science & Technology Pillar Program in the Eleventh Five-year Plan Period (No.2006BAI08B01-04) and China Postdoctoral Science Foundation.(No.20080430678).

Development of Body Constitution Questionnaire (BCQ) in Traditional Chinese Medicine

Jui-Shan Lin, and Yi-Chang Su

School of Chinese Medicine, China Medical University, Taichung

Since traditional Chinese medicine (TCM) is broadly used in academic field and medical science nowadays, how to integrate the diagnosis and treatment strategies of TCM with the mainstream modern medicine has become an important issue in this century. Therefore, it is quite necessary to set up a useful platform or bridge tool between modern medicine and TCM. The individual constitution uniqueness and tailored treatment for each patient is highly stressed in TCM, since it is considered the occurrence of disease is originated from the attenuation of one's constitution, and the manifestation diversity of the patients with the same disease is also derived from the difference among each patient's constitution characteristics.

In order to provide an objective, reliable and rigorous diagnostic tool for clinical practice and research using TCM, we conducted the following steps to develop this objective measurement tool: (1) to form the research team and select a panel of 26 experts for Delphi method; (2) to generate items for questionnaire by literature review and Delphi process, then convert the items into colloquial questions and evaluate the face and content validity by Delphi process again; (3) to evaluate the difficulty of answering the questionnaire by pilot study with 81 subjects whose age were between 20-60; (4) to perform the pilot test, formal test, and criterion-related validity test, 4 groups of eligible participants, aged between 20-60, no disease onset in the past month, and receiving health examination in teaching hospitals were recruited. The BCQ was developed and psychometrically evaluated to ascertain and validate the factor structures of the BCQ. Reliability and validity of BCQ were also examined.

Finally, 44 items in BCQ were developed. BCQ is an objective, accurate, and easily-applied tool for TCM a valid and potentially useful tool for evaluating the "Yang-Xu", "Yin-Xu" and "Stasis" constitution types in clinical practices and researches.

Image Processing for Tongue Diagnosis in TCM

Hen-Hong Chang, Wei-Chin Hsu, Li-Shen Chou, Kang-Ping Lin

*Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taipei
Department of Electrical Engineering, Chung Yuan Christian University, Taipei
Formosan Association of Clinical Diagnosis in Traditional Chinese Medicine, Taipei
Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Taipei*

Studies in the standardization and computerization of TCM's tongue diagnosis have provided not only teaching and researching materials but a transition from traditional to modern diagnoses. The tongue diagnosis instrument developed by Chang Gung TCM center has adopted digital imaging, standard capture environment, color management and standard color calibration processing techniques in collecting tongue images followed by normalization with Thin Plate Spline Mappings method to set up a tongue pattern database that creates a tongue model with areas of organ correspondences. This serves to increase reliability and efficiency in clinical tongue diagnosis and to test the organ correspondence theory.

To expand the database, tongue information like color, coating, shape, spirits and overlying fluids were collected and reviewed by experts to establish typical tongue patterns for a complete tongue diagnosis system. The database is further equipped with search engines that identifies similar tongue images by their color features obtained from K-means algorithm analysis of segment images.

To test the organ correspondence theory in tongue diagnosis, we recruited chronic hepatitis patients with liver qi stagnant and blood stasis patterns and compare their tongue patterns with those of healthy individuals. Eight different surface-partition models were used and compared. We found: (1) two of eight partitions model have significant differences between chronic hepatitis patients and health individuals. (2) Significant differences in brightness scale of liver area were seen between chronic hepatitis and control groups.

The Nailfold Capillary Microscopic Patterns of Raynaud's Phenomenon in Patients with Systemic Lupus Erythematosus and Progressive Systemic Sclerosis --- An Integrative Medicine View

Yu-Chin Chen, Hen-Hong Chang

Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taipei
Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Taipei

Nailfold Capillary microscope is a helpful instrument for diagnosing Raynaud's phenomenon (RP). Physician can observe the abnormalities and the circulatory function of the capillaries directly by eyes. In our study, we intend to analyze the microcirculation of RP in systemic lupus erythematosus (SLE) and progressive systemic sclerosis (PSS) patients.

From the aspect of traditional Chinese medicine (TCM), "Heat" is the major pattern for SLE, and "cold" is the major pattern for PSS, while RP is recognized as "reversal". We recruit patients from Rheumatology out patient clinic who were diagnosed as SLE or PSS, the images of their microcirculation by nailfold capillary microscope will be taken. All the patients will be interviewed by TCM physician according to TCM pattern identification. We will compare and analyze the images from the patients with RP and those who are not.

By observing the morphology of these patients' nailfold capillaries, we are trying to set up a potential diagnosis model for TCM "reversal" pattern and figure out the the difference between the hot and the cold pattern in TCM with microscopic findings.

Voice Analysis on Patients with Obstructive Sleep Apnea Syndrome

Huang Po-Yu¹, Chang Hen-Hong², Chen Ning-Hung³, Lin Kang-Ping⁴, Wu Wen-Hsiang⁵

¹Department of Chinese medicine, Taipei city hospital, Linsen Branch, Taipei

²Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taipei

²Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Taipei

³Sleep center, Chang Gung Memorial Hospital, Taipei

⁴Department of Electrical Engineering, Chung Yuan Christian University, Taipei

⁵Department of Healthcare Management, Yuanpei University of Science and Technology, Hsinchu

Objective: Listening examination is one of the important diagnosis methods in traditional Chinese medicine. Since there maybe some changes in the larynx and pharynx of the patients with obstructive sleep apnea syndrome, we hypothesize that their voice were different from others probably. The aim of this study is to assess the voice in patients with obstructive sleep apnea syndrome.

Methods: Twenty male patients and eleven male volunteers were enrolled for voice analysis. Subjects were asked to phonating the vowel /a/ and /i/ for 10 seconds by sitting and lying positions. The voice signals were record and analyzed by the portable device.

Results: The results of voice analysis revealed there were significant different in formant energy ratio between the two groups.

Conclusions: Our results suggest that voice analysis might be a screen tool on obstructive sleep apnea syndrome. However, the sensitivity and feasibility need further evaluation.

Quantitative Evaluation of Pathological and Pharmacological Effects on Meridians with Harmonic Pulse Apparatus

Yucheng Kuo, Geoge Hsiao
Taipei Medical University, Taipei

In 1991, our research team found that the phenomenon of resonance in the arteries which has always been missing in current dynamics. Subsequently in 1997, we derived a radial resonance equation to describe the property of the blood pressure wave propagating and transmitting in the arteries. From the resonance theory and the results obtained from both animal and clinical experiments, we verified the one-coin two sides aspects of the pulse diagnostic method and devised a pulse apparatus according to this principle.

Through the pulse diagnostic apparatus, we could quantitatively analyze the patient pathological excess or deficiency of the meridians and five zang-organs and six fu organs (pathological matrix). In addition, a series of pharmacology research analyses of acupuncture, Chinese herbs, herbs prescription formula and western medicine on the reinforcing or reducing effect of meridians were being carried out. Meanwhile, with matrix operation, we were able to simulate the whole make up function of several herbs in a prescription formula (pharmacological matrix).

In clinic, we found that the pathological indicator in pulse diagnostic apparatus-H.C.V. (Coefficient of Variations of Harmonics Magnitude) could quantitatively show the severity of diseases and evaluate the outcome of patients after treatment. Combined the pathological and pharmacological reverse pair matrixes, we could identify the indication of the prescription formulae recorded in *Shang Han Za Bing Lun*, such as the white tiger and green dragon formula. The scientific study of the basic Chinese medicine is the basis of the integrative medicine.

Analysis of Regional and Seasonal Variation of Triterpene and Flavonoid Contents in *Centella asiatica* by High Performance Liquid Chromatography and Chemometric Analysis

Ali Alqahtani¹, Wannit Tongkao-on¹, Kong M Li², Valentina Razmovski-Naumovski¹, Benjamin Kimble¹, Kelvin Chan^{1&3}, George Q Li^{1*}

¹Faculty of Pharmacy, The University of Sydney, Australia

²Department of Pharmacology, The University of Sydney, Australia

³College of Science & Health, University of Western Sydney, Australia

Centella asiatica (L) Urban (Apiaceae/Umbelliferae) is a popular medicinal plant, extensively used in traditional medicine for wound healing and variety of inflammatory disorders. The aim of this study is to determine the content of triterpenoid derivatives (asiaticoside, madecassoside, asiatic acid and madecassic acid) and phenolic components (chlorogenic acid, rutin, quercetin and kaempferol) in the plant grown in different countries and harvested in different seasons and to evaluate the variation of the chromatographic fingerprints. Methanolic extracts of *C. asiatica* collected from different countries and in different months were subjected to qualitative and quantitative analysis. HPLC was performed by using a gradient mobile phase consisting of 0.3% phosphoric acid in water and acetonitrile. The eluents from the HPLC column were detected via UV-DAD and monitored at 210 nm. The HPLC chromatographic data was processed by chemometric analysis, in particular, similarity and pattern recognition. The results indicated that HPLC provided high resolution and excellent linearity for all standard compounds. The Australian sample has been shown to contain high amount of the investigated compounds. The HPLC quantitative analysis combined with chemometric analysis provides a new method for quality control of *C. asiatica*.

Simultaneous Determination of Lithospermic Acid, Rosmaric Acid, Salvianolic Acid B and Salvianolic Acid A in Dog Plasma by HPLC-MS/MS

Li LIN, Jian-xun LIU, Ying ZHANG, Cang-ling DUAN

Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing

A sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was developed and validated for the quantification of Lithospermic Acid (LA), Rosmaric Acid (RA), Salvianolic Acid A (SAA) and Salvianolic Acid B (SAB) in dog plasma. The analytes were extracted by liquid-liquid extraction, separated on a Luna C18 column (50mm*4.6mm, I.D. 5µm particle size), and detected by tandem mass spectrometry with a API 4000 QTRAP. The mobile phase consisted of 0.1% formic acid and gradient-acetonitrile/methanol resulted at a flow rate of 0.60 mL · mL⁻¹. Quantification was performed using multiple reaction monitoring (MRM) of the transitions m/z537.2→m/z493.2 for LA, m/z359.2→m/z161.0 for RA, m/z493.2→m/z295.1 for SAA and m/z717.2 →m/z519.1 for SAB respectively. The method showed excellent linearity over the concentration range 0.3125–200 ng/mL of the four components ($r = 0.997$ for LA, $r = 0.998$ for RA, $r = 0.990$ for SAA, $r = 0.998$ for SAB). The low limits of quantification were both 0.3125 ng · mL⁻¹. The extract recoveries of analytes were from 53 to 62% for LA, from 55 to 67% for RA, from 33 to 45% for SAA and from 50 to 55% for SAB. The precisions, the accuracy and the stability of the analytes meet the requirements. The method was applied to a pharmacokinetic study of LA, RA, SAA and SAB after intraduodenal administration of the Danshen extraction.

Simultaneous Determination of Berberine, Dehydrocorydaline, Palmatine and Tetrahydropalmatine in Dog Plasma by HPLC-MS/MS

Jian-xun LIU, LIN Li, Ying ZHANG, Cang-ling DUAN

Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing

A sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was developed and validated for the quantification of Berberine(Ber), dehydrocorydaline(DHC), Palmatine(Pal) and tetrahydropalmatine (THP) in dog plasma. The compounds were simply pretreated by protein precipitation using acetone. Chromatographic separation was achieved on a Luna C18 column(50mm*4.6mm,I.D. 5 μ m particle size) with the mobile phase of acetonitrile-0.1% acetic acid and step gradient elution resulted at a flow rate of 0.60 mL \cdot mL⁻¹. A tandem mass spectrometer equipped with electrospray ionization source was used as detector and operated in the positive ion mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions m/z336.2 \rightarrow m/z320.2 for Ber., m/z356.2 \rightarrow m/z191.9 for DHC, m/z352.2 \rightarrow m/z336.2 for Pal and m/z366.2 \rightarrow m/z350.2 for THP respectively. The method showed excellent linearity over the concentration range 0.0488–50/100 ng/mL of the four components ($r = 0.996$ for Ber, $r = 0.997$ for DHC, $r = 0.994$ for Pal, $r = 0.994$ for THP). The low limits of quantification were both 0.0488 ng \cdot mL⁻¹. The extract recoveries of analytes were from 89 to 92% for Ber, from 98 to 107% for DHC, from 71 to 88% for Pal and from 75 to 110% for THP. The precisions, the accuracy and the stability of the analytes meet the requirements. The method was applied to a pharmacokinetic study of multiple components of Yanhusuo (traditional Chinese medicine).

On the Clinic-combined Standardization of Chinese Medicines

Xiao-he Xiao, Jia-bo Wang, Chun-mei Dai, Yong-yan Wang, Pei-gen Xiao

PLA Institute of Chinese Materia Medica, Beijing

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing

The standardization for Chinese medicines (CM) is one of the great topics on modernization and globalization as well as sustainable development of TCM. However, the clinic-oriented researches for standardization of CM, including dosage, usage, efficacy and medicinal property of CM, has always been paid little attention by both medical and pharmaceutical researchers who have been mainly focusing the upper and middle stages in their own fields, but not the clinic-combined researches jointing with the two fields. In this paper, the clinic-combined researches for standardization of CM were specially highlighted in order to establish the clinic-combined CM standards system, including the medicinal property standard, quality standard, dosage standard and good usage practice (GUP), which would directly serve the rational and scientific application of TCM in clinic. In summary, the clinic-combined researches for standardization of CM would be one of the key aspects and strategic directions of TCM modernization in the future.

Investigation on HPLC Fingerprint of TCM Prescription "Xiaoyanlidan Tablet" (XYLD) on Market

Zhi-hong Yao^{1,2)}, Yu-ming Pan^{1,2)}, Yi Dai^{1,2)}, Yan Chen^{1,2)}, Feng-juan Tu^{2,3)}, Hao Gao^{1,2)}, Xin-sheng Yao^{1,2,3)}

¹Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangdong

²Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangdong

³College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang

Xiaoyanlidan Tablet (XYLD) was a traditional Chinese medicine (TCM) prescription on market being consisted of *Rabdosia serra*, *Picrasma quassioides*, *Andrographis paniculata* for the therapy of acute cholecystitis, angiocholitis et al. Up to now, there were 133 domestic manufactures for XYLD, with poor quality standards. Most of the studies on its quality control methods focused on the assay of dehydroandrographolide in XYLD, while the investigation on HPLC fingerprint as specific identification method reflecting its profile constituents has not yet been reported. In order to develop an HPLC fingerprint chromatogram of XYLD, an HPLC method was developed on a C18 column (250 mm×4.6 mm, 5 μm) by gradient elution with acetonitrile-water (both containing 0.1% (V/V) formic acid) as mobile phase at a flow rate of 1.00 mL·min⁻¹ with the detected wavelength at 254 nm and column temperature at 25°C. The HPLC fingerprint chromatogram of XYLD was established with good stability, precision and reproducibility. Thirty-one out of forty detected peaks in the chromatogram was traced to the source by comparing with overlaid chromatograms of its compositive crude herbs (Fig.1) and seven of them were identified with reference substance (Fig.2). Furthermore, the qualities of 24 batches of XYLD from 3 different manufactures were evaluated by TCM fingerprint similarity evaluation system. The similarity of XYLD tablet were no less than 0.954 within the same manufacture and were 0.845 to 0.987 within different manufactures, but it dropped to 0.478 to 0.896 within the cut chromatogram (0 to 69 minute). Our investigation on HPLC fingerprint chromatogram, which represented the profile of multiple chemical substances in XYLD tablet, would contribute to its quality control.

This work was supported by project of Guangzhou modernization of TCM (2004Z1-E5011).

Thanks are given to Guocai Wang, Weihua Jiao and Chenyang Li for providing the reference substances.

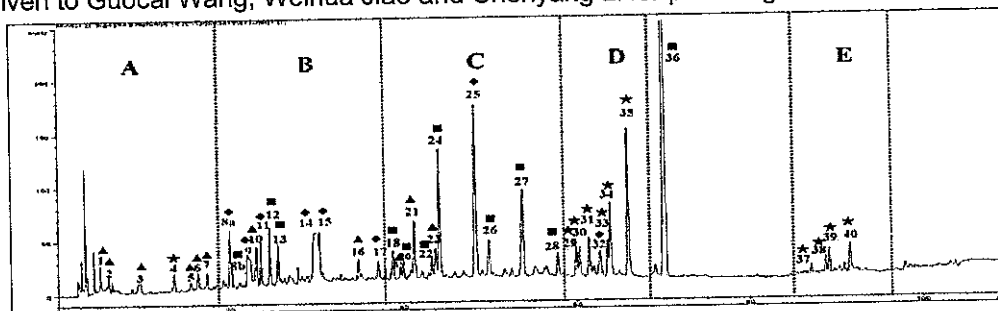


Fig.1 The HPLC fingerprint chromatogram of XYLD

(▲ peaks original from *Rabdosia serra* (Maxim.) Hara extract; ◆ peaks original from *Picrasma quassioides* (D. Don) Benn. extract; ■ peaks original from *Andrographis paniculata* (Burm.f.) Nees extract; ★ uncertain original peaks)

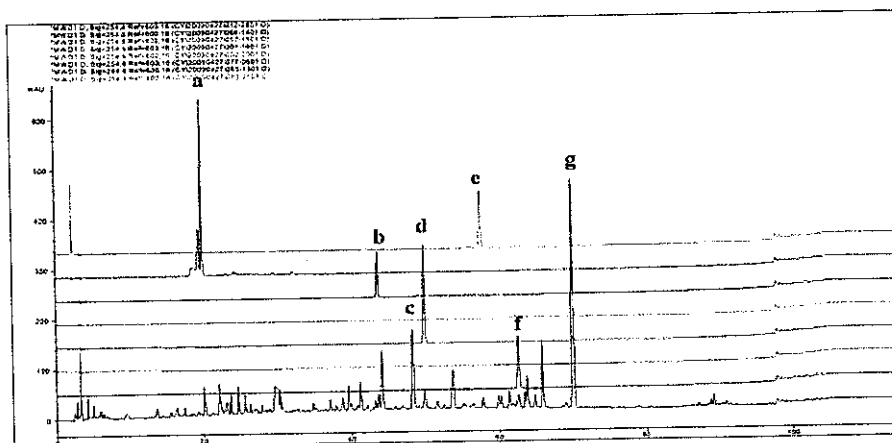


Fig.2 The overlay chromatogram of XYLD and seven reference substances: a- 1-hydroxymethyl-β-carboline, b- rosmarinic acid, c- 4-methoxy-5-hydroxycanthin-6-one, d- andrographolide, e- neoandrographolide C, f- 4,5-dimethoxycanthin, g- dehydroandrographolide

Development And Validation of a LC-MS/MS Method for Simultaneous Determination of Six Schisandrins in Wuzhi Tablet

Xiao Ling Qin¹, Hui Chang Bi¹, Xue Ding Wang¹, Guo Ping Zhong¹, Ying Wang¹, Xin Ping Xue¹, Jia Li Li¹, Chang Xi Wang², Le Jia Xu¹, Yi Tao Wang³, Xiao Chen², and Min Huang^{1*}

¹School of Pharmaceutical Sciences, Sun Yat-sen University, Guangdong

²the First Affiliated Hospital, Sun Yat-sen University, Guangdong

³Institute of Chinese Medical Sciences, University of Macau, Macau

Background: We recently reported that the blood concentrations of Tacrolimus (FK506) in renal transplant recipients and rats were markedly increased following co-administration of Wuzhi Tablet (WZ), a preparation of ethanol extract of Wuweizi (*Schisandra sphenanthera*). WZ contains 7.5 mg Schisantherin A per tablet, but the amount of other major active schisandrins including Schisandrin A, Schisandrin B, Schisandrin C, Schisandrol A, and Schisandrol B is not clear. Thus, the current study was aimed to develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously determine the 6 schisandrins in WZ. **Method:** Schisandrins and FK520 (internal standard) were subjected to LC-MS/MS analysis using positive electro-spray ionization (ESI⁺) under multiple reaction monitoring (MRM) mode. The MRM transitions of m/z 417.2→402.2 for Schisandrin A, m/z 401.3→300.4 for Schisandrin B, m/z 385.3→285.3 for Schisandrin C, m/z 433.1→384.1 for Schisandrol A, m/z 417.2→368.2 for Schisandrol B, m/z 599.1→415.1 for Schisantherin A and m/z 809.5→756.4 for FK520 were selected to obtain maximum sensitivity. Chromatographic separation of all analytes was performed on a Hypersil BDS C₁₈ column. The mobile phase consisted of methanol-water (containing 2 mM ammonium acetate) (95:5, v/v), pumped at a flow rate of 200 $\mu\text{L}\cdot\text{min}^{-1}$. The running time was 2 min. **Results:** The validation results were met the requirements of SFDA with high selectivity, acceptable accuracy, precision and sensitivity. The amounts ($\text{mg}\cdot\text{g}^{-1}$) of Schisandrins in WZ were: Schisantherin A 8.883 ± 0.938 $\text{mg}\cdot\text{g}^{-1}$; Schisandrin A 6.186 ± 0.378 $\text{mg}\cdot\text{g}^{-1}$; Schisandrol B 0.672 ± 0.144 $\text{mg}\cdot\text{g}^{-1}$; other schisandrins were much lower than the above contents. **Conclusion:** These results indicated that the method was rapid and sensitive enough to simultaneously determine 6 schisandrins in WZ. Schisantherin A and Schisandrin A were the two highest contents in WZ, and may play an important role in the interaction with tacrolimus.

Acknowledgement: The work was supported by the National Major Projects for science and technology development (grant number 2009ZX09304-003 and 2008ZX09312), and the National Basic Research Program (Grant No. 2009CB522707) from Science and Technology Ministry of China.

Determination on the Contents of Tannins of *Sanguisorba officinalis* L.

Yue Cheng¹, Jia-Sheng Chen¹, Jian-Ping Chen², Dong-Mei Wang^{1*}

¹School of Pharmaceutical Sciences, Sun Yat-sen University, Guangdong

²School of Chinese Medicine, The University of Hong Kong, Hong Kong

Diyu (*Sanguisorba officinalis* L.) is a traditional Chinese Medicine with high content of tannins. In this study, in order to set up the quality control methodology of Diyu, the quantification methods for different types of tannins in Diyu were established. The HPLC method was used for the determination of the contents of hydrolysable tannin, because Gallic acid and Ellagic acid are the main tannins after hydrolyzation. The Vanillin-H₂SO₄ method was employed for the determination of the contents of condensed tannin. The results showed that these methods proved to be simple, sensitive, reproducible, and accurate, and the contents of hydrolysable tannin and condensed tannin in the reference Diyu crude drug were measured as 39.19 mg/g and 87.20 mg/g respectively. Therefore, the HPLC method and Vanillin-H₂SO₄ method for determination of the contents of hydrolysable and condensed tannins could be integratedly used as quality control methods of *Sanguisorba officinalis* L.

Separation and Quantification of Monosaccharide Compositions of Astragalus Polysaccharide by Precolumn Derivation HPLC

Siu-kit Yung, Jing Liu, Zhong-zhen Zhao, Tao Yi, Hu-biao Chen*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Radix Astragali (RA), or "Huangqi" is the dried root of *Astragalus membranaceus* (Fisch.) Bge var. *mongholicus* (Bge.) Hsiao (MGHQ) or *Astragalus membranaceus* (Fisch.) Bge (MJHQ). RA is claimed to be useful to benefit the deficiency of 'Qi' (the vital energy). Polysaccharides are reported to be the activity constituents in RA in recent years. In our study, a precolumn derivation reversed-phase high-performance liquid chromatographic (HPLC) method is developed to simultaneously separate and quantify the monosaccharide compositions of three polysaccharides including MGAPS (extracted from MGHQ) and MJAPS (extracted from MJHQ). The polysaccharides were isolated from RA by water extraction-alcohol precipitation. They were hydrolyzed by trifluoroacetic acid into monosaccharides and then labeled with 1-phenyl-3-methyl-5-pyrazolone (PMP). The labeled monosaccharides derivatives were separated by a reverse phase C-18 column and monitored by UV absorbance at 245 nm. The composition analysis of monosaccharides of hydrolyzed MGAPS and MJAPS could be achieved by using a set of monosaccharide standards. The results showed the hydrolyzed polysaccharides of MGHQ and MJHQ were consisted with arabinose, galacturonic acid, galactose, glucose, mannose and rhamnose in a ratio of 2.72: 7.65: 5.88: 206.64: 1: 5.49 and 1.54: 9: 6.7: 311.43: 1: 9.21 respectively. Quantitative recoveries of the component monosaccharides in Astragalus polysaccharides were in the range of 99.71-101.91% and the RSD values were lower than 1.81%. The results demonstrated that the precolumn derivation HPLC method was precise and practice for the analysis of Astragalus polysaccharides.

Chemical Authentication of Sweet and Bitter *Gynostemma Pentaphyllum* (Thunb.) Makino

Lin Zhu, Zhi-Hong Jiang*

School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

Gynostemma pentaphyllum (Thunb.) Makino, a Chinese herb possessing antitumor and antioxidant activities, contains saponins as its main and active components.

Sweet and bitter Gp have been used in China. However, of the existing studies on Gp, we can hardly get clear information about the chemical differences of the two kinds of Gp. On the other hand, because of the same botanic resource, the two herbs are very similar in morphology, which make the identification of them using conventional methods impossible. Therefore, to find out the differences of the two types of herbs, the necessity for an authentication based on chemical identification of the characteristic constituents is urgent.

In the present study, an UPLC-ESI-Q-TOF-MS method was developed to distinguish sweet Gp from the bitter one. By comparing the retention time and accurate molecular weight with the standards, three protopanaxadiol (PPD) type saponins, namely Rb₁, Rb₂ and R_d were determined in all of the sweet Gp samples. However, none of the three PPD saponins was detected in the bitter ones using the proposed method. Moreover, the present finding was further confirmed by the hydrolysis experiment. After the sweet Gp samples was refluxed in 30% HCl at 70 °C for 3 hours, panoxadiol (PD), a hydrolysate of PPD type saponins, was determined using HPLC coupled with ELSD. PD was not detected in the bitter Gp.

In summary, it was first found that the characteristic constituents were PPD type saponins in sweet Gp. The present method which utilize Rb₁, Rb₂ and R_d, as well as the hydrolysate (PD) as the markers is reliable for the chemical authentication of sweet Gp and bitter one.

Unveiling the Significance of the Constituents Towards Bioactivity in Natural Products

Foo-tim Chau¹, Olav M. Kvalheim², Hoi-yan Chan¹, Tsui-yan Lau¹

¹Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong

²Department of Chemistry, University of Bergen, Norway

Recently, we reported an application of the Quantitative Pattern-Activity Relationship (QPAR) approach to a Herbal Medicine (HM), Radix Puerariae Lobatae (YG)¹. Two different kinds of data were involved. One was related to antioxidant bioactivity and the other one was chemical composition based on the chemical fingerprint of the HM. The QPAR model thus obtained provides crucial information about the prediction of total bioactivity and the discovery of the bioactive regions in the HPLC-UV chromatographic fingerprint. There is no doubt that the first piece of information can help to upgrade the existing quality control of HM to a higher level as both chemical and pharmacological properties are considered at the same time. As for the second one, it is useful for drug development as well as assisting the bioassay-guided fractionation to be carried out more efficiently and cost effectively.

Up to now, we have applied our developed QPAR method to two different systems, the herbal medicine, YG, as mentioned above as well as a mixture system (MIX) with twelve chemical compounds having known chemical property and antioxidant activity (the so called white system). Sixty mixture samples of MIX were prepared systematically for our study by mixing all these chemicals at different concentration levels according to uniform design. One of our algorithm TP&SR worked out successfully to find out the five known active compounds from the data obtained. Furthermore, their relative strengths of the activity were sorted out either². Hence, their extents in contributing to the total bioactivity were exposed.

With regard to YG, 78 extracts were prepared from different YG sources. Their HPLC-UV fingerprints and total antioxidant capacities via the FRAP method were gained. In addition, three fractions of YG extract were prepared and their antioxidant capacities were measured also. In this way, their strengths of antioxidant activities were known. When TP&SR was applied to analyze the two sets of data of the original or crude extract mentioned above, three active profile regions in the fingerprint were identified and they all fell into the most active fraction as acquired before. Another QPAR method QPAR3 has been developed recently with the same outcome.

Reference:

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Gold-Nanoparticles Assisted Laser Desorption/Ionization Imaging Mass Spectrometric Analysis of American Ginseng and Cordyceps

Ho-Wai Tang; Chi-Ming Che; Kwan-Ming Ng*

Department of Chemistry and Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Hong Kong

Imaging Mass Spectrometry (IMS) is an emerging molecular-specific imaging technique. IMS provides two levels of chemical information, which are the identities and distributions of the compounds of interest within the given sample. With these advantages, IMS is now advancing the research in various fields such as medical and biological sciences. Chemical analysis is an important part for quality monitoring of raw herbal materials. IMS has great potential to be applied for the histochemical analysis of raw herbal materials in the solid state. The key of success in applying IMS for herbal analysis is the matrix. Matrix-Assisted Laser Desorption/Ionization (MALDI)-based IMS is generally chosen for biological tissue analysis. MALDI is good for large peptides/proteins detection but may not be good enough for the detection of small phytochemicals, because of the matrix interference effect in the low molecular mass region. We have successfully applied IMS for the analysis of American Ginseng and Cordyceps by replacing the conventional MALDI matrix with gold nanoparticles (AuNPs). The AuNPs can be homogeneously coated on the herbal materials for IMS analysis. AuNPs do not create matrix interference problem and is chemically stable, hence the sample chemical information will not be complicated. Furthermore, the electrical conductivity of AuNPs simultaneously allows the use of scanning electron microscopy for the examination of sample morphology of herbal materials. In conclusion, we have introduced the use of AuNPs acting as a dual-function matrix allowing the chemical analysis and microscopic examination of raw herbal material in the solid state.

Acknowledgement: We acknowledge the financial support from the Area of Excellence Scheme (AoE/P-10/01) administered by the University Grant Council (HKSAR, China) and the Strategic Research Theme on Drug administrated by the University of Hong Kong.

Quality Assessment of RCTs on Danshen Dripping Pills (DSP) in Treating Angina Pectoris

Yongliang Jia, Siu-wai Leung

Institute of Chinese Medical Sciences, University of Macau, Macau

Background: All available sampled literature surveys found that the quality of RCTs on traditional Chinese medicine (TCM) has been extremely poor. The efficacy validation of TCM flagship products such as DSP may suffer from the poor conduct of RCTs on TCM.

Objectives: To assess the quality of RCTs on DSP in treating coronary heart disease angina pectoris (CHDAP) and find the factors explaining the overall effects.

Methods: RCT reports were retrieved from CNKI, WanFang Data, PubMed, China Master Theses Full-text Database, Chinese Electronic Periodical Services, Social Sciences Citation Index, Science Direct, Cambridge Journals Online, and EBM reviews. Their quality was independently assessed by two reviewers according to CONSORT 2010, the Jadad Scale, and our refined Jadad Scale. Kendall correlations between basic characteristics and the quality of the RCTs were analyzed.

Results: The median (95% CI) of the CONSORT compliance score (0 to 37 points) and Jadad score (0 to 5 points), and refined Jadad score (-1 to 7 points) of the 76 included RCTs were 15 ([14.99 to 15.50]), 2.00 ([1.99 to 2.00]), and 3.00 ([2.99 to 3.50]), respectively. Only 9.21% (7/76) of RCTs were of high quality (Jadad score ≥ 3). Total CONSORT compliance percentages were 41% (CONSORT 2010) and 48% (CONSORT 2005). These RCT quality measures were correlated with individual items of reporting, e.g., reporting of treatment dates ($\tau=0.28$, $P=0.006$).

Conclusions: The quality of RCTs on DSP was significantly better than other RCTs on general TCM but has not been improved significantly since 2005.

Free Radical Scavenging Activity and their Contents of Seven Flavonoids in Eagle Tea

Qiong Meng, Zheng-ming Qian, Shao-ping Li*

Institute of Chinese Medical Sciences, University of Macau, Macau

Eagle tea, a well-known traditional beverage in south China, has attracted more attention because of its health-beneficial effect. In this study, a pressurized liquid extraction (PLE) and high performance liquid chromatography coupled with diode array detector (HPLC-DAD) was developed for simultaneous determination of seven flavonoids, including hyperin (quercetin-3-O-β-D-galactopyranoside), isoquercitrin (quercetin-3-O-β-D-glucopyranoside), kaempferol-3-O-β-D-galactopyranoside, quercitrin (quercetin-3-O-α-L-rhamnopyranoside), astragalin (kaempferol-3-O-β-D-glucopyranoside), quercetin, kaempferol, in Eagle tea. The separation was performed on a Zorbax SB C18 column (250 mm × 4.6 mm i.d., 5 μm) with gradient elution of water and methanol. All calibration curves showed good linearity ($R^2 > 0.999$) within the test ranges. The overall intra- and inter-day variations (RSDs) of seven analytes were less than 3.90% and 4.59%, respectively, and the recoveries were between 91.6% and 105.6%. Moreover, online HPLC-DAD-MS coupled with 2,2'-azinobis (3-ethylbenzthiazolinesulfonic acid) diammonium salt (ABTS) assay were employed for evaluating free radical scavenging activity of the investigated flavonoids in Eagle tea. Twenty-three chromatographic peaks were detected, and nineteen components, including five out of seven investigated flavonoids (hyperin, isoquercitrin, quercitrin, quercetin, kaempferol), catechins, chlorogenic acid and epicatechin, had free radical scavenging activity.

Study on Qualitative Analysis of Polysaccharides from Chinese Medicines

Jia Guan and Shao-ping Li*

Institute of Chinese Medical Sciences, University of Macau, Macau

Polysaccharides isolated from Traditional Chinese Medicines (TCMs) exhibit multiple pharmacological activities. However, quality control of polysaccharides is a challenge because of their complicate structure and macro-molecular mass. In this presentation, saccharide mapping based on specific enzymatic digestion of polysaccharides and chromatographic analysis was proposed for qualitative analysis of polysaccharides from different TCMs. Endo-carbohydrase such as glucanase, arabinanase, xylanase, galactanase, cellulase, amylase and pectinase were used for enzymatic digestion of polysaccharides from TCMs, including *Panax ginseng*, *P. notoginseng*, *P. quinquefolium*, *Cordyceps sinensis*, *C. militaris*, *Ganoderma lucidum*, *G. sinense*, *Astragalus membranaceus* and *Angelica sinensis*. By using high performance size exclusion chromatography (HPSEC) as well as HPLC-MS analysis after derivatization, the enzymatic hydrolysis properties of polysaccharides and their saccharide mapping were determined. The polysaccharides from different TCMs could be successful distinguished based on their characteristic saccharide mapping, which is helpful to improve the quality control of polysaccharides.

Quality Control of the Commonly Used Traditional Chinese Medicine in Taiwan

Shu-Jen Chang^{1*}, Chao-Lin Kuo², Yih-Chiang Yuan³ and Sheng-Sing Lin⁴

¹School of Pharmacy, China Medical University, Taichung

²School of Chinese Medicine Resources, China Medical University, Taichung

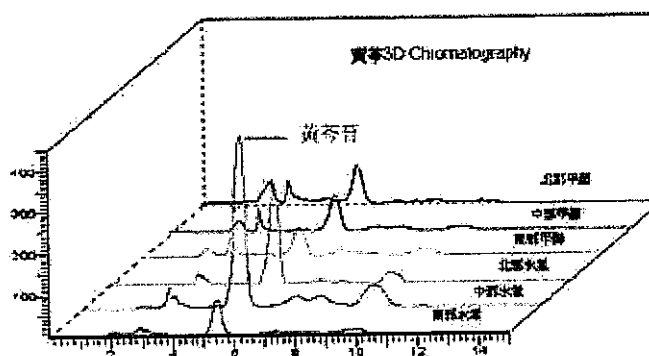
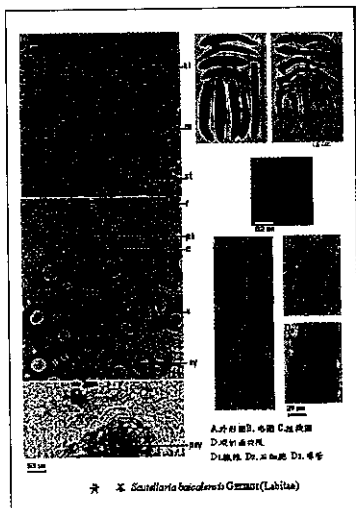
³Department of Information Management, Hsiuping Institute of Technology, Taichung

⁴China Medical University Hospital, Taichung

Over 80% population have been used traditional Chinese medicine (TCM), most of the TCM sold in Taiwan markets were imported from Mainland China, the quality of the TCM may vary due to different sources. Along with popular concept of "medicine and food have the same origin" and trend of medicinal edible in the markets, the quality control for TCM materials become increasingly important.

The main focus of this study is to set up a scientific and systematic methodology for quality control, fifty kinds of commonly used TCM materials were collected from market, including northern, middle and southern regions of Taiwan. Scientific and systematic methodology for quality control, such as microscopic examination, thin-layer chromatography and as well as high performance liquid chromatography were used. In this study we will report part of our results, and give some comments for quality control of TCM. And the results will be provided as references for GMP Pharmaceutical factories.

Acknowledgement: This study were financially supported by the Committee of Chinese Medicine and Pharmacy, Department of health, Executive Yuan and China Medical University of the Republic of China (CCMP95-TP-022, CMU95-042, 43, & CMU96-90, 91).



Multivariate Data Analysis Using High Performance Liquid Chromatographic, Nuclear Magnetic Resonance Spectroscopic and Infrared Spectroscopic Methods on the Quality Control of *Danshen* Products

Huiying Zhao and Peter J Hylands

Department of Pharmacy, King's College London, UK

There are difficulties in the standardisation of traditional Chinese medicine (TCM) because of the complexity of TCM preparations. The main function of the widely-used Chinese medicinal plants *Danshen* and *Sanqi* is improvement of cardiovascular function. *Fufang Danshen* medicinal products mainly contain extracts of these two plants. High performance liquid chromatographic (HPLC), nuclear magnetic resonance (NMR) and infrared (IR) spectroscopic methods are used for attempted quality control.

10 batches of the raw material *Danshen* and *Sanqi*, intermediate products, finished products of *Fufang Danshen* formulation 1 (FD1) and 7 batches of 3 different brands of *Danshen* tablets were studied. The extracts were subjected to HPLC, and NMR and IR spectroscopy. Exported chromatographic and spectroscopic data were analyzed by principal component analysis and partial least squares-discrimination analysis.

Different brands of *Danshen* finished products were successfully discriminated by PCA and hierarchical cluster analysis. Repeatability was higher between batches of FD1 than batches of Compound *Danshen* tablets. *Danshen* grown in Shandong has a higher content of salvianolic acid B than those grown in Shanxi. HPLC or IR-PCA method cannot discriminate between batches of *Sanqi* plant whereas NMR-PCA can.

NMR, IR spectroscopy and HPLC can be used as complementary methods depending on the nature of the compounds concerned. Differences between batches can be distinguished in raw plant material, intermediate products and finished commercial products. Correlated batches cluster similarly in *Danshen* raw and intermediate products. No correlations were detected between FD1 and samples of materials at different stages of production. *Danshen* roots of geographically different origins can be distinguished.

Further studies are planned attempting to link these results with to proteomic studies (MALDI-TOF MS, solid state NMR etc.) to further evaluate the quality of TCM by providing a measure of functional bioactivity. It is to be hoped that this work may contribute to the eventual licensing of TCM preparation as traditional medicines

Acknowledgement: We are grateful to the College of Pharmaceutical Sciences, Zhejiang University and pharmaceutical company 1 who kindly provided all the herbal materials.

Determination of Tanshinone IIA, Salvianolic Acid B and Sodium Danshensu in *Salvia Miltiorrhiza* Bunge with Different Preparation Methods

Jue Zhou and Peter J Hylands

Department of Pharmacy, King's College London, UK

As part of ongoing studies on the quality control of traditional Chinese medicines (TCM), different methods of preparation of *Salvia miltiorrhiza* Bunge extracts were compared. The nature of the different extracts was assessed by measuring the content of tanshinone IIA, salvianolic acid B and sodium danshensu (assayed by HPLC and LC/MS). The results show that there is significant difference of the content of these bioactive constituents according to the method of preparation of the extract. This work can serve as a guide for the quality control of TCM and also for patients in their method of preparation of their own TCM.

Keywords: *Salvia miltiorrhiza* Bunge; tanshinone IIA; salvianolic acid B; sodium danshensu

Is there a Possibility to Register Chinese TCM in Europe?

Liya Ju

Sino-European Therapeutics R&D, France

According to WHO statistics, the global herbal market size is close to 40 billion U.S. dollars, and increases around 10% every year. In recent years, the plant (THM) in Europe, more and more attention and favor, non-prescription herbal market has great potential, its growth rate has been higher than the chemicals, the world's plant in Europe accounted for 40% of market share. WHO emphasis to motivate herbal or traditional medicine research and notes that 119 plant-derived medicines are used in modern medicine and Major pharmaceutical companies are now in research on plant materials for their potential medicinal value.

The Herbal Medicinal Products Working Party of the EMEA aims to facilitate the Mutual Recognition Procedure for traditional herbal remedies be in use for more than 15 yrs in any of EU member & 30 yrs in country of origin. However, the European Directive 2004/24/EC released in 2004 by the European Parliament, known as a 'draconian directive', will be a barrier of the registration of Chinese TCM in Europe after its application after March 2011.

How the Chinese TCM companies to find the strategies and solutions to reply the highly demanding approaches in the evaluating quality, safety, and efficacy of Chinese Herbal medicine?



香港浸會大學
HONG KONG BAPTIST UNIVERSITY

School of **中醫藥學院**
Chinese Medicine



