Effect of acupuncture on target tissue distribution of *Schisandra* lignans

Tu-Lin Lu,1,2 Xiao-Yan Wu,1 Yu Song,1 Hao Chen,3 Bin Xu,3 Yuan Zhou,1 Zhi-Jun Huang,1 Yong Sun,3 Chun-Qin Mao1

**ABSTRACT**

**Background** Recently, the combination of acupuncture and Chinese medicine as a practical strategy to treat diseases is receiving considerable attention worldwide as they are usually found to exhibit intriguing therapeutic effectiveness. The current study aimed to study the adjunct effect of acupuncture on target tissue distribution of *schisandra* lignans when acupuncture is combined with *Schisandra chinensis*.

**Methods** A simple and reliable high performance liquid chromatography-electrospray tandem-mass spectrometry (HPLC-ESI-MS) method for simultaneous analysis of three bioactive lignans (schisandrin, deoxyschisandrin and schisandrin B) in rat tissues was established. Using this analytical method we evaluated whether acupuncture had a synergistic effect on the tissue distribution of *schisandra* lignans.

**Results** Tissue concentrations of the three lignans in the group receiving acupuncture were significantly higher than those in the *schisandra* only group, suggesting that acupuncture may potently increase tissue concentrations of *schisandra* lignans. The highest concentrations of the three lignans occurred in the liver compared with other tissues, and tissue concentrations in the heart, spleen, lungs and kidneys were increased by 315%, 203%, 250% and 224%, respectively. In addition, retention times of the lignans in tissues were prolonged for a relative long time.

**Conclusions** Our data indicate that the combined use of acupuncture and *Schisandra chinensis* could produce a synergistic effect which could play a beneficial role on promoting the tissue distribution of lignans. This has supported our initial hypothesis. The HPLC-MS method showed good sensitivity in quantifying the three *schisandra* lignans in different tissues.

**INTRODUCTION**

Acupuncture is an accessible, non-confrontational therapy that appears to be effective and was traditionally used in oriental medicine. The number of users of acupuncture worldwide has steadily increased over the past 40 years.1 Acupuncture shows therapeutic effects for certain diseases, such as chronic pain, asthma, rhinitis and rheumatoid arthritis.2-4 For example, acupuncture has been mostly investigated as a therapy for liver diseases because stimulation of ST36 is believed in Traditional Chinese Medicine (TCM) to activate the blood circulation to dissipate blood stasis.5 Therefore, ST36 is one of the most studied points. Recently, combination therapy of acupuncture and medication as a practical strategy to treat diseases has gained increasing importance in both experimental and clinical investigations.6 Increasing amounts of data indicate that acupuncture therapy may exhibit synergistic actions when combined with Chinese medicine for disease therapy.7

Despite the combination therapy being popular, there is still no universally accepted research evidence for the effectiveness of acupuncture in combination with Chinese medicines, and the literature on the combination of acupuncture and medicine is sparse. In the present study, we combined acupuncture with *Schisandra chinensis* to study the effect of acupuncture on target tissue distribution in rats. *Schisandra chinensis*, one of the well known TCM in the Chinese Pharmacopoeia, is derived from the dried fruit of *Schisandra chinensis* (Turcz.) Baill. It has been used clinically for thousands of years for the treatment of jaundice, hepatitis, spontaneous diaphoresis, nocturnal diaphoresis and other liver diseases.8 Its usefulness in protecting the liver has been clinically validated.9

Studies have shown that *Schisandra chinensis* alcohol extracts have a significant protective effect on injured liver cells caused by chemical toxicants. Schisandra lignans are the major bioactive agents of *Schisandra chinensis*, in which the most abundant ingredients include schisandrin, deoxyschisandrin and schisantherin B. Modern research demonstrated that these lignans have a special affinity with the liver cells and have shown hepatoprotective effects. Increasingly, numerous studies have reported that these lignans exhibited beneficial bioactivities, such as antihypotensive, anti-HIV, anticancer, antioxidant and antitumor activities, platelet activating factor antagonistic and CNS protective activities.

We hypothesised that acupuncture may play a beneficial role in combination to promote the tissue distribution of lignans. To our knowledge, the effect of acupuncture on tissue distribution of schisandra lignans has not yet been clarified. Most of the current research is from clinical areas, such as theoretical and experimental role in combination to promote the tissue distribution of lignans.

A sensitive and selective high performance liquid chromatography-electrospray tandem-mass spectrometry (HPLC-ESI-MS) method was developed for simultaneous determination of the three schisandra lignans in biological samples. Using this method, we evaluated whether acupuncture had any effect on the distribution of schisandra lignans. The method was validated in terms of selectivity, sensitivity, accuracy, precision and recovery, and successfully applied for assay of the distribution of schisandra lignans. This is the first report which has studied the effect of acupuncture on tissue distribution of schisandra lignans by simultaneously measuring three schisandra lignans in rat tissues after oral administration of *Schisandra chinensis* extract with or without acupuncture. This study may provide useful knowledge on the mechanism of action and clinical applications of the herbal medicine combined with acupuncture.

**METHODS**

**Study design**

In this report we describe the development of a chemical analysis method (HPLC-ESI-MS) for simultaneous analysis of bioactive lignans in rat tissues. Schisandrin, deoxyschisandrin and schisandrin B were selected as the iconic bioactive ingredients of schisandra lignans. It is important to ensure that the method was reasonable for simultaneous analysis of the three bioactive lignans. Then, the animal experiments, which assessed the effect of combined use of acupuncture and *Schisandra chinensis* on tissue distribution, were carried out. This assay is ideally suited for studying the effect of acupuncture on target tissue distribution of schisandra lignans in clinical laboratories.

**Subjects**

The complete experimental protocol was carried out under the guidelines on the use of living animals in scientific investigations. All studies were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People’s Republic of China. The animals were male and female healthy adult Sprague–Dawley rats, weighing 250–300 g at 3 months of age. They were maintained under a 12 h light/dark cycle at a controlled temperature (25°C and 55–60% relative humidity) with free access to food and tap water until the day of the experiment. Ten healthy rats were divided into two groups of five.

**Chemical and reagents**

Schisandrin, deoxyschisandrin and schisandrin B (purity≥99%) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Nanjing, China). Testosterone (≥99.5%) was obtained from Dr Ehrenstorfer GmbH (Germany) for use as an internal standard (IS) for biosample analysis. *Schisandra chinensis* (Turcz.) Baill was identified by Professor Tu-lin Lu of Nanjing University of Chinese Medicine. Voucher specimens were deposited at the College of Pharmacy of Nanjing University of Chinese Medicine. Formic acid (98%) (analytical reagent grade), acetonitrile (analytical grade) and methanol (HPLC grade) were purchased from Tedia Company (USA) and Shandong Yuwang Industrial Co Ltd (Yucheng, China). HPLC quality water (resistivity of 18.2 MΩ cm) was from Milli-Q system (Millipore, Bedford, Massachusetts, USA). All other reagents were of analytical grade and were used as received.

**Preparation of standard and quality control samples**

Stock standard solutions of schisantherin, deoxyschisandrin and schisandrin B were prepared by dissolving the accurately weighed individual compounds in methanol. The solutions were then serially diluted with methanol to make a series of standard mixture working solutions of 0.496, 0.992, 1.98, 3.97, 7.94 and 15.88 μg/ml for schisantherin; 0.129, 0.259, 0.518, 1.04, 2.07 and 4.14 μg/ml for deoxyschisandrin; and 0.109, 0.218, 0.435, 0.870, 1.74 and 3.48 μg/ml for schisandrin B. The stock solution of IS was diluted with acetonitrile to make a working solution of IS (2.88 μg/ml). The stock solutions of the analyte or IS were stored at −80°C, and the working solutions were kept at −20°C. Quality control samples (QC samples) were prepared at low, medium and high concentrations at 0.071, 0.141 and 0.565 μg/ml for schisantherin, 0.023, 0.045 and 0.182 μg/ml for deoxyschisandrin, and 0.029, 0.057 and 0.229 μg/ml for schisandrin B in the same manner as the calibration standards, and used to assess accuracy and precision of the assay method.
Method validation

Specificity
Selectivity was assessed by analysis of five different samples of blank matrix with and without spiking with mixed standard of schisancherin, deoxyschisandrin, schisandrin B and IS. There was no significant interference at the measured mass transitions and retention times of the analytes, which provide evidence of no endogenous interferences in the drug free tissue samples.

Linearity and lower limit of quantification
The calibration curves were constructed by plotting the peak area ratio of schisancherin, deoxyschisandrin and schisandrin B to internal standard versus their respective concentrations in rat tissues. The squared correlation coefficients (r) for the calibration curves were all >0.995, which indicated that there was excellent linearity between the peak area ratio and concentration of each compound within the linear range. The results were fitted to linear regression analysis.

Accuracy and precision
Accuracy and precision of the established method were determined by analysing QC samples at three different concentrations (low, medium and high) on the same day. Accuracy ranged from 1.5% to 5.87%, while precision (relative standard deviation) ranged from 4.4% to 7.8% for tissue homogenates of rats, which indicated that the present method has a satisfactory accuracy, precision and reproducibility.

Recovery and matrix effect
Extraction recoveries of the three target lignans at all three concentrations in rat tissue homogenates were less than 15%, which indicated that the recoveries of schisandrin, deoxyschisandrin and schisandrin B were consistent and precise. Matrix effect data were acceptable, with relative standard deviation values <9.9% at different concentrations, suggesting that the relative matrix effect for the analyte was not significant. Thus the present analytical method was considered reliable.

Stability
Stabilities of three lignans were assessed by comparing the mean concentration of the stored QC samples with the mean concentration of freshly prepared QC samples. Stability samples were considered stable if bias was within ±15% of the actual value.

Experimental protocols
All rats were randomly divided into two groups of 15 (five rats were used in the distribution phase, five were used in the equilibrium phase and the other five rats were used in the elimination phase), corresponding to 2 h, 4.5 h and 6 h. The tissue distribution study was conducted with oral administration of schisandra alone or oral administration of schisandra combined with acupuncture simultaneously. Rats in the schisandra only group were administered a single oral dose of 5.0 g/kg of Schisandra chinensis extract (schisandrin 4.02 mg/ml, deoxyschisandrin 1.72 mg/ml and schisandrin B 1.88 mg/ml) dissolved in sodium carboxy methyl cellulose (CMC) by oral gavage. Rats in the combination group received Schisandra chinensis extract orally according to the described methods after stimulating the Zusanli point (ST36).

All groups were dosed once a day at 08:00 h for 7 days, and rats were sacrificed at the predetermined sampling time. Then, samples from a number of tissues (heart, liver, spleen, lung and kidneys) were quickly dissected. Tissues were quickly excised, rinsed well with ice cold saline, blotted dry and weighed. Samples were then homogenised with physiological saline solution (0.9%, w/v) to prepare 0.5 g/ml homogenates. Blank tissue homogenates were prepared by the same method as mentioned above using rats without prior exposure to schisandra. Tissue homogenates were kept frozen at −20°C until analysis.

Acupuncture protocols
Rats in the combination group (n=15) were treated by acupuncture by inserting stainless steel needles (0.25 mm diameter, Nanjing, China) bilaterally at ST36 to a depth of approximately 3 mm. The needles were connected to a G-6505 electroacupuncture instrument to stimulate the acupuncture points for 20 min with parameters of 50 Hz, 4 V and 1–3 mA (increased gradually). Electrical intensity was just strong enough to elicit slight twitches of the forelimbs. To control for the effects of needle insertion, sham acupuncture was performed by needle insertion at ST36 without electrical stimulation.

RESULTS
Concentrations of schisancherin, deoxyschisandrin and schisandrin B in rat tissues after oral administration of schisandra with or without acupuncture are shown in figures 1–3, respectively.

Within 2 h of administration of the schisandra lignans, considerable amounts of schisandra lignans were detected in all tissues analysed. These results show that the three bioactive ingredients of Schisandra chinensis extract underwent a rapid and wide distribution into tissues during the time course investigated. Tissue concentrations in the combination group (with acupuncture) were slightly higher than those in the schisandra only group, but most of these differences were insignificant in the absorption phase. The lowest concentrations of the three lignans were in the spleen, which indicates that the spleen might not be the primary absorbent organ of lignans. The highest tissue level of the schisandra lignans appeared in the liver and lung, followed by the heart, kidney and spleen.

Concentrations of the three lignans 4.5 h after administration of acupuncture were higher than those in the schisandra only group. The highest
concentrations occurred in the liver, suggesting that acupuncture promotes the distribution of lignans in the liver, prolonging the retention time for a relative long time. Tissue concentrations of schisantherin, deoxyschisandrin and schisandrin B in the heart, spleen, lungs and kidneys were increased by 315%, 203%, 250% and 224%, respectively. This may be because acupuncture produced a synergistic effect on tissue distribution of the three lignans.

Tissue concentrations in the schisandra only group were found to decrease at 6 h in the order of the kidneys > spleen > heart > liver > lungs, while tissue concentrations in the group who received acupuncture decreased in a similar order (kidneys > spleen > heart > lungs > liver). Although tissue concentrations of the lignans were significantly decreased in the elimination phase, the concentrations of the three lignans in the group with acupuncture were still higher than those of the schisandra only group, especially in rat liver and lungs.

DISCUSSION

In recent years there has been an increasing interest in using acupuncture in combination therapy, both among the public and medical profession. Combined therapy of acupuncture and medication as a practical strategy to treat diseases has gained increasing importance in both experimental and clinical investigations. However, there have been few previous studies clarifying the therapeutic effectiveness of the combination. The effect of acupuncture on the tissue distribution of schisandra lignans has not yet been clarified.

In TCM, the five zang-fu organs consist of the heart (including the pericardium), lung, spleen, liver and kidneys and have frequently been investigated for tissue distribution in modern pharmacology. Their functions are believed to produce and store essence (blood and body fluid). ST36 is one of the acupuncture points that have been used in medicinal practice for thousands of years. Although the physiological nature of ST36 has not been clarified, stimulation at ST36 has been used successfully to study the effects of acupuncture. For instance, acupuncture at ST36 is frequently used to study the effects of acupuncture on various physiological regulatory mechanisms. One report claimed that the therapeutic potential of ST36 may be related to the high proportion of A-3 afferent fibres at the site of this point in rats, containing more myelinated fibres compared with other non-acupoint sites. Therefore, we selected ST36 to investigate the effect of acupuncture on tissue distribution.
In the animal experiments, male and female rats were chosen to investigate the mechanism of action of acupuncture in tissue distribution. The results of our preliminary pharmacokinetic studies in rats suggested that female rats’ menstrual cycles had little effect on the pharmacokinetics of the lignans. Thus it could be argued that choosing female rats in this study was reasonable and made the study more comprehensive. In addition, we used CMC as solubilising agents for dissolving the *Schisandra chinensis* extract. To avoid any interference produced by CMC, our laboratory has extensively investigated the effect of CMC on tissue distribution of lignans. Our studies indicated that CMC had no pharmacological effect on rats when used as a blank control.

To our knowledge, this is the first report to clarify the effect of acupuncture on tissue distributions of the three lignans in rats. From our data it appeared that the three lignans mainly distributed in the liver and lung, followed by the kidneys and heart, with the lowest distribution in the spleen. Tissue concentrations of schisandra lignans in the liver were highest, suggesting that acupuncture may produce a distribution trend to the liver. This could be associated with the effect of acupuncture which led to significant liver histological improvements. Tissue concentrations of the three lignans in the group who received acupuncture were significantly higher than those of the schisandra only group, implying that acupuncture at ST36 has a synergistic effect on the tissue distribution of the three lignans. It promoted the tissue distribution activity of the schisandra lignans, although related reports have not been seen to date.

The results of the present study, showing extensive distribution of lignans in tissues, provides a reasonable explanation for the synergistic effects of acupuncture and schisandra lignans. The present method not only provides a scientific system for further research of the effect of acupuncture on herbal medicine (determining target tissues and evaluating the drug–acupuncture relationship) but has also laid the foundation for the clinical application of acupuncture combined with *Schisandra chinensis* extract.

**CONCLUSION**

Acupuncture is a non-confrontational therapy that appears to be effective and is well received in TCM. For the first time, a sensitive and selective HPLC-ESI-MS method was developed for the study of the effect of acupuncture on target tissue...
The results of the tissue distribution study supported our initial hypothesis and demonstrated that combination therapy (acupuncture and schisandra) may change the distribution trends of the three lignans, promote the distribution of the three lignans, delay the peak time of the three lignans and prolong the mean residence time of the three lignans in rats. Acupuncture had a synergistic effect on tissue distribution that enhanced drug distribution when combined with TCM. These findings provided novel insights into the preventive strategies for clinical treatment. Our data suggested that the effectiveness of acupuncture and its combination with schisandra should be investigated further as a possible adjuvant therapy in the liver.

Summary points

- Schisandra lignans is a herbal therapy for liver disease.
- We tested the effect of acupuncture as a combination therapy.
- Acupuncture changed its distribution, prolonging its presence in several tissues.

Funding  The research was supported by the Major Programme of State Commission of Science Technology of China (No 2009ZX09308-004), Educational Commission of Jiangsu Province of China (No 09KJA360001), Key Laboratory of Acupuncture of Jiangsu Province of China (No KJA200906) and Open Topics of Advantage Disciplines of Jiangsu Province (No 2011ZYX2-003).

Contributors  Z-JH designed the experiments. X-YW analysed the experimental data and wrote the article. BX provided technical guidance in acupuncture experiment. HC and YZ collected the samples and the data. C-QM and T-LL served as scientific advisors. YSo and YSu critically reviewed the study proposal.

Competing interests  None.

Provenance and peer review  Not commissioned; externally peer reviewed.

REFERENCES


Effect of acupuncture on target tissue distribution of *Schisandra* lignans

Tu-Lin Lu, Xiao-Yan Wu, Yu Song, Hao Chen, Bin Xu, Yuan Zhou, Zhi-Jun Huang, Yong Sun and Chun-Qin Mao

*Acupunct Med* 2013 31: 207-213 originally published online February 28, 2013
doi: 10.1136/acupmed-2012-010266

Updated information and services can be found at:
http://aim.bmj.com/content/31/2/207

These include:

References
This article cites 17 articles, 3 of which you can access for free at:
http://aim.bmj.com/content/31/2/207#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/