Effect of stimulating the acupoints Feishu (BL 13) and Dazhui (GV 14) on transdermal uptake of sinapine thiocyanate in asthma gel


**Abstract**

**OBJECTIVE:** To investigate the effect of stimulating the acupoints Feishu (BL 13) and Dazhui (GV 14) on the transdermal uptake of sinapine thiocyanate contained in a gel used for the management of asthma.

**METHODS:** Thirty Sprague-Dawley rats were randomly divided into three equal groups using a random number table: the Feishu (BL 13) acupoint group, the Dazhui (GV 14) acupoint group, and the nonacupoint group or control group. Using microdialysis technology, preprocessed skin probes were implanted into the rats at Feishu (BL 13), Dazhui (GV 14), and a nonacupoint site. Asthma gel was then placed on the skin at Feishu (BL 13), Dazhui (GV 14) acupoints, and the nonacupoint for all groups. Dialysate was collected every 30 min for 12 h. The normalized concentration of sinapine thiocyanate in the skin was determined by high-performance liquid chromatography.

**RESULTS:** The rat in vivo transdermal experiment demonstrated that the quantity-time equation showed a good linear correlation with zero-order kinetics \((r > 0.99)\). The transdermal behavior was in accordance with the first-order rate open model in which the transdermal penetration rates and the accumulative amounts of sinapine thiocyanate in the skin at the acupoint sites were greater than those through the skin of the nonacupoint site. The systemic maximum concentration and the area under the curve of sinapine thiocyanate in the skin determined by high-performance liquid chromatography.

**CONCLUSION:** Stimulating the acupoints promotes the percutaneous absorption of sinapine thiocyanate and also controls its release, reducing concentration fluctuations in the blood.

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**Keywords:** Asthma; Acupuncture; Point BL13 (Feishu); Point GV14 (Dazhui); Microdialysis; Skin absorption; Pharmacokinetics
INTRODUCTION

In Traditional Chinese Medicine (TCM), the majority of prescriptions for the management of asthma consist of five herbs: Xixin (Herba Asari Mandshurica), Shengjisang (Rhizoma Zingiberis Recens), Baijiezi (Semen Sinapis), Yanhusuo (Rhizoma Corydalis Yanhusuo), and Gansui (Radix Kansui), in a respective weight ratio of 1:1:2:2:1. Baijiezi (Semen Sinapis), Yanhusuo (Rhizoma Corydalis Yanhusuo), and Gansui (Radix Kansui) are the principal drugs, Xixin (Herba Asari Mandshurica) is the official drug, Gansui (Radix Kansui) is the adjuvant drug, and Shengjisang (Rhizoma Zingiberis Recens) is the ministerial drug. This prescription for the clinical treatment of asthma is from Cold Asthma Recipe in Zhang Shi Yi Tong, written by Zhang Lu during the Qing Dynasty, which also includes the external application of Baijiezi (Semen Sinapis) for treating asthma due to the TCM symptom pattern of cold. The principal herb in asthma gel is Baijiezi (Semen Sinapis), which is pungent, warm, and shows meridional troppism in the lung. As an expectorant, Baijiezi (Semen Sinapis) disperses stagnation, dredges the meridians, and relieves pain. Recently, various preclinical and clinical studies have demonstrated that Baijiezi (Semen Sinapis) is an effective antitussive, anti-asthmatic, and anti-inflammatory agent. The active ingredient in Baijiezi (Semen Sinapis) is sinapine thiocyanate, which has multiple pharmacological activities (Figure 1). Previous research has shown that sinapine thiocyanate, which is the primary active compound of white mustard, can pass through the skin and significantly enhances the release of interleukin-1β and tumor necrosis factor-α. A further mechanistic study revealed that topical white mustard treatment resulted in morphometric changes of Langerhans cells and local cytokine release, which might explain the efficacy of white mustard extract in treating asthma and bronchitis. In addition, the release rate of sinapine thiocyanate is reportedly much greater than the transdermal absorption rate. Thus, it is of great importance to improve the transdermal absorption rate of sinapine thiocyanate in asthma gel.

Figure 1 Structure of sinapine thiocyanate

Acupuncture treatment was initially documented in 52 Bing Fang (Recipes for Fifty-Two Ailments) and is based on a holistic concept, main and collateral channels, and the acupoint-accumulating 'Qi' (Qi in TCM frequently refers to natural energy, life force, or energy flow) and blood of the Zang-Fu organ system. Acupuncture allows the absorption of medication and promotes the movement of the channel Qi. Acupuncture plus herbal medicine effectively improves the symptoms of asthma. In addition, evaluations of both rat plasma pharmacokinetics and mouse lung distribution of tetrahydropalmatine found that needling Feishu (BL 13) allowed greater absorption of a cold asthma recipe extract into the blood circulation and distribution in target tissue. These results might provide a rational explanation for acupoint selection, and help to elucidate the underlying mechanism of this treatment. A previous study showed that acupuncture plus Chinese herbal medicines could balance T helper I/T helper 2 cells and effectively reduce the infiltration of eosinophils, which might contribute to its therapeutic effect in relieving asthma.

The common acupoints used to treat asthma and bronchitis in humans include Feishu (BL 13), Dingchuan (EX-B 1), Xinshu (BL 15), and Dazhui (GV 14). In addition, meridian transdermal drug delivery plays a role in the percutaneous penetration of drugs, but its mechanism remains to be elucidated. Therefore, the current study aimed to investigate the effect of stimulating the acupoints Feishu (BL 13) and Dazhui (GV 14) on the transdermal penetration of sinapine thiocyanate contained in a gel used for the management of asthma.

MATERIALS AND METHODS

Chemicals

Sinapine thiocyanate standard was purchased from Chengdu Herbpurify Company (Chengdu, China). Methanol and acetonitrile were purchased from Beijing Dikema Company (Beijing, China). Potassium dihydrogen phosphate, sodium chloride, potassium chloride, sodium sulfide, and anhydrous calcium chloride were purchased from Tianjin Kemiu Chemical Reagent Company (Tianjin, China). Heparin sodium injection were purchased from Tianjin Biochem Pharmaceutical Company (Tianjin, China). Urethane was purchased from Shanghai Sinopharm Chemical Reagent Company (Shanghai, China). Ringer’s solution (147 mM NaCl, 1.2 mM CaCl₂, and 4 mM KCl) was used as received. The resistance of the ultrapure water used was 18.2 Ω/cm.

Instruments

An LC-20A high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan), an AUW120D five-decimal super-precision electronic balance (Shimadzu, Kyoto, Japan), a microdialysis BAS system (BASi, West Lafayette, IN, USA), a CMA 30 MD linear probe (CMA Company, Stockholm, Sweden), and surgical instruments (Shanghai Medical Equipment Co., Ltd., Shanghai, China) were used in this study.
Preparation of asthma gel
All raw herbal materials used in the current study were purchased from Guangzhou Zhixin Herbal Slices Co., Ltd., (Guangzhou, China) and were authenticated by Prof. Li Wei (Department of TCM Identification, School of Pharmacy, Guangzhou University of Chinese Medicine). The volatile oil was extracted from Xixin (Herba Asari Mandshurica) and Shengjiang (Rhizoma Zingiberis Recens) with 10 times the amount of water for 6 h. After the first extraction, Xixin (Herba Asari Mandshurica), Shengjiang (Rhizoma Zingiberis Recens), and other herbs including Baijiezi (Semen Sinapis), Yanhusuo (Rhizoma Corydalisl Yanhusuo), and Gansui (Radicis Kansui) were subsequently extracted twice with six times the amount of 70% ethanol, for 2 h each time. After hot filtration, the secondary extract was concentrated to 1 g/mL. A total of 5 g of 1% carbopol was used as the A phase. Polyvinylpyrrolidone (0.5 g) dissolved in 2 mL of water supplemented with 0.5 mL of the abovementioned volatile oil and 4 mL of concentrated extract was used as the B phase. Polypolypropylene acid (NP-700; 0.5 g) and 0.015 g of dihydroxyaluminum aminoacetate dissolved in 3 g of glycerol was used as the C phase. The B phase was mixed with the A phase, then the C phase was slowly added to the mixture of A and B, and finally 0.01 g of EDTA-2 was added to the above mixture to produce the asthma gel. The amount of sinapine thiocyanate in the asthma gel was analyzed by HPLC to be 12.77 mg·cm⁻³.

Animal and experimental protocol
Thirty healthy 3-month-old male Specific pathogen free grade Sprague-Dawley rats weighing 200-250 g were purchased from the Guangzhou University of Chinese Medicine Laboratory Animal Center. All animal experiments were approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine (certificate No. SCXK 2008-0020). The 30 rats were randomly divided into three equal groups using a random number table: the Feishu (BL 13) acupoint group, the Dazhui (GV 14) acupoint group, and the nonacupoint group or control group. To anesthetize the rats, 20% urethane (0.6 mL/100 g) was injected into the abdominal cavity. A skin probe was implanted under the skin of the acupoint or non-acupoint site with the aid of a puncture needle. Feishu (BL 13) in rats is located on the back between the third thoracic ribs on both sides, while Dazhui (GV 14) is located on the back between the middle of the seventh cervical rib and the first thoracic rib (Figure 2A). The nonacupoint site was positioned about 1 cm below Dazhui (GV 14) on the rat back. The skin probe was kept in the skin, and its inlet and outlet positions were fixed. During the whole operation procedure, perfusion was conducted with blank Ringer’s solution at a flow rate of 1.0 µL/min. In addition, the probe membrane was kept in a wet state during the procedure. After the probe was implanted, the rats were administered 2.5 g of asthma gel over a 1.0 cm × 1.0 cm area of shaved skin (Figure 2B). The sinapine thiocyanate content in the dialysate collected from the skin probes was determined by HPLC.

Microdialysis
Pretreatment of microdialysis probes: the skin microdialysis probes (MAB6.14.4, 150000D, MAB, Sweden) were first washed with Ringer’s solution for 2 h, followed by artificial cerebrospinal fluid (containing 147 mM NaCl, 2.7 mM CaCl₂, 2.7 mM KCl, and 0.85 mM MgCl₂) at 2.0 mL/min for another 2 h. Before starting the experiment, the probes were perfused again with Ringer’s solution and artificial cerebrospinal fluid containing 1% sodium heparin at 0.5 mL/min for 20 min. In vivo recovery by retrodialysis: perfusion balance was achieved and maintained for 1 h with blank Ringer’s solution at a flow rate of 1.0 µL/min after the skin probe was implanted, in order to balance the physiological environment of the skin around the probe and obtain a stable baseline. After that, a new balance was reached and maintained for 1 h with 5.82 µg/mL sinapine thiocyanate instead of blank Ringer’s solution. Thereafter, 30 µL of dialysate was collected every 30 min for a total of six times. Next, the concentration of sinapine thiocyanate in each dialysate was determined by HPLC. The relative loss of sinapine thiocyanate as measured by the skin probe was calculated according to the following equation:

\[
RL = \frac{C_{	ext{blank}} - C_{	ext{dialysate}}}{C_{	ext{blank}}} \times 100\%
\]  

where RL: relative loss, \( C_{	ext{dialysate}} \): mass concentration of
sinapine thiocyanate in the dialysate, and $C_{\text{reference}}$: mass concentration of sinapine thiocyanate in the perfusat.

According to the equation $C = \frac{C_{\text{reference}}}{RL}$, the calculated relative loss (RL) rate was used to normalize the skin concentration of sinapine thiocyanate at each timepoint.

Sample collection by skin microdialysis: with the in vivo relative loss of sinapine thiocyanate determined above, blank Ringer’s solution was used to achieve perfusion balance. Before dosing, three consecutive dialysates of 30 µL were collected as negative controls. After that, the same dose of asthma gel was applied on the skin at Feishu (BL-14), Dazhui (GV 14), and the non-acupoint site. After drug administration, 30 µL of dialysate was collected every 30 min for 12 h and analyzed by HPLC. The whole experiment was conducted with the rats under general anesthesia.

**HPLC analytical method validation**

HPLC conditions: an Agilent TC-C18 column (250 mm x 4.6 mm, 5 µm) with a mobile phase of acetonitrile and 0.1 M potassium dihydrogen phosphate (14: 86) was used for compound separation. The detection wavelength, flow rate, and column temperature were 326 nm, 1.0 mL/min, and 30 °C, respectively. The theoretical plate number as calculated by the sinapine thiocyanate peak was not less than 3000.

Specificity: blank dialysate, sinapine thiocyanate standard, sinapine thiocyanate standard in blank dialysis fluid, and dialysate samples were analyzed. The chromatographic peaks of the analytes were identified based on their retention times.

Linear relationship examination: nine calibration standards consisting of 0.0328, 0.0656, 0.164, 0.328, 0.656, 1.64, 3.28, 6.56, and 16.4 µg/mL sinapine thiocyanate were prepared. The concentration of the standard solution was measured by the HPLC method. The standard curve of sinapine thiocyanate was plotted with linear regression.

Precision and accuracy: three concentrations of reference substance (QC samples), including low (0.0656 µg/mL), medium (0.656 µg/mL), and high (6.56 µg/mL), were measured within a day. Each measurement was repeated six times within 1 day for the determination of intra-day precision. A 3-day inter-day precision was also calculated. Precision was expressed as the percentage of relative standard deviation (RSD%) of the analyte peak. Accuracy was determined from the three QC samples, and expressed as the average content percentage of sinapine thiocyanate.

Stability: the same sample solution was measured by HPLC every 4 h over a 40-h period.

**Data analyses**

The data was expressed as the mean ± standard deviation (x ± s). The pharmacokinetic parameters were calculated using DAS 2.1 (Mathematical Pharmacology Professional Committee of China, Shanghai, China). One-way analysis of variance and t-tests were conducted to test the differences between the groups. $P < 0.05$ were considered to denote significant differences.

**RESULTS**

**Validation of the HPLC method**

Specificity: Figure 3 shows the HPLC chromatographs of blank dialysate, sinapine thiocyanate reference, sinapine thiocyanate in blank dialysis fluid, and dialysate samples. The results indicated that there was no interference with sinapine thiocyanate.

Linearity, precision, and accuracy: the calibration curve for sinapine thiocyanate was linear from 0.0328 to 16.4 µg/mL ($r = 0.97034.25 + 1470.2x, R^2 = 0.9992$). The accuracy was 95.5%-105.0%. The intra-day RSD and inter-day RSD were less than 2.9% and 3.5%, respectively.

Stability: the stability results showed that for up to 40 h, the remaining percentage of sinapine thiocyanate in the dialysate was 95.3%-105.4%, suggesting a good solution stability for sinapine thiocyanate in the current study.

**Microdialysis results**

There was no significant difference in the in vivo skin
probe recovery rate between the acupoint sites and the nonacupoint site. The average skin recovery rates of Feishu (BL 13), Dazhui (GV 14), and the nonacupoint site were 46.13%, 45.78%, and 46.16%, respectively, indicating that the current microdialysis technology was suitable to evaluate the pharmacokinetics of asthma gel when the acupoints were stimulated.

The sinapine thiocyanate in the asthma gel passed rapidly through the skin in all three groups. The concentrations of sinapine thiocyanate in the acupoint groups were significantly higher than that in the nonacupoint group ($P < 0.05$). Moreover, it was observed that the absorption of sinapine thiocyanate in the acupoint groups continued to increase for a longer period of time, while the absorption in the nonacupoint group decreased quickly after the maximum concentration was reached (Figure 4).

Figure 5 shows the correlation of the relative accumulative intake of transdermal drug ($Q$) against time. The slope of the regression equation represents the drug transdermal rate (Table 1). The lag time ($T_{lag}$) was obtained by extrapolating the X-intercept of the linear regression. The $in vivo$ transdermal penetration rate of sinapine thiocyanate remained almost constant over the 12-h period (Figure 5, Table 1). The release of the active ingredients in the asthma gel lasted for a long time, which is characteristic of controlled release and a long-acting transdermal drug delivery system. After transdermal administration of asthma gel, the $Q$-$t$ equations of sinapine thiocyanate in the acupoint groups showed a good linear relationship ($r > 0.99$). The $in vivo$ transdermal behavior was consistent with zero-order kinetics. The transdermal rates of the Feishu (BL 13), Dazhui (GV 14), and nonacupoint groups were 0.1147, 0.0832, and 0.0224 µg/h, respectively (Table 1). The accumulated transdermal amounts of sinapine thiocyanate in the Feishu (BL 13) and Dazhui (GV 14) groups, which were 1.134 and 0.956 µg/cm², respectively, were significantly higher than that of the nonacupoint group (0.238 µg/cm²). In addition, a drug lag time was found in the two acupoint groups, but not in the nonacupoint group ($P < 0.05$). Both the transdermal penetration rate and the accumulative transdermal amount in the Feishu (BL 13) group were significantly higher than those of the Dazhui (GV 14) group.

### Pharmacokinetic parameters

The pharmacokinetic parameters of the area under the curve (AUC), peak time ($T_{\text{peak}}$), and maximum concentration ($C_{\text{max}}$) were calculated using the noncompartmental model.

The drug concentration reached its peak rapidly within 4 h in both the acupoint and nonacupoint groups, indicating that sinapine thiocyanate could pass through the skin quickly by percutaneous administration. Compared with the nonacupoint group, the $C_{\text{max}}$ and AUC values were higher in the acupoint groups. The mean residence time and $t_{1/2}$ were also longer in the acupoint groups than in the nonacupoint group ($P < 0.05$), suggesting that the skin tissue around the acupoint site could enhance drug absorption and extend drug release for a long period, with less drug concentration fluctuation (Table 2).

### DISCUSSION

Percutaneous microdialysis technology can be used to detect drug concentration in the dermal extracellular fluid, and can continuously monitor the change in drug concentration around the location at which the probe is implanted in real time. It has the advantages of lowering the number of experimental animals required, causing minimal trauma to the animals, and simplifying sample processing. Percutaneous microdialysis technology applied to the transdermal drug delivery system with acupoint application can easily reflect the dynamic changes in the drugs percutaneous penetration and clarify the relationship between acupuncture meridians and drug percutaneous absorption. Thus, using microdialysis technology to illuminate the mechanism of acupoint therapy in the transdermal delivery system is a breakthrough in the pharmaceutical
In the current study, in order to achieve the analysis requirements and also to monitor the drug concentration changes, the flow rate of the perfusate was set at 1.0 µL/min and the sampling interval was 30 min.

In in vivo microdialysis, there is an assumption of directional independence of the analyte through the dialysis membrane. In our preliminary studies (data not shown), in vitro recoveries were not significantly different for sinapine thiocyanate, indicating that this compound exhibited in vitro directional independence. Based on this finding, in vivo recovery by the retrodialysis method was used to calculate the extracellular drug concentrations. The sinapine thiocyanate recovery as determined by the skin probe was stable for up to 10 h; therefore, the established microdialysis method of measuring sinapine thiocyanate levels could be further used in in vivo experiments.

There was a lag time associated with drug penetration in both acupoint groups, while no lag time was observed in the nonacupoint group. This indicates that acupoint application enabled the controlled release of sinapine thiocyanate in the skin. The skin absorption of sinapine thiocyanate in the acupoint groups remained steady for a longer time, while the skin absorption in the nonacupoint group fell rapidly after reaching the $C_{\text{max}}$, suggesting that the skin after acupoint treatment could result in the sustained release of the active ingredients in the asthma gel, as well as promoting transdermal drug intake, which is consistent with previously reported findings, and different skin acupoints have different physiological functions and characteristics. Therefore, in the clinic, acupoint selection should be made according to the specific diagnosis and the condition of the patient. The sinapine thiocyanate levels of the last three samplings of microdialysates in the nonacupoint group were below the limit of quantitation.

In addition, percutaneous absorption of sinapine thiocyanate in both the acupoint and nonacupoint groups was in accordance with the first-order rate and the one-compartment open model; however, the degree of curve fitting was poor. As the absolute bioavailability of sinapine thiocyanate is low, the pharmacokinetics of this transdermal drug delivery system has its unique characteristics and advantages. It is reportedly not suitable to use the general house models; therefore, in the current study, extravascular administration analysis of the noncompartmental model was applied to calculate the statistical moment parameters.

In conclusion, stimulating the acupoints Feishu (BL 13) and Dazhui (GV 14) can promote the percutaneous absorption of sinapine thiocyanate, and can also control its release to reducing fluctuations of the drug concentration in the blood and improve its efficacy in treating asthma.

**REFERENCES**


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**Table 1** Concentration-time regression equation of sinapine thiocyanate in the acupoint and nonacupoint groups

<table>
<thead>
<tr>
<th>Location</th>
<th>$Q-t$</th>
<th>$J$ (µg/h)</th>
<th>$R^2$</th>
<th>$T_m$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feishu acupoint (BL13)</td>
<td>$y = 0.1147x - 0.0047$</td>
<td>0.1147</td>
<td>0.9976</td>
<td>0.401</td>
</tr>
<tr>
<td>Dazhui acupoint (GV 14)</td>
<td>$y = 0.0832x+0.0194$</td>
<td>0.0832</td>
<td>0.9920</td>
<td>0.228</td>
</tr>
<tr>
<td>Nonacupoint</td>
<td>$y = 0.0224x+0.0373$</td>
<td>0.0224</td>
<td>0.9039</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: $Q-t$: concentration-time; $T_m$: lag time.

**Table 2** Pharmacokinetic parameters of sinapine thiocyanate in the three group ($\bar{x} \pm s$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Feishu acupoint (BL 13) ($n = 10$)</th>
<th>Dazhui acupoint (GV 14) ($n = 10$)</th>
<th>Nonacupoint ($n = 10$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_0-\infty$ (mg·L$^{-1}·$h$^{-1}$)</td>
<td>21.5±2.2</td>
<td>15.6±1.9</td>
<td>3.9±0.6</td>
</tr>
<tr>
<td>$AUC_0-\infty$ (mg·L$^{-1}·$h$^{-1}$)</td>
<td>79.9±19.5</td>
<td>35.8±10.1</td>
<td>5.2±1.2</td>
</tr>
<tr>
<td>$MRT_0$ (h)</td>
<td>6.1±0.8</td>
<td>5.9±0.7</td>
<td>4.1±0.5</td>
</tr>
<tr>
<td>$t_{\text{lag}}$ (h)</td>
<td>22.3±3.3</td>
<td>12.3±2.6</td>
<td>5.3±2.0</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4.0±0.9</td>
<td>3.5±0.8</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mg/L)</td>
<td>2.6±0.5</td>
<td>2.1±0.5</td>
<td>1.2±0.3</td>
</tr>
</tbody>
</table>

Notes: $AUC$: area under the curve; $MRT$: mean residence time; $T_{\text{max}}$: peak time; $C_{\text{max}}$: systemic maximum concentration. $^*$P < 0.05, compared with the nonacupoint group.


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