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Transdermal permeation of geniposide in the herbal complex liniment in vivo and in vitro

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ABSTRACT

Zhongtong Caji, a kind of liniment, is a traditional Chinese medicinal formula that is widely used for clinical treatment of inflammation and sprains. In this study, the principal effective compound of this formula, geniposide, was used as a criterion to represent the transdermal permeability of the whole formula. A passive diffusion of Zhongtong Caji through the stratum corneum was discovered by an in vitro experiment. The dosage–content relationship detected in subcutaneous tissue after in vivo drug administration was further evidence of its permeation. Blood analysis after different dosages showed that the geniposide could be absorbed and accumulated by subcutaneous tissue within 1 h after drug administration, and it would be eliminated by blood circulation 1 h after drug treatment.

1. Introduction

Transdermal drug delivery (TDD) is a commonly used and convenient method of drug administration enabling physicians to provide controlled delivery of drugs to patients with local injury, such as a wrench or sprain. Zhongtong Caji is a kind of liniment for external transdermal medicine that is made from more than ten kinds of various herbal drugs, such as gardenia fruit, borneol, schefflera arboricola hayata, ampelopsis delavayana, menthol and so on. Gardenia fruit is the principal effective element in this formula. This formula has been widely used among the Yi population of southwest China for its anti-inflammatory activities and analgesic effects.

Gardenia fruit has been traditionally used as a Chinese medicine for centuries in China, as well as in other Asian countries. It has been included in traditional medical formulas for the treatment of inflammation, sprain, jaundice headache, edema, fever, hepatic disorders, and hypertension (Aburada et al., 1976; Chen et al., 2009; Miyasita, 1976; Tseng et al., 1995). Modern clinic pharmacological research has revealed that gardenia fruit has several other activities, such as anti-inflammatory properties, cytotoxic effects, as well as protective activity against oxidative damage (Tseng et al., 1995; Lee et al., 2009; Jung et al., 2009; Jagedeeswaran et al., 2000).

Geniposide (Fig. 1), one of the major iridoid glycosides and active compounds of gardenia fruit, was previously shown to inhibit 5-lipoxygenase (Nishizawa et al., 1988), ovalbumin-induced junction permeability, and inhibit recovery of transepithelial electrical resistance in guinea pig trachea, which shows its potential as an anti-asthma therapeutic drug (Liaw and Chao, 2001). Additionally, geniposide was shown to have activity against the tumor-promoting factor, 12-O-tetradecanoylphorbol-13-acetate (TPA), which activates protein kinase C (Lee et al., 1995), as well as display anti-angiogenic activity (Park et al., 2003; Koo et al., 2004). Recent reports have demonstrated that geniposide can prevent PC12 cells from oxidative damage via the MAP kinase pathway (Liu et al., 2007). It is also reported that geniposide has anti-inflammatory activities in carrageenan-induced rat paw edema and it can also inhibit vascular permeability induced by acetic acid in a dose-dependent manner (Koo et al., 2006).

To find the pharmacological performance of this formula, geniposide was used as a criterion to evaluate its transdermal penetration of this liniment. The permeability of geniposide of the liniment was first observed in vitro. The distribution and concentration of geniposide in subcutaneous tissue was determined in a dose-dependent manner in vivo. Finally, high performance liquid chromatography (HPLC) was used to observe the blood concentration of geniposide after external use of the liniment with different dosages and to explore the correlation between geniposide concentrations in both blood and subcutaneous tissue at different dosages and time.

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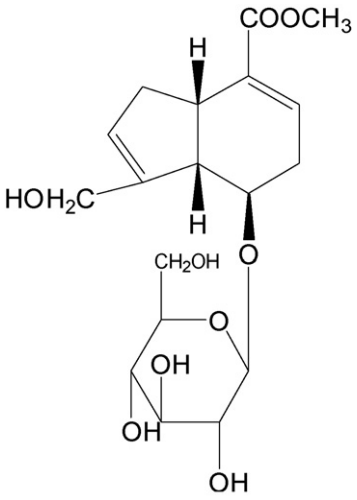

Fig. 1. Chemical structure of geniposide.

2. Materials and methods

2.1. Animals

ICR mice (male, weighing 25–28 g) were purchased from Vital River Experimental Animal Center, license number: SCXK 2005-0003. The experimental protocol was in accordance with the guidelines of China for animal care, which was conformed to the internationally accepted principles in the care and use of experimental animals. All experiments were performed in accordance with international standards on the ethical treatment of animals, and the minimum number of animals was used to minimize suffering. Mice were housed under climate-controlled conditions with a 12-h light/dark cycle and provided standard food and water ad libitum. To eliminate the effect of epidermal hairs on drug absorption, 25% (m/v) Na_2S was used to remove the epidermal hair at the dorsal region 2 days before experimentation.

2.2. Chemicals

Geniposide standard was obtained from the National Institute for the Control of Pharmaceutical and Biological Products, China (batch number: 110749-200613); methanol and acetonitrile were purchased from the Beijing Chemical Reagent Company; Zhongtong Caji (the liniment) was obtained from the Institute of Materia Medica of Yunnan Province (batch number: 090721), containing geniposide 0.064% (w/v) detected by HPLC assay; purified water was made from distilled water.

2.3. Drug administration and sampling

2.3.1. The permeation in vitro

Drug administration and sampling in vitro was modified based on previous references (Li et al., 2009; Sonavanea et al., 2008). Full-thickness mouse skin was excised from the dorsal region of the 25% Na_2S treated mouse. The subcutaneous fat, tissue and blood vessel were carefully removed. The treated skin was mounted on the donor compartment with the stratum corneum side facing downward into the receptor compartment. 15 mL of normal saline (pH 7.4) was used as the receptor medium. The donor compartment was filled with 5 mL of drug solution. The available diffusion area between compartments was 2.98 cm^2. The temperature of the whole system was kept at 37 °C. To stabilize the whole system, two compartments were connected to each other 15 min before drug solution was administered into the donor partition, and 1 mL of receptor medium was sampled before the moment of drug administration as control. 0.25, 0.5, 1, 2, 4 and 6 h were set as time points to collect samples from the receptor compartment from the moment of drug administration. Every sample was 1 mL of the receptor medium, and 1 mL of normal saline was added into the receptor compartment after each sampling. Each time point included triplicate analysis.

2.3.2. The permeation in vivo

Drug administration and sampling in vivo was modified based on a previous reference (Morris et al., 2009). The mice were divided into 4 groups; each group had 3 mice. 1 group was control, which was administered with normal saline. And the others were trial groups, which were liniment-administered 1, 2 and 3 times, respectively. The drug was administered on the epidermal hair-removed skin, and each administration had intervals of 0.5 h. The animal was killed and the subcutaneous tissue of the drug-administered region was collected immediately 0.5 h after the last drug administration. Samples were weighed, and stored at −20 °C to prepare for analysis. The experimental procedure was as displayed in Table 1.

2.4. Determination of geniposide in blood

The groups of mice and the method of drug administration were the same as above. For each group, the blood of each mouse was collected 30 min after the last administration. Blood plasma was isolated from the blood sample and stored at −20 °C for analysis.

2.5. HPLC analytical methods (Lv et al., 2008)

2.5.1. HPLC system

The HPLC system for geniposide analysis included a Waters 600 pump, a Waters 7725 sample processor, and a Waters 2487 double-UV–vis detector. A Zorbax SB-C18 reverse column (5 μm, 4.6 mm × 150 mm, Agilent) was used. The mobile phase was an acetonitrile:purified water (15:85) mixture at a flow rate of 1.0 mL/min. The detector was set to 238 nm, and the column temperature was controlled at 25 °C. The volume of each injection was 10 μL.

2.5.2. Preparation of samples for HPLC analysis

0.4 mL of plasma from each sample was added to 2 mL of methanol in glass test tubes to denature and deposit protein, and then the mixture was vortexed for 1 min and was subject to 30 min of ultrasonic treatment followed by a 10-min centrifuge at the speed of 1500 rpm. The supernatant was removed to a clear penicillin bottle. After the methanol evaporated at 37 °C, the residue was dissolved in 200 μL of methanol. The solution was moved to an eppendorf tube to centrifuge at 14,000 rpm for 20 min, after which then the supernatant was injected into the HPLC system to be analyzed.

About 300 mg of subcutaneous tissue from each sample was mixed with 2 mL of methanol and homogenized. The homogenate was subject to ultrasonic treatment in a 10 mL glass test tube for 30 min followed by a 15-min centrifuge at a speed of 1500 rpm. The supernatant was removed to a penicillin bottle. The previous step was repeated again with 2 mL of methanol, and the supernatant was removed to the same penicillin bottle. Methanol was evaporated at 37 °C, and the residue was dissolved in 200 μL of methanol. The supernatant, after a 20-min centrifuge at the speed of 14,000 rpm, could be injected into the HPLC system to be analyzed.

2.5.3. Presentations of testing criteria

To determine the quantity of drug release (μg/cm^2) (Wang et al., 2008), a linear equation was used to compute the released drug quantity of each sample. The data from the in vitro experiment was

Table 1
The experimental procedure for geniposide permeation in vivo.

Group	Time (h)			
	0 1st admin.	0.5 2nd admin./sampling	1 3rd admin./sampling	1.5 Sampling
1. Control	Saline	−/+ (1 mouse)	−/+ (1 mouse)	+ (1 mouse)
2. Drug	+	−/+ (3 mice)		
3. Drug	+	+/−	−/+ (3 mice)	
4. Drug	+	+/−	+/−	+ (3 mice)

"+": administrated or sampling; "−": not administrated or not sampling. "Admin." means administrated.

presented as μg/cm^2, and the data from the in vivo experiment was presented as μg/g. The transdermal rate of the drug was presented as μg/(cm^2 h) (Gao et al., 2009):

$$J = \frac{C_n - C_{n-1}}{t_n - t_{n-1}}$$

J is the transdermal rate of release of the drug at each time point; $C_n - C_{n-1}$ is the difference of concentration between two samples; $t_n - t_{n-1}$ is the time interval between samples (hours). The percentage of drug release (Si) (%) = (the quantity of drug in each sample/the gross quantity administered) × 100%. And the percentage of accumulated drug release (S) (%) was computed by the following equation:

$$S = \sum_{i=1}^{n} Si,$$

where S is the percentage released at different times of sampling and Si is the percentage released at the times of sampling.

2.5.4. Calibration curve

The geniposide standard was dissolved in methanol and diluted to give concentrations of 0.0176, 0.088, 0.44, 2.2, 11 and 55 μg/mL. The calibration curve was constructed from the peak heights of geniposide against the concentrations using un-weighted linear regression. The concentrations of geniposide in the samples were determined using the regression parameters obtained from the calibration curve. Calibration standards were included in every analytical batch of samples. 10 μL volumes of geniposide standards were quantified by HPLC and a standard curve was derived.

2.5.5. Limit of detection and quantification

The limit of detection (LOD) was determined as the lowest concentration that could be detected with acceptable accuracy and precision, which was achieved from the plot three times of the noise level. The limit of quantification (LOQ) in plasma was defined as the lowest concentration on the calibration curve for which the assay precision (coefficient of variation, CV) was lower than 10% and was 10 times of the LOD.

2.5.6. Recovery

The recovery of geniposide from the subcutaneous tissue was evaluated using two different concentrations (11 and 55 μg/mL), and the recovery of geniposide from the blood sample was evaluated using 4 different concentrations (0.44, 2.2, 11 and 55 μg/mL); all concentrations were within the linear range of the curve. After the samples were processed according to the method mentioned above, the resulting peak heights were compared to the geniposide standard carried in the mobile phase to provide the recovery values.

2.5.7. Precision and accuracy

The precision of a quantitative method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It was measured by repeatedly injecting a ready-made sample pool and expressed as the relative standard deviation of the results. Analyses with three different concentrations (0.088, 2.2 and 55 μg/mL) of geniposide were performed. To determine the intra-day variance, the assays were carried out on the same samples at different times during one day. Inter-day variance was determined by assaying the spikes samples over 3 consecutive days at the same time each day. Coefficients of variation (CV) were calculated from these values.

2.6. Statistical analysis

All values were expressed as mean ± SD. Data were statistically analyzed by one-way ANOVA followed by Dunnet's test. $P < 0.05$ was accepted as statistical significance.

3. Results

3.1. Calibration curve

The calibration curve was linear across the concentration range of 0.0176, 0.088, 0.44, 2.2, 11 and 55 μg/mL. The correlation coefficient (R^2) was 0.999 with a good linear relationship between peak areas and concentrations of geniposide. The equation of the calibration curve was as follows: $y = 9 \times 10^{-5}x - 0.119$, where y is the concentration of geniposide, and x is the area of the peak. The retention time of geniposide in the system we used was 4.2 min (Fig. 2).

3.2. Recovery and precision

The loss of geniposide due to the extraction process was determined by comparing the data obtained by the direct injection of standard dissolved in mobile phase to those obtained after the whole extraction procedure. For the isolation of geniposide from the subcutaneous tissue, methanol was employed because geniposide was easily dissolved in it. The average recovery of 11 and 55 μg/mL from subcutaneous tissue was 74.55%, while the average recovery of 0.44, 2.2, 11 and 55 μg/mL from blood plasma was 69.85%.

The intra-day precision, inter-day precision and the accuracy when using three different concentrations were evaluated. The intra-day imprecision of 0.088, 2.2 and 55 μg/mL were 18.3%, 3.0% and 2.3%, respectively, while the inter-day imprecision of the three concentrations were 16.1%, 7.6% and 6.3%, respectively. The accuracy of the three dosages were 93.6%, 98% and 97.2%, respectively, indicating that the method was quite precise.

3.3. Penetration in vitro

During the in vitro experiment, an accumulation of geniposide in the receptor compartment was observed (Fig. 3). The correlation between the content of geniposide in the receptor compartment

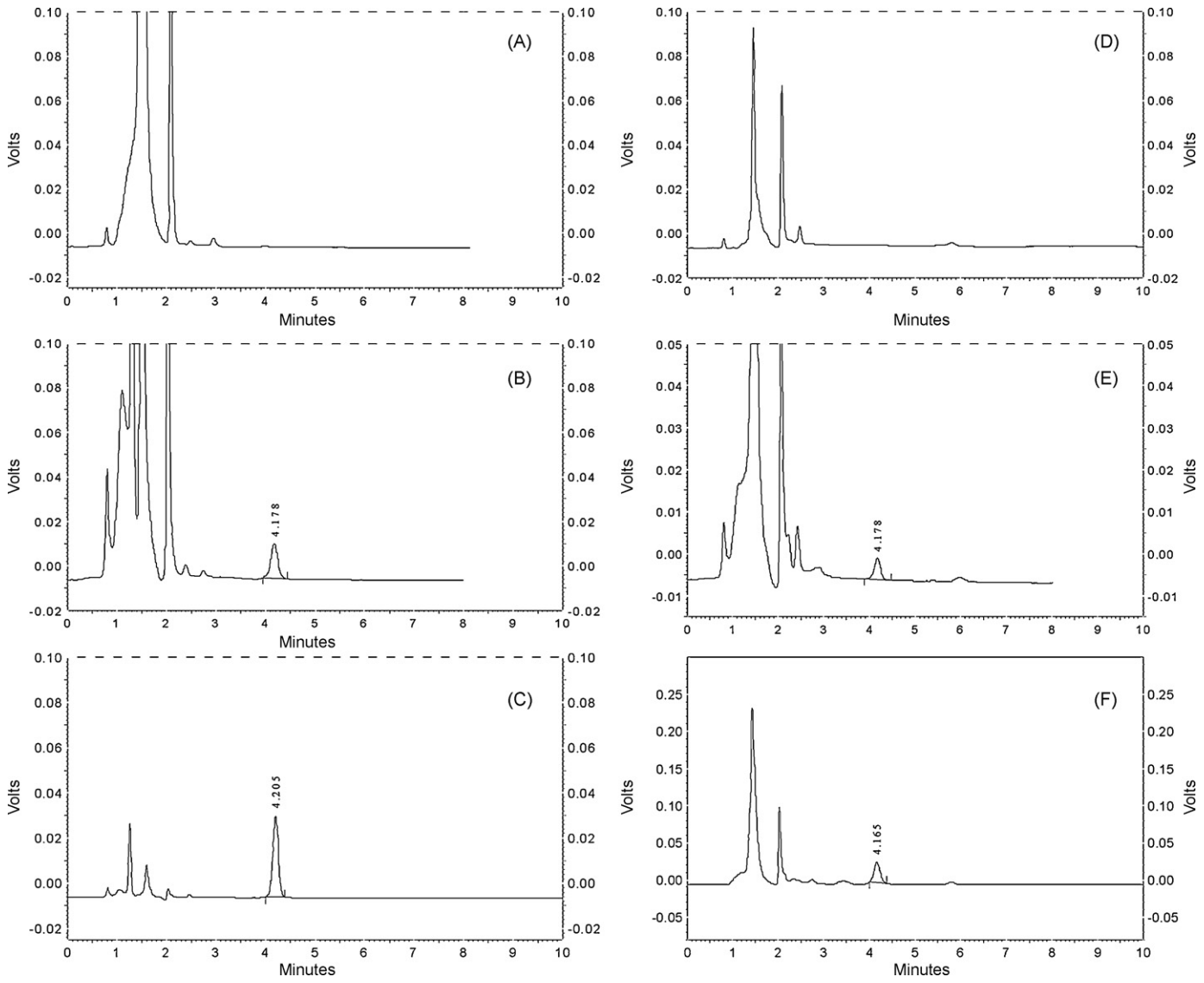

Fig. 2. HPLC chromatogram of geniposide. (A) Blank subcutaneous tissue; (B) geniposide detected in subcutaneous tissue 30 min after drug administration; (C) geniposide control; (D) blank serum; (E) geniposide detected in blood serum 30 min after drug administration; (F) geniposide detected in receptor medium 30 min after drug administration.

and time could be represented as $y = 0.567x + 2.9654$, $R^2 = 0.9748$, where x is the minutes used and y is the content of geniposide that passed through every square centimeter of the stratum corneum (Fig. 4). There was a sharp increase in transdermal rate in the first 60 min and the rate was maintained at a relatively high level (40 μg/cm^2 h) during the subsequent 180 min, when the transdermal percentage reached about 25%; then it slowly decreased during the last 120 min (Fig. 5).

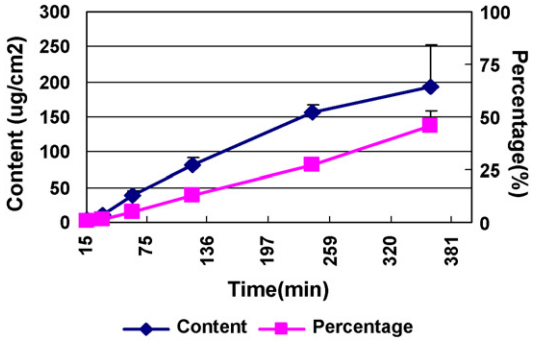

Fig. 3. Content and percentage of geniposide of the liniment in administrated drug pass though the stratum corneum in vitro ($\bar{x}$, $n = 3$).

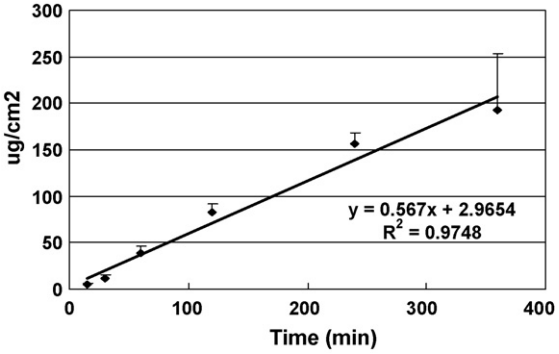

Fig. 4. The curve of transdermal content of geniposide–time. Linear equation: $y = 0.567x + 2.9654$, $R^2 = 0.9748$, y is the content of geniposide passed though every square centimeter of stratum corneum, x is the time passed by ($\bar{x}$, $n = 3$).

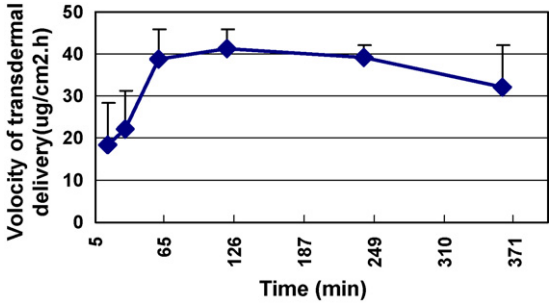

Fig. 5. The velocity rate of penetration of geniposide after using the liniment in vitro (μg/cm^2 h) ($\bar{x}$, $n = 3$).

3.4. Penetration in vivo

In order to understand the meaning of the results from the in vitro system, in vivo research was conducted to investigate the dynamic pharmacological action of geniposide of the liniment after its transdermal process. First, we analyzed the content of geniposide in the subcutaneous tissue after treatment with different dosages to support the results from in vitro experiments. In addition to the existence of geniposide in the subcutaneous tissue after drug administration, a correlation, though not very strict, was found between the times of administration and the content of geniposide in subcutaneous tissue. Subsequent blood analysis demonstrated that the content of geniposide in blood increased with the times of administration (Fig. 6). Furthermore, it was found that 30 min after the third administration, the content of geniposide in subcutaneous tissue decreased significantly while the content of geniposide in blood increased (Fig. 7); the transdermal rate of geniposide during the third administration was significantly lower than that in the second administration (Fig. 8). The ratio of the content of geniposide in subcutaneous tissue compared to the content in the administered drug was much lower than the ratio for the second administration (Fig. 9).

4. Discussion and conclusion

According to the results from in vitro experiments in this study, the geniposide could permeate the stratum corneum within 30 min after drug administration, and the content of its penetration increased with time. The percentage of geniposide permeation 6 h after administration was about 45%. According to the observation of the dynamic alternation, the rate of penetration increased sharply within 1 h after drug administration, and it remained at a relatively high level for the subsequent 180 min, and then decreased slightly when the content of geniposide in the receptor compartment reached a certain level, where 25% of geniposide in the administered drug permeated into the receptor medium. The phenomena above indicates that the transport of geniposide through the stratum corneum was via a passive route and that the rate of permeation of this formula after administration would reach its highest limit within 1 h.

For these reasons, the formula has been used to cure local inflammation. The existence of the effective compound in local

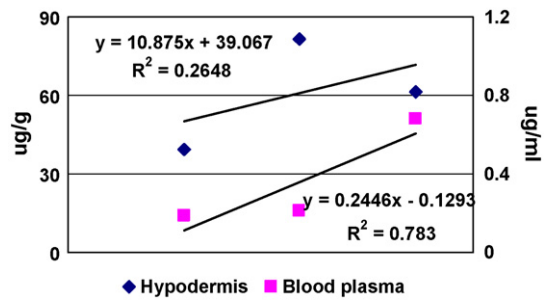

Fig. 6. The content of geniposide in subcutaneous tissue and blood after different times of the liniment administration. The equation of content of geniposide in subcutaneous tissue is $y = 10.875x + 39.067$, $R^2 = 0.2648$. The equation of content of geniposide in blood serum is $y = 0.2446x - 0.1293$, $R^2 = 0.783$. x is the time of administration, y is the content of geniposide detected ($\bar{x}$, $n = 3$).

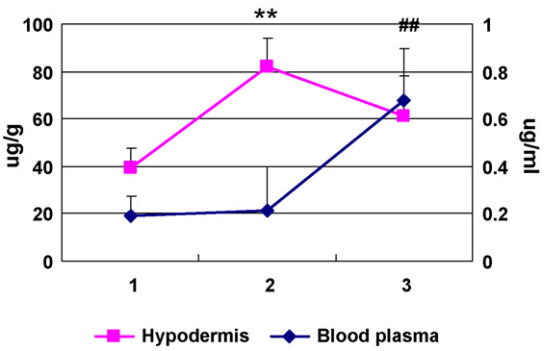

Fig. 7. The variation of content of geniposide in subcutaneous tissue and blood after different administrations. 1, 2 and 3 in this figure means the time of administration, and the interval between two administrations was 0.5 h. Compared with the data of the first administration of hypodermis, $^{**}P < 0.01$. Compared with the data of the first and second administration of blood plasma, $^{\#\#}P < 0.01$ ($\bar{x}$, $n = 3$).

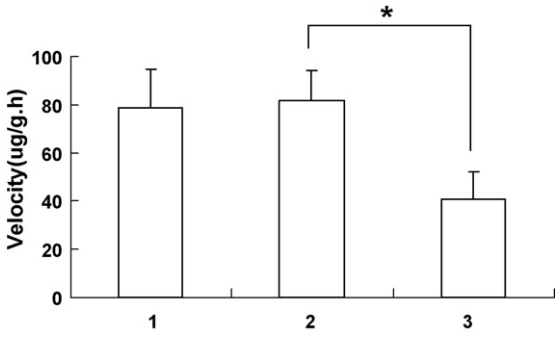

Fig. 8. The velocity rate of penetration of geinposide after different times of the liniment administration. 1, 2 and 3 in this figure means the time of administration, and the interval between two administrations was 0.5 h. The transdermal rate of geniposide during the third administration was significantly lower than that of the second time, $^{*}P < 0.05$ ($\bar{x}$, $n = 3$).

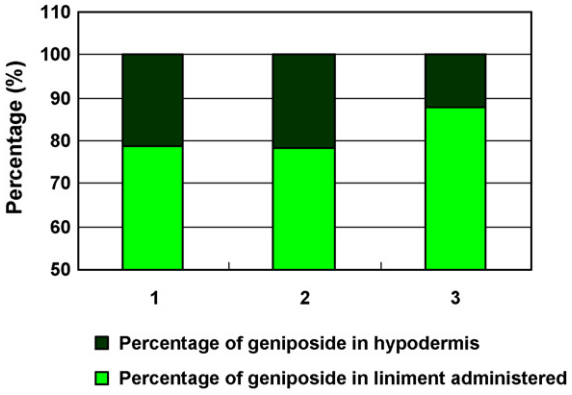

Fig. 9. Percentage of geniposide permeated into subcutaneous tissue to the content of geniposide administrated the liniment after different times of administration. 1, 2 and 3 in this figure means the time of administration, and the interval between two administrations was 0.5 h ($\bar{x}$, $n = 3$).

subcutaneous tissue after administration was considered to be a critical criterion in its pharmacological function. The existence of geniposide with content of 26.61 ± 5.29% was detected 30 min after one drug administration in mice. Interestingly, this percentage decreased, as the time of treatment increased, to 13.76 ± 3.86% 30 min after the third administration (90 min after the first administration). However, blood analysis after dermis administration showed that the content of geniposide in blood increased with time since the first drug treatment. The concentration of geniposide was at the same level in the first two times of administration (30 and 60 min after the first administration, respectively), and it increased significantly 30 min after the third drug administration (90 min after the first administration). This indicates that the transdermal geniposide in subcutaneous tissue would be taken to the whole body by blood circulation, which is also called local clearance (Mills and Cross, 2006).

Previous research in vitro reported that a single geniposide compound could hardly penetrate the stratum corneum within 12 h after administration (Wei et al., 2008). Combining the dynamic alternation in content of geniposide in subcutaneous tissue, a conclusion was made that the absorption of geniposide of the liniment after its administration was prompted by other ingredients in the liniment and the complex process needed cooperation of more than one physiological system. After its penetration, absorption and distribution of geniposide by some unknown, complicated mechanism was conducted in the live dermal system. And the micro-blood circulation system within subcutaneous tissue would conduct its transport function to transport materials absorbed by live tissue to other parts of the body and clear it locally.

The transdermal permeability of geniposide in the liniment, Zhongtong Caji, was demonstrated by experiments in vitro and in vivo as stated above. It is believed that the effective compound of this formula, geniposide, could permeate into the body quickly once it is administered. The accumulation of effective factors by subcutaneous tissue was conducted within 1 h after drug administration. The micro-blood circulation system would transport it to other parts of the body and clear the drug locally 1 h after administration. Considering that this liniment formula is used to treat local injury-induced inflammation, it is, therefore, preferably used for ankle sprain and wrench. Based on the alteration of genipiside in the subcutemias and the blood, a recommendation for the clinical usage of this liniment is that only repeated drug administration within 1 h would be helpful for the accumulation of the effective compound at the treated region.

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References

Aburada, M., Sasaki, H., Harada, M., 1976. Pharmacological studies of gardenia fruits. II. Contribution of the constituent crude drugs to choleretic activity of 'Inchinkoto' in rats. Yakugaku Zasshi 96, 147–153.

Chen, Q.C., Zhang, W.Y., Youn, Y.J., Kim, H.J., Lee, I.S., Jung, H.J., Na, M.K., Min, B.S., Bae, K.H., 2009. Iridoid glycosides from Gardeniae Fructus for treatment of ankle sprain. Phytochemistry 70, 779–784.

Gao, C.H., Guo, W.Y., Zhang, Y.Q., Zhao, Y., 2009. Promoting effect of Clove bud oil and Galangal oil on percutaneous absorption of ferulic acid. Chin. J. New Drugs 18, 844–846.

Jagedeeswaran, R., Thirunavukkarasu, C., Gunasekaran, P., Ramamurty, N., Sakthisekaran, D., 2000. In vitro studies on the selective cytotoxic effect of crocetin and quercetin. Fitoterapia 71, 395–399.

Jung, W.S., Chae, Y.H., Kim, D.Y., Seo, S.W., Park, H.J., Bae, G.S., Kim, T.H., Oh, H.J., Yun, K.J., Park, R.K., Kim, J.S., Kim, E.C., Hwang, S.Y., Park, S.J., Song, H.J., 2009. Gardenia jasminoides protects against cerulein-induced acute pancreatitis. World J. Gastroenterol. 40, 6188–6194.

Koo, H.J., Lee, S., Shin, K.-H., Kim, B.C., Lim, C.J., Park, E.H., 2004. Geniposide, an anti-angiogenic compound from the fruits of Gardenia jasminoides. Planta Med. 70, 467–469.

Koo, H.J., Lim, K.H., Jung, H.J., Park, E.H., 2006. Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. J. Ethnopharmacol. 103, 496–500.

Lee, J.H., Lee, D.U., Jeong, C.S., 2009. Gardenia jasminoides Ellis ethanol extract and its constituents reduce the risks of gastritis and reverse gastric lesions in rats. Food Chem. Toxicol. 47, 1127–1131.

Lee, M., Hsu, J., Wang, C., 1995. Inhibition of 12-O-tetradecanoyl-1-phorbol-13-acetate-caused tumor promotion in benzopyrene-initiated CD-1 mouse skin by geniposide. Anticancer Res. 15, 411–416.

Li, G.H., Badkar, A., Nema, S., Kolli, C.S., Banga, A.K., 2009. In vitro transdermal delivery of therapeutic antibodies using maltose microneedles. Int. J. Pharm. 368, 109–115.

Liaw, J., Chao, Y.C., 2001. Effect of in vitro and in vivo aerosolized treatment with geniposide on tracheal permeability in ovalbumin-induced guinea pigs. Eur. J. Pharmacol. 433, 115–121.

Liu, J.H., Yin, F., Zheng, X.X., Jing, J.J., Hu, Y.H., 2007. Geniposide, a novel agonist for GLP-1 receptor, prevents PC12 cells from oxidative damage via MAP kinase pathway. Neurochem. Int. 51, 361–369.

Lv, H., Sun, H., Sun, W., Liu, L., Wang, P., Wang, X., Cao, H., 2008. Pharmacokinetic studies of a Chinese triple herbal drug formula. Phytomedicine 15, 993–1001.

Mills, P.C., Cross, S.E., 2006. Transdermal drug delivery: basic principles for the veterinarian. Vet. J. 172, 218–233.

Miyasita, S., 1976. A historical study of Chinese drugs for the treatment of jaundice. Am. J. Chin. Med. 4, 239–243.

Morris, A.P., Brain, K.R., Heard, C.M., 2009. Skin permeation and ex vivo skin metabolism of O-acyl haloperidol ester prodrugs. Int. J. Pharm. 367, 44–50.

Nishizawa, M., Izuhara, K., Kaneko, K., Fujimoto, Y., 1988. 5-Lipoxgenase inhibitors isolated from Gardeniae fructus. Chem. Pharm. Bull. 36, 87–95.

Park, E.-H., Joo, M.-H., Kim, S.-H., Lim, C.-J., 2003. Antiangiogenic activity of Gardenia jasminoides fruit. Phytother. Res. 17, 961–962.

Sonavanea, G., Tomoda, K., Sanoa, A., Ohshima, H., Terada, H., Makino, K., 2008. In vitro permeation of gold nanoparticles through rat skin and rat intestine: effect of particle size. Colloids Surf. B Biointerfaces 65, 1–10.

Tseng, T.H., Chu, C.Y., Huang, J.M., Shiow, S.J., Wang, C.J., 1995. Crocetin protects against damage in rat primary hepatocytes. Cancer Lett. 97, 61–67.

Wang, X.H., Li, L., Shen, Q.F., Zhang, L., Qi, G., 2008. Effects of different dosage form of paracetamol on its percutaneous absorption in vitro. China Pharm. 19, 2189–2191.

Wei, F.L., Wu, X., Cui, G., 2008. Transdermal study of geniposide. Herald Med. 27, 1380–1382.