

## *In Vivo* and *in Vitro* Evaluation of Essential Oils from *Ligusticum chuanxiong* HORT on the Transdermal Delivery of Flurbiprofen in Rabbits

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The present study was designed to evaluate skin permeation enhancement effect of essential oils from *Ligusticum chuanxiong* HORT (chuanxiong oil) in rabbits and to compare the *in vivo* absorption and *in vitro* permeation using flurbiprofen as a model drug. *In vivo* results demonstrated that chuanxiong oil showed a rapid and marked permeation enhancement effect. The group with 10% oil exhibited the highest value of area under the curve (AUC) of  $418 \pm 124 \mu\text{g/ml} \cdot \text{h}$ , which was 2.43 times the high of control. The AUC value of 3% oil group ( $245 \pm 81.6 \mu\text{g/ml} \cdot \text{h}$ ) was similar to that of 5% oleic acid group ( $235 \pm 74.5 \mu\text{g/ml} \cdot \text{h}$ ). Whereas *in vitro* results indicated the enhancement of chuanxiong oil was relatively weak. The group with 3% oil appeared to the highest flurbiprofen flux ( $84.9 \pm 19.3 \mu\text{g/cm}^2/\text{h}$ ), to some extent lower than 5% oleic acid group ( $107 \pm 5.85 \mu\text{g/cm}^2/\text{h}$ ). At 10% and 15% concentrations, chuanxiong oil even decreased the flux of flurbiprofen compared with the control. Both *in vitro* results with pretreated skin and flurbiprofen content accumulated in skin indicated the potential mechanism for the *in vitro* enhancement of chuanxiong oil was the weakened barrier function by improving in the partitioning of flurbiprofen to the stratum corneum. The discrepancy was noted between the *in vivo* and *in vitro* results, indicating only about the weakened barrier function was not enough to explain the sharply increment of *in vivo* absorption of flurbiprofen by chuanxiong oil. The GS-MS results indicated phthalides identified from chuanxiong oil might mainly contribute to enhance *in vivo* absorption of flurbiprofen because of its large quantities (91.15%).

**Key words** skin permeation enhancement; essential oil; chuanxiong; flurbiprofen; phthalide

The efficiency of the stratum corneum as a barrier to drug transport has led to the development of a variety of chemical permeation enhancers to increase the potential systemic application of transdermal drug delivery, such as Azone and its analogues, pyrrolidones, polyunsaturated fatty acids, alkanols, non-ionic surfactants and cyclodextrin.<sup>1–3)</sup> All the above enhancers have been reported to increase significantly the number of candidates suitable for transdermal delivery. Despite the large number of different chemical entities identified, few is available in the market place due to various limitations such as skin irritation.<sup>1)</sup> So, the search for new enhancers with excellent characteristics has been continuing in the last two decades. Recently, considerable research is in progress on the use of essential oils derived from natural products as permeation enhancers to improve drug permeation due to their high permeation enhancement abilities and low irritancy potential. Most investigations on this respect have focused on the fatty acid, terpenes from essential oils as permeation enhancers.<sup>4–6)</sup>

*Ligusticum chuanxiong* HORT (chuanxiong) is one of the most frequently used drugs in the prescriptions of traditional Chinese medicine for treating cardiovascular diseases.<sup>7)</sup> Chuanxiong is rich in essential oils, which thought to be the component with important biologically activities of chuanxiong.<sup>8)</sup> The extract of chuanxiong has been used in the balneotherapy for a long time in Japan and it can facilitate some drugs to permeate through human skin.<sup>9)</sup> Namba *et al.* investigated the influence of the various extracts (water, ester, methanol) from *Ligusticum chuanxiong* HORT on the permeation of benzoic acid through excised rat skin. It was found that the essential oils from *Ligusticum chuanxiong* HORT (chuanxiong oil) could enhanced markedly *in vitro* transder-

mal delivery of benzoic acid. The mechanism for the permeation enhancement might be due to the improvement in the partitioning of benzoic acid to the stratum corneum.<sup>9)</sup>

Although *in vitro* permeation experiments are valuable for studying the rate and the mechanisms of percutaneous absorption of drugs, they sometimes do not reflect the *in vivo* profile. This is due to a difference between *in vitro* and *in vivo* circumstances, especially cutaneous blood flow.<sup>10)</sup> Previous investigations indicated that the main constituents identified from chuanxiong oil such as ligustilide and butylidenephthalide were significantly effective to inhibit platelet aggregation, improve local blood flow, and induce vasodilation.<sup>7,11,12)</sup>

In this study, we first investigated the *in vivo* skin permeation enhancement effect of chuanxiong oil formulated in Carbopol gel in rabbits. Flurbiprofen was chosen as a drug model. It is a suitable candidate for transdermal drug delivery system and widely studied for evaluating new chemical permeation enhancers.<sup>13–15)</sup> To distinguish whether the skin permeation enhancement effects are due to the weakened stratum corneum barrier function, *in vitro* experiments with excised rabbit skin were also performed.

### MATERIALS AND METHODS

**Chemicals** Flurbiprofen and ketoprofen were supplied by Sanwei Pharmaceutical (Shanghai, China). *Ligusticum chuanxiong* HORT was purchased from traditional Chinese Medicine Store (Shanghai, China). Oleic acid was purchased from Shanghai Chemical Company (Shanghai, China). Carbopol 980NF was a gift sample from BF Goodrich Company (U.S.A.). Other chemicals and solvents were of the highest

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purity available commercially.

**Animals** Male New Zealand white rabbits weighing 2.0–2.2 kg were obtained from the Animal Center of Second Military Medical University (Shanghai, China). The experimental protocol was approved by the Committee on Animal Research of our university. Also, all the animal experiments were performed in compliance with Guiding Principles for the Care and Use of Laboratory Animals, Second Military Medical University, China.

**Supercritical Fluid Extraction** The dry rhizome of chuanxiong was pulverized (10–20 mesh). A 10 kg amount of the pulverized sample was packed into a 20 l sample cartridge. Supercritical fluid extraction was performed on a HA-48 supercritical fluid extractor (Hua'an SFE Equipment Co., China). The extraction temperature was set at  $30 \pm 2^\circ\text{C}$ . Liquid carbon dioxide at high pressure  $7.3 \pm 0.1\text{ MPa}$  was then allowed to flow into the sample cartridge. The extraction process was run for 2 h. Chuanxiong oil was obtained by reducing pressure of supercritical carbon dioxide. The final amount of yellow liquid was approximately 450 g.

**Preparation of Gel** The gel was composed of flurbiprofen (1% w/w), Carbopol 980NF (2%), ethanol (15%), and triethanolamine (3%). Carbopol powder was dispersed into the water phase and set for 24 h at room temperature, followed by adding triethanolamine for neutralization under 200 rpm agitation to form a homogeneous gel. Flurbiprofen mixed with chuanxiong oil or oleic acid if necessary was dissolved in ethanol. The alcoholic solution was slowly added to the vortex of agitated gel. The remaining water phase was added to the gel with continuous stirring.

**In Vivo Percutaneous Absorption** The rabbits were housed individually over 2 weeks in a temperature-controlled ( $25 \pm 1^\circ\text{C}$ ) and relative humidity-controlled (50–60%) environment and had free access to a standard diet and water 1 week prior to the experiments. The rabbits were fasted for 24 h and then whose dorsal regions were carefully depilated (approx.  $100\text{ cm}^2$ ) by Veet® (Cedex, France) depilatory cream and the skins were cleaned by wiping with water containing cotton under pentobarbital anesthesia. The rabbits were randomly divided into four groups of six each: two of the control (no enhancer and 5% oleic acid), two of chuanxiong oil (3, 10%). The gel was applied to a  $9 \times 8\text{ cm}^2$  area of the depilated dorsal area of the rabbit, covered with a non-porous silicone membrane and wrapped with an elastic adhesive bandage. The amount of gel used was equivalent to a dose of 50 mg/kg flurbiprofen.

A 1.2 ml of blood sample was withdrawn from the central ear artery at predetermined times over 36 h. After taking a blood sample, heparinized physiological saline (95 IU/ml) was inserted into the set in order to prevent blood coagulation. The homeostasis of the rabbits was maintained by injecting the same volume of physiological saline *via* the ear vein. The blood samples were centrifuged at 3000 rpm for 10 min to obtain 0.5 ml of the plasma, which was stored at  $-20^\circ\text{C}$  prior to analysis.

**In Vitro Skin Permeation** Modified Franz vertical diffusion cell systems containing 8 cells was used. The donor cell was filled with 1.0 g of test gel and occluded with aluminum foil. The receiver solution was 4.2 ml of phosphate-buffered saline at pH 7.2 (PBS). The effective surface area available for permeation was  $0.952\text{ cm}^2$ . Full-thickness dorsal skin,

whose hair had been previously removed, was excised from anesthetized rabbits. Subcutaneous fat was carefully removed with a scalpel and washed with saline solution. The excised skin was mounted between the donor and receptor cells with epidermal side facing the donor cell. The temperature and stirring rate were maintained at  $37 \pm 0.5^\circ\text{C}$  and 300 rpm. All the receiver solution was withdrawn at predetermined times over 12 h and replaced with an equivalent volume of fresh buffer. The samples were stored at  $-20^\circ\text{C}$  prior to analysis.

To study the effect of pretreatment of rabbit skin on the permeation of flurbiprofen, 1.0 g of the gel with chuanxiong oil at different concentrations (containing no drug) was filled into the donor cell for 12 h. At the end of 12 h, the gel was discarded. The donor cell was thoroughly washed with water and then filled with 1.0 g of the control gel (containing no enhancer or oil). The permeation experiments were performed as described in the above paragraph.

**In Vitro Skin Content** After 12 h of sampling, the applied site of skin was removed from the cells and washed briefly in methanol, using a previously reported procedure with minor modification.<sup>16</sup> The skin was weighed, cut with scissors, and then homogenized for 5 min using a tissue homogenizer containing 5 ml of PBS. The homogenate was centrifuged for 10 min at 10000 rpm. The supernatant was stored at  $-20^\circ\text{C}$  prior to analysis.

**HPLC Analysis of Flurbiprofen** The quantitative determination of flurbiprofen was performed by HPLC (liquid chromatograph with two LC-10AT<sub>VP</sub> pumps and 20  $\mu\text{l}$  Rheodyne injector, SPD10A detector and computer integrating system, Shimadzu, Kyoto, Japan). The chromatographic column was a Hypersil C<sub>18</sub> ODS column ( $250 \times 4.6\text{ mm i.d.}$ , 5  $\mu\text{m}$  particle size) obtained from Applied Chemistry Research Institute (Dalian, China). The mobile phase consisted of 40% v/v 9.74 mM phosphoric acid, 56% v/v acetonitrile, and 4% v/v tetrahydrofuran. The flow rate, wavelength for determination, and temperature of the column were 1.0 ml/min, 247 nm, and  $25^\circ\text{C}$ , respectively. The retention times of ketoprofen (internal standard) and flurbiprofen peak were 4.6 and 6.7 min, respectively.

A 400  $\mu\text{l}$  of the plasma and 50  $\mu\text{l}$  of ketoprofen (equal to 1  $\mu\text{g}$ ) were pipetted into a 10 ml polypropylene centrifuge tube, and vortex-mixed for 10 s. A 200  $\mu\text{l}$  of 3.0 M hydrochloric acid was added and vortex-mixed for 30 s. The mixture was extracted with 5 ml of ethyl acetate for 3 min using vortex mixer. After centrifuged at 2000 rpm for 5 min, 3 ml of the organic solvent phase was transferred to another capped tube and evaporated to dryness under nitrogen gas at  $60^\circ\text{C}$ . The residue was reconstituted in 200  $\mu\text{l}$  of the mobile phase for 1 min using vortex mixer and 20  $\mu\text{l}$  of the supernatant was then injected into the chromatograph after centrifuging at 15000 rpm for 15 min.

**Gas Chromatography-Mass Spectrometry Analysis** The GC-MS system consisted of a GC (Agilent 6890N), a mass selective detector (HP 5973), and an HP ChemStation Data analysis system. Chuanxiong oil of 1  $\mu\text{l}$  was injected manually into a HP-5 MS capillary column ( $29.8\text{ m} \times 0.25\text{ mm i.d.}$ , 0.25  $\mu\text{m}$  phase thickness, 5% phenyl methyl siloxane) using the following temperature program. The initial temperature was  $80^\circ\text{C}$  for 2 min, then increased to  $200^\circ\text{C}$  at  $12^\circ\text{C}/\text{min}$ , hold 4 min, and finally increased to  $224^\circ\text{C}$  at  $12^\circ\text{C}/\text{min}$ , hold for 14 min. The injection tempera-

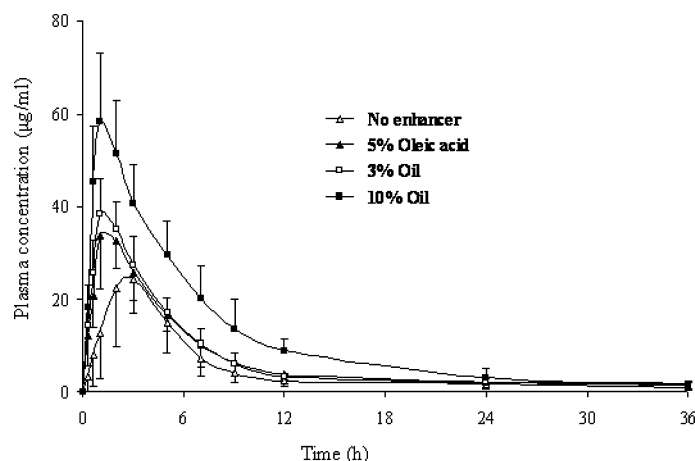


Fig. 1. Plasma Concentration Profile of Flurbiprofen after the Transdermal Administration of Carbopol Hydrogel to Rabbits at a Dose of 50 mg/kg  
All data represent six experiments (mean  $\pm$  S.D.).

ture was 230 °C, the MS source temperature was 230 °C, and the column flow of the carrier gas (He) was 1.0 ml/min with split ratio of 50 : 1. The full-scan acquisition mode was used for detection. Solvent delay was set as 2.0 min. Chromatographic peaks were checked for homogeneity with the aid of mass chromatograms with characteristic ion fragments. The Wiley275.I (Mass Spectra Library) database was used for automatic identification of the peaks.

**Data Analysis** The plasma concentration of flurbiprofen at different time intervals was subjected to pharmacokinetic analysis. Various parameters such as maximum plasma concentration ( $C_{\max}$ ), time to reach maximum plasma concentration ( $T_{\max}$ ) and area under the curve ( $AUC$ ) were calculated. The values of  $C_{\max}$  and  $T_{\max}$  were directly read from the arithmetic plot of time *versus* plasma concentration of flurbiprofen. The value of  $AUC$  was calculated by the trapezoidal rule. The cumulative amount of flurbiprofen permeated through the skin was plotted as a function of time. The slope of the linear portion of the plot from 0 to 12 h was calculated as the flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ ). The data were subjected to one-way analysis of variance (ANOVA) followed by Student's *t*-test to determine level of significance between various groups. The data were expressed by mean  $\pm$  S.D. and considered to be significant at  $p < 0.05$ .

## RESULTS

**In Vivo Percutaneous Absorption** Oleic acid was used as a control for chuanxiong oil to accelerate percutaneous absorption of flurbiprofen and its concentration in gel was fixed at 5%. Placebo (containing no enhancer) was also applied in a group of rabbits. The plasma concentrations of flurbiprofen after the transdermal administration of gels with chuanxiong oil at 3% and 10% concentrations to rabbits are shown in Fig. 1. Some pharmacokinetic parameters are summarized in Table 1. Chuanxiong oil showed a marked permeation enhancement to transdermal delivery of flurbiprofen through skin barrier. The group with 10% oil exhibited the highest  $AUC$  value of  $418 \pm 124 \mu\text{g}/\text{ml} \cdot \text{h}$ , which was 2.43 times the high of control ( $172 \pm 39.3 \mu\text{g}/\text{ml} \cdot \text{h}$ ). The  $AUC$  value of 3% oil group ( $245 \pm 81.6 \mu\text{g}/\text{ml} \cdot \text{h}$ ) was similar to that of 5% oleic acid group ( $235 \pm 74.5 \mu\text{g}/\text{ml} \cdot \text{h}$ ).

Table 1. Pharmacokinetic Parameters of Flurbiprofen in Rabbits of Flurbiprofen after the Transdermal Administration of Carbopol Hydrogel to Rabbits at a Dose of 50 mg/kg

| Formula       | $AUC$ ( $\mu\text{g}/\text{ml} \cdot \text{h}$ ) | $C_{\max}$ ( $\mu\text{g}/\text{ml}$ ) | $T_{\max}$ (h)    |
|---------------|--|--|-------------------|
| No enhancer   | $172 \pm 39.3$                                   | $30.2 \pm 9.60$                        | $2.67 \pm 0.52$   |
| 5% Oleic acid | $235 \pm 74.5^*$                                 | $35.1 \pm 9.87$                        | $1.56 \pm 0.69^*$ |
| 3% Oil        | $245 \pm 81.6^*$                                 | $39.5 \pm 11.6$                        | $1.33 \pm 0.52^*$ |
| 10% Oil       | $418 \pm 124^*$                                  | $62.5 \pm 17.3^*$                      | $1.22 \pm 0.62^*$ |

Each value represents the mean  $\pm$  S.D. ( $n=4$ ). \*  $p < 0.05$  higher than the control.

Chuanxiong oil also appeared a higher and rapider permeation enhancement compared with the control. As listed in Table 1, 10% oil group exhibited the highest  $C_{\max}$  value ( $62.5 \pm 17.3 \mu\text{g}/\text{ml}$ ) and the shortest  $T_{\max}$  value ( $1.22 \pm 0.62$  h). The effects group with 3% oil were similar to those of 5% oleic acid group in  $C_{\max}$  value ( $39.5 \pm 11.6 \mu\text{g}/\text{ml}$  vs.  $35.1 \pm 9.87 \mu\text{g}/\text{ml}$ ) and  $T_{\max}$  value ( $1.33 \pm 0.52$  h vs.  $1.56 \pm 0.69$  h).

**In Vitro Skin Permeation** The effect of chuanxiong oil and oleic acid at 5% concentration on the permeation of flurbiprofen from Carbopol gel is shown in Fig. 2. Figure 2 shows that the permeation of flurbiprofen through excised rabbit skin followed zero order release kinetics and accorded with the Fick's first diffusion law. The flurbiprofen flux of 5% oil group was  $82.1 \pm 17.7 \mu\text{g}/\text{cm}^2/\text{h}$ , which was significantly higher ( $p < 0.05$ ) than the control of  $55.5 \pm 16.9 \mu\text{g}/\text{cm}^2/\text{h}$ . The flurbiprofen flux of 5% oleic acid group was  $107 \pm 5.85 \mu\text{g}/\text{cm}^2/\text{h}$ , which was higher ( $p < 0.05$ ) than chuanxiong oil group at the same concentration.

The effect of chuanxiong oil at different concentrations (1, 3, 5, 10, 15%) on the flux of flurbiprofen is listed in Table 2. Only at 3% and 5% concentrations, chuanxiong oil exerted a significant enhancement to the flux of flurbiprofen through excised rabbit skin. The enhancement did not show a concentration-dependent manner. As shown in Table 2, at 3% oil concentration, the flux of flurbiprofen was highest ( $84.9 \pm 19.3 \mu\text{g}/\text{cm}^2/\text{h}$ ), which was 1.53 times the high of the control. Then, the flux of flurbiprofen was declined with increasing oil concentration. Interestingly, at higher concentrations (10, 15%), chuanxiong oil even slightly decreased the flux of flurbiprofen through excised rabbit skin compared

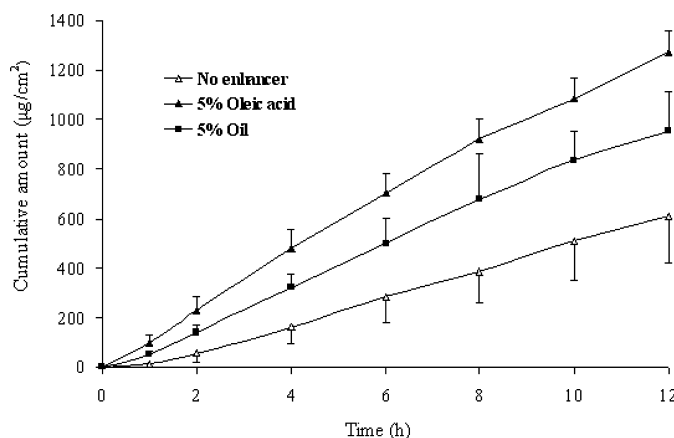


Fig. 2. Effect of Chuangxiong Oil and Oleic Acid at 5% Concentration on the Permeation of Flurbiprofen through Excised Rabbit Skin

All data represent four experiments (mean  $\pm$  S.D.).

Table 2. Effect of Chuanxiong Oil on the Flux through Excised Rabbit Skin and Skin Content of Flurbiprofen

| Formula     | Flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ ) | Skin content ( $\mu\text{g}/\text{mg}$ ) $\times 10^2$ |
|-------------|---|--|
| No enhancer | 55.5 $\pm$ 16.9                             | 172 $\pm$ 20.8   |
| 1% Oil      | 70.9 $\pm$ 13.2                             | 268 $\pm$ 33.0*  |
| 3% Oil      | 84.9 $\pm$ 19.3*                            | 316 $\pm$ 70.5*  |
| 5% Oil      | 82.1 $\pm$ 17.7*                            | 279 $\pm$ 25.8*  |
| 10% Oil     | 53.7 $\pm$ 12.6                             | 225 $\pm$ 63.2*  |
| 15% Oil     | 47.6 $\pm$ 10.4                             | 158 $\pm$ 57.2   |

Each value represents the mean  $\pm$  S.D. ( $n=4$ ). \* $p<0.05$  higher than the control.

with the control.

The effect of chuanxiong oil through pretreated excised rabbit skin at different concentrations (3, 10, 15%) on the flux of flurbiprofen is presented in Table 3. The permeation of flurbiprofen also followed zero order release kinetics and accorded with the Fick's first diffusion law. All three pretreated groups increased the flux of flurbiprofen significantly ( $p<0.05$ ) compared with the control. In addition, unlike *in vitro* experiments with untreated skin with chuanxiong oil (Table 2), with the pretreated skin, no decline in the flux of flurbiprofen was observed at higher oil concentrations. However, there was no significant difference ( $p>0.05$ ) in the flux of flurbiprofen among three pretreated groups.

The flurbiprofen content accumulated in the skin tissue was also measured after the permeation experiments for 12 h. As shown in Tables 2 and 3, all untreated and pretreated concentrations groups increased the content of flurbiprofen in skin along with the flux of flurbiprofen except 15% oil group. In the untreated and pretreated groups, the correlation coefficients between the increment of residual drug content in skin and the increment of drug flux were 0.9344 and 0.9140, respectively.

**Identification and Quantification of 16 Compounds from Chuanxiong Oil** The complete list of principal identified compounds from chuanxiong oil under supercritical fluid extraction is given in Table 4. Nineteen compounds were separated, and sixteen of them were identified according to the mass spectrum of each constituent. By comparing the mass spectra data of the sample with literature data,<sup>17,18)</sup> peaks 9, 10, 12, 13, 14, and 15 were identified as 3-*n*-

Table 3. Effect of Chuanxiong Oil on the Flux through Pretreated Excised Rabbit Skin and Skin Content of Flurbiprofen

| Formula     | Flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ ) | Skin content ( $\mu\text{g}/\text{mg}$ ) $\times 10^2$ |
|-------------|---|--|
| No enhancer | 62.2 $\pm$ 13.7                             | 197 $\pm$ 10.4   |
| 3% Oil      | 87.5 $\pm$ 19.2*                            | 260 $\pm$ 33.8*  |
| 10% Oil     | 96.9 $\pm$ 12.1*                            | 268 $\pm$ 42.5*  |
| 15% Oil     | 101.5 $\pm$ 24.0*                           | 264 $\pm$ 18.1*  |

Each value represents the mean  $\pm$  S.D. ( $n=4$ ). \* $p<0.05$  higher than the control.

Table 4. Identification and Quantification of Principal Compounds from Chuanxiong Oil under Supercritical Fluid Extraction in GC-MS

| Peak no. | Retention time (min) | Compound                    | Relative content (%) |
|----------|----------------------|-----------------------------|----------------------|
| 1        | 3.44                 | <i>p</i> -Cymene            | 0.23                 |
| 2        | 3.74                 | $\gamma$ -Terpinene         | 0.37                 |
| 3        | 4.05                 | Terpinolene                 | 0.35                 |
| 4        | 5.01                 | Terpinen-4-ol               | 0.63                 |
| 5        | 7.70                 | $\alpha$ -Cedrene           | 0.64                 |
| 6        | 8.37                 | $\beta$ -Selinene           | 1.92                 |
| 7        | 8.45                 | $\alpha$ -Selinene          | 0.57                 |
| 8        | 9.30                 | Spathulenol                 | 0.48                 |
| 9        | 10.02                | 3- <i>n</i> -Butylphthalide | 5.16                 |
| 10       | 10.23                | Butylidenephthalide         | 4.93                 |
| 11       | 10.42                | 1,3,5-Undecatriene          | 1.65                 |
| 12       | 10.73                | Senkyunolide                | 26.37                |
| 13       | 10.79                | Neocnidilide                | 5.21                 |
| 14       | 10.90                | <i>Z</i> -Ligustilide       | 48.36                |
| 15       | 11.67                | <i>E</i> -Ligustilide       | 1.12                 |
| 16       | 17.01                | Ethyl linoleate             | 0.35                 |
| 17       | 4.75                 | Unknown                     | 0.47                 |
| 18       | 9.87                 | Unknown                     | 0.38                 |
| 19       | 10.28                | Unknown                     | 0.80                 |

butylphthalide, butylidenephthalide, senkyunolide, neocnidilide, *Z*-ligustilide, *E*-ligustilide, respectively. These compounds were classified as phthalides and the total of their relative content was 91.15%. Peaks 1, 2, 3, 4, 5, 6, 7, and 8 were identified as *p*-cymene,  $\gamma$ -terpinene, terpinolene, terpinen-4-ol,  $\alpha$ -cedrene,  $\beta$ -selinene,  $\alpha$ -selinene, and spathulenol. All of them were terpenes, including four monoterpenoids (peaks 1, 2, 3, 4), three sesquiterpene (peaks 5, 6, 7), and one sesquiterpene alcohol (peak 8). The eight terpenes

made up 5.19% of the oil. The remaining portion was low levels of benzene, 1,3,5-undecatriene, ethyl linoleate, and other three unknown compounds. The mass spectra of peaks 9, 14, and 15 were similar because of similar structures and all of them were identified as 3-*n*-butylphthalide. According to the reported GC-MS separation of chuanxiong oil,<sup>17,18)</sup> peaks 14 and 15 were tentatively identified as two isomers of ligustilide, which were *Z*-ligustilide and *E*-ligustilide, respectively.

## DISCUSSION

*In vivo* results demonstrated that chuanxiong oil showed a rapid and marked effect enhancing the absorption of flurbiprofen in rabbits. Oleic acid was reported to possess high transdermal enhancing ability for flurbiprofen *in vitro* and *in vivo*.<sup>16,19)</sup> The skin permeation enhancement effects of 3% oil group and 5% oleic acid group were similar. Chuanxiong oil could also enhance the absorption of flurbiprofen in a concentration-dependent manner up to 10%. The *AUC* value of 10% oil group was 178% compared with 5% oleic acid group, indicating that the much greater efficiency of chuanxiong oil to enhance the absorption of flurbiprofen than did oleic acid.

Surprisingly, *in vitro* results indicated chuanxiong oil showed a relatively weak enhancement effect through excised rabbit skin compared with oleic acid. Of five concentrations studied, 3% oil group appeared the highest flux of flurbiprofen, which was only 79.3% of that of 5% oleic acid group. Furthermore, unlike *in vivo* results, the *in vitro* enhancement of chuanxiong oil did not show a concentration-dependent manner. At higher concentrations (10%, 15%), chuanxiong oil even suppressed the permeation of flurbiprofen compared with the control.

Pretreatment studies can verify whether the enhancers act directly on the stratum corneum barrier function or not.<sup>14)</sup> It is clear from Table 3 that chuanxiong oil was effective in perturbing the barrier function of the stratum corneum. In addition, the explanation for the declining permeation of flurbiprofen by chuanxiong oil at higher concentrations was probably due to the gradual decrease of drug thermodynamic activity in gel. In this study, the solubility of flurbiprofen in chuanxiong oil and water at 25 °C was measured by HPLC. The value of solubility in chuanxiong oil was  $579 \pm 20.5 \mu\text{g/ml}$ , significantly higher than that in water ( $53.5 \pm 3.81 \mu\text{g/ml}$ ). At a constant drug concentration, drug thermodynamic activity was reduced with increasing its solubility in gel. For this reason, the flurbiprofen activity was reduced with increasing oil concentration formulated in gel, resulting in the poorer efficiency of drug partitioning from the gel into the stratum corneum.

In addition, the linear relationships between the increment of residual flurbiprofen content in skin and the increment of flurbiprofen flux were found in the *in vitro* experiments with untreated and pretreated skin (correlation coefficient,  $r=0.9344$  and  $0.9140$ , respectively), indicating the potential mechanism for the *in vitro* permeation enhancement of chuanxiong oil was the weakened stratum corneum barrier function by improving in the partitioning of flurbiprofen to the stratum corneum, which supported the previous reports.<sup>9)</sup>

A general theory for the mechanism of action of perme-

ation enhancers was to reversibly alter the barrier function of the stratum corneum by disrupting highly ordered lipid structure between the corneocytes, or improving in the partitioning of the drug or a coenhancer into the tissue.<sup>20)</sup> It is noteworthy that a discrepancy between the *in vitro* and *in vivo* results was found, indicating that only the weakened stratum corneum barrier function was not enough to explain the sharply increment of *in vivo* absorption of flurbiprofen. In this study, the permeation enhancement effect of chuanxiong oil seemed to follow another mechanism except for the weakened stratum corneum barrier function.

The GC-MS results (Table 4) showed six phthalides (91.15%) and eight terpenes (5.19%) made up of chuanxiong oil. Although a variety of terpenes such as limonene appeared to high transdermal enhancing ability at low concentrations,<sup>21)</sup> the permeation enhancement effect of terpenes identified from chuanxiong oil under this study, hitherto unreported, except *p*-cymene, terpinen-4-ol, terpinolene.<sup>22,23)</sup> Furthermore, because of its small amounts compared with phthalides in the oil, the influence of terpenes on flurbiprofen permeability might be negligible. Therefore, it is most probably that the effects of the oil on enhancing transdermal delivery of flurbiprofen are attributed to phthalides. While to date, there is no literature available for phthalides the skin enhancing effect.

It is noteworthy that the application sites of the rabbit were observed for any signs of skin irritation at the end of the *in vivo* experiment. There were no signs of skin irritancy potential after the application of the gel with chuanxiong oil at various test concentrations for 36 h. In addition, the studies performed in rabbits revealed no acute (48 h) skin irritancy potential for the flurbiprofen-free gel with chuanxiong oil at 15% concentration (data not shown). The results indicated that chuanxiong oil might possess low skin irritancy. However, the possible skin irritation on long-term usage of the gel with chuanxiong oil needs to be verified.

In conclusion, our results have demonstrated that chuanxiong oil could enhance the transdermal delivery of flurbiprofen significantly with greater efficiency *in vivo* absorption experiments than did *in vitro* permeation experiments. It will be very important and interesting to further investigate the mechanisms of the sharply increment of *in vivo* absorption of flurbiprofen by chuanxiong oil in rabbits.

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