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Percutaneous absorption effects of Chinese herbal medicine

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This study was carried out to evaluate the effects of Chinese herbal transdermal ointment on mastitis in the dairy cow and to select the best efficient formula for treating the disorder. With chlorogenic acid as a marker, using high performance liquid chromatography (HPLC), the contents of chlorogenic acid in permeate liquid were determined. Different skin permeation enhancing effects of Chinese herbal medicinal formulations and various herbal transdermal promoters were evaluated in the form of ointment. The results indicated that ultrafined powder (UFP) of Chinese herbal medicine (CHM) directly adding to medication and 4.5% borneol plus 4.5% Azone as skin permeation enhancers showed the best skin-penetrating effect.

Key words: Chinese herbal medicine, mastitis, high performance liquid chromatography (HPLC), transdermal promoter.

INTRODUCTION

There have been more and more reports on efficacy of transdermal patch in treating both human and animal diseases (Plosker, 2011; Zhang et al., 2011; Dou et al., 2011). Use of Chinese Herbal Medicine (CHM) as transdermal agents is a new combination of Traditional Chinese Medicine (TCM) with modern pharmaceutical technology (He et al., 2009; Qian et al., 2011). The present study was conducted to evaluate the skin permeation effects of different dosage forms of CHMs and different transdermal enhancers by measuring the chlorogenic acid content as a marker, using high performance liquid chromatography (HPLC), and to establish the kinetic curves of the chlorogenic acid percutaneous absorption. Evaluation of the transdermal effects of CHMs as skin permeation enhancers will potentially provide safe and effective drugs for veterinary clinical usage.

MATERIALS AND METHODS

Chemicals and reagents

The herbs honeysuckle, Chinese angelica, dandelion, safflower and borneol were bought from Qizhou Herbal Pharmaceuticals (Baoding city, China). Stearic acid ester, vaseline, liquid paraffin and nitrogen ketones were obtained from Huaxin Instrument Co. Ltd (Beijing, China).

Equipments

Agilent 1200 liquid chromatography was manufactured by Hewlett-Packard Company (USA). TT-6 (B) transdermal absorption meter was from Zhengtong Technology Co.Ltd (Tianjin, China).

Preparation of transdermal ointment of CHM

The herbal formula consists of Chinese angelica, Motherwort, Caulis Lonicerae, dandelion, Bunge Corydalis Herb, Mongolian snakegourd fruit, safflower, Submature bitter orange, Great burdock fruit, radix scutellariae, Szechwan Lovage Rhizome, immature tangerine fruit, Akabia stem, Cowherb seed, Dan-shen root, borneol, dried toads venom, rice paper plant pith, Polygonum
The herbals were produced by Dikma, Beijing, China. Dermal enhancers were added to the matrix to refine; after 18°C, as well as color, 0.05). Flow rate, 1.0 ml °C incubator for 6°C. Glycerin and distilled water were heated to 39°C and kept in 4°C. The abdominal skin of mice was employed with the subcutaneous fat tissues being removed, soaked in saline and kept in 4°C refrigerator for three months in a constant temperature incubator of 40 ± 2°C. The pretreated skin was unfolded on the Franz diffusion device and then heat test. Cold and heat test

The transdermal CHM agents were placed in test tube with plug, kept in a 55°C incubator for 6 h and -15°C refrigerator for 24 h to observe whether the layered, demulsification, mildew, as well as color and uniformity of change occurs (Wu et al., 2008).

Accelerating test

The transdermal CHM agents were placed in test tube with plug, kept for three months in a constant temperature incubator of temperature (40 ± 2°C) and relative humidity of 75% to observe whether the stratification, demulsification, mildew, as well as color, uniformity of change occurs(Wu et al., 2008).

In vitro diffusion

Skin treatment

The abdominal skin of mice was employed with the subcutaneous fat tissues being removed, soaked in saline and kept in 4°C refrigerator for further use.

Transdermal test

The pretreated skin was unfolded on the Franz diffusion device and fixed. Two gram (2 g) of the testing drugs was put in the delivery room using 6 ml normal saline as the receiving liquid. The whole device was kept in 39°C water bath with thermostatic control and magnetic stirring. An appropriate receiving liquid as the sample and measurement of the content of chlorogenic acid according to the previously mentioned chromatographic condition was taken (Ge et al., 2006).

Calculation of the amount of cumulative infiltration

\[ M = C_nV + \sum_{i=1}^{n-1} C_i V_i \]

M, Total amount of transdermal penetration; \(C_n\) the sampling points of the concentration of sample solution; \(V\), receive pool size; \(C_i\), the sampling point the concentration of sample solution; \(V_i\), sampling volume (Ge et al., 2006).

Method of HPLC

Chromatographic conditions

Chromatogram columns used is Diamonsil C_18 (4.6 mm×250 mm×5.0 μm) produced by Dikma, Beijing, China. Mobile phase is acetonitrile - water - phosphoric acid (10:90:0.05). Flow rate, 1.0 ml / min; Injection volume, 20 μl; column temperature, 25°C; detection wave, 327 nm.

Establishment of standard curve

5 mg standardized sample of chlorogenic acid was accurately weighed and dissolved in 50% methanol to make 50 ml standard liquid of 100 μg/mL. Precisely, 0.025, 0.1, 0.4, 1.6 and 6.4 ml of the standard liquid was taken in 50 ml volumetric flask, the samples was diluted to desired volume with methanol and shaken thoroughly. Each time, 20 μl of sample liquid was injected. Standard curve and regression equation were calculated using chlorogenic acid standard peak area (A) and drug concentration (C) (Chen and Zhang, 2003).

Precision test

20 μl chlorogenic acid standard liquid was accurately taken. After continuous injection of five times, chlorogenic acid peak area and relative standard deviation (RSD) were calculated.

Influences of different preparations of herbals on the contents of CHM

Preparation of test liquid

One gram (1 g) of sample was put into a conical bottle with plug. Precisely, 25 ml of 50% methanol was added. Extraction was done ultrasonically for 30 min after mesa and weighing. When it becomes cool, some methanol was added until the original weight.. The obtained liquid was filtrated using 0.45 μm microsporous membrane filtration (Huang et al., 2009).

Determination of samples

Respectively drawing reference substance solution and sample solution, 20 μl was injected into the solution and determined according to the chromatographic conditions, after which the chromatography was recorded and the results were calculated.
The transdermal CHM agent stability test results showed there was no layered, emulsion-breaking, mildew, as well as changes in color or uniformity in the centrifuge test, cold and heat test and accelerated test. The transdermal CHM agent has a good stability, the stability and satisfactory. Three (3) months conservation at a temperature 37 to 40°C and relative humidity above 75% indicates the product stability, and can be tentatively valid for 2 years. Therefore, the valid period of the drug was set for 2 years.

Preparation of standard curve

The chlorogenic acid standard was prepared into a series of concentrations of liquid. Measurement was carried out according to the mentioned chromatographic conditions. Of abscissa regression, the regression equation was obtained using peak area value as the vertical axis and chlorogenic acid content as the horizontal axis: \( y = 64.944x - 5.3596 \) (\( R^2 = 0.9992 \)), indicating that range of linearity was good at the concentration range of 0.05 to 12.8 \( \mu \text{g/ml} \). (Figure 1).

Precision test

20 \( \mu \text{l} \) chlorogenic acid standard liquid of 0.2 \( \mu \text{g/ml} \) was withdrawn accurately, continuous nebulization five times, the peak area of chlorogenic acid RSD was 0.98%, indicating that sampling accuracy and precision of instrument are both good.

**Results**

Quality evaluation of transdermal CHM agent

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Effects of various preparations on the contents of transdermal CHM

The results showed that the content of chlorogenic acid in group A was 174 μg/g, the content of chlorogenic acid in group B being 88 μg/g and the content of chlorogenic acid in group C being 62.1 μg/ g, indicate that group A was the best (Figure 2).

Effects of various transdermal promoters on transdermal rates of CHM

The results showed that the amount of chlorogenic acid in the preparation containing 9% of borneol as transdermal promotors was 5.94μg, the amount of chlorogenic acid in the preparation containing 4.5% borneol + 4.5% Azone was 6.6 μg. The amount of chlorogenic acid in the preparation containing 9% Azone was 4.02 μg. The preparation containing 4.5% borneol + 4.5% Azone showed the best result as transdermal promotors (Figure 3).

Kinetic curve of transdermal rates of the best transdermal preparation

The amount of chlorogenic acid was increasing with time increase with the total accumulation of 2.726 μg at 7 h post starting point (Table 1). The transdermal rate was rising from the first hour through the fourth hour. The transdermal rate became constant at the fifth hour, showed characteristics similar to zero order kinetics. The result showed that the transdermal agent had a certain penetration effect in vitro and was able to maintain a certain delivery rate (Figure 4)

DISCUSSION

Research on Chinese medicinal herbals as transdermal agents

Since the transdermal CHM ointment consisted of many herbals, it is not easy to detect the transdermal rates of all the herbal components. Therefore, it is quite common to use just one or more biologically active ingredient in the compound preparation as the indicators to evaluate the transdermal effect of a compound preparation (Ning et al., 2007). Honeysuckle is the main drug, while which chlorogenic acid is the main component. By measuring the amount of chlorogenic acid in vitro, the transdermal effects of the different CHM formulations and different various transdermal promotors can be determined and of symbolic and practical significance (Zhang and Gu, 2005).

There are two methods to test the transdermal effects, that is, in vitro and in vivo. It is difficult to do the in vivo transdermal studies because of the complexity of transdermal CHM contents. That is why the present study was carried out in vitro. In in vitro studies, the common used animal skins are from mice, rats, guinea pigs, rabbits and so on. Different animal skins had different permeability. According to the characteristics of the breast skin of the cow, mouse skin was often used as skin models for a moderate permeability in vitro diffusion (Xian et al., 2006).

Advantages of transdermal ointment of ultrafinened herbal powder

The ultrafining technology has advantages when applied in Chinese herbal preparation. The advantages include increased herbal absorption rate and bioactivity, hence, increased drug effects and biological utility compared with the conventional herbal preparation procedure. Chinese herbals are quite often used in formula which contains more than one herb. That is why the Chinese herbal formula contains so complicated components. In some studies, morphocytology of UFP of Cortex Cinnamomi was compared with its common powder. The physical parameters of the UFP particles and the transdermal amount of cinnamaldehyde through rat skin in vitro were determined with an improved Franz diffuse cell. The diameter of the UFP particles detected was small (D_50 =12.72 μm) and a typical microscopic characters of Cortex Cinnamomi powder was not observed. The Cortex Cinnamomi UFP has good transdermal effects. There existed the linear relationship between transdermal amount of cinnamaldehyde and acting time. The highest transdermal rate (0.25 mg/cm²/h) was within the first hour at the beginning, and then the rate became lower and sustained. The accumulating transdermal amount of the UFP was 1.0 mg/cm totally in 8 h (Zhao et al., 2002).

HPLC was applied to determine the concentrations of plamsa baicalin in two groups of rabbits after administristering the ultramicroculverised powder and the ordinary powder of HLJDS (in water suspension) by gastrogavage, respectively. Pharmacokinetical characteristics of both the ultramicroculverised powder and ordinary powder of HLJDS were compared. The results showed that the best pharmacokinetic model of baicalin in either group was two compartment open model. As compared to the baicalin from ordinary powder, the relative bioavailability of baicalin from ultramicroculverised powder was increased by 30.26% (Ma et al., 2007).

Selection of transdermal promotors

Ordinarily, combination of two or more transdermal promotors works synergistically in promoting drug
Figure 2. HPLC patterns of transdermal CHM agent with different preparation forms of CHM.
Figure 3. HPLC patterns of different transdermal CHM agent with different transdermal promoters enhancers.

A. Chlorogenic acid standard

B. Ointment containing 9% borneol.

C. Ointment containing 4.5% borneol + 4.5% Azone

D. Ointment containing 4.5% Azone

E. Control group
penetration through the skin with much better results than one promoter alone. Thereby, combined use of promoters will reduce the amount used of each promoter with lower toxicity. Nitrogen ketones and borneol are the common used penetration enhancers, and we have two kinds of penetration enhancing effects of promoters studied. From this experiment, we can see that borneol and nitrogen mixed ketones showed the best penetration or transdermal promoting effects, indicating that borneol and Azone in the transdermal play a synergistic role in helping the drug penetrate the skin. Borneol alone and Azone alone, however, showed less transdermal effects. The role of borneol was stronger than Azone, suggesting a leading role of borneol and Azone as a supporting partner. Complicated ingredients of Chinese herbal compound, borneol partial fat-soluble and water-soluble Azone, the two combined is conducive to the overall absorption of active ingredients (Pan et al., 2006; Lin et al., 2007).

The dynamic characteristics of transdermal CHM

The results showed that CHM active ingredients in the transdermal agent could effectively pass through the skin, which can more accurately reflect the drugs absorption process in the practical application of the transdermal, providing the basis for clinical application. The kinetic curves obtained in this study are similar to zero order kinetics which indicated that transdermal agent had maintained constant blood concentration or pharmacological effect and extended the duration time. These results were similar to transdermal results of other CHM ingredients in vitro (Zhang et al., 2008).

Conclusions

The results of the present study indicate that UFP of CHM directly adding to medication with 4.5% borneol plus 4.5% Azone as skin permeation enhancers gives the best skin-penetrating effect.

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REFERENCES


