Modernization of Traditional Medicine

In vitro evaluation of transdermal permeation effects of Fu's cupping therapy via six diffusion kinetics models

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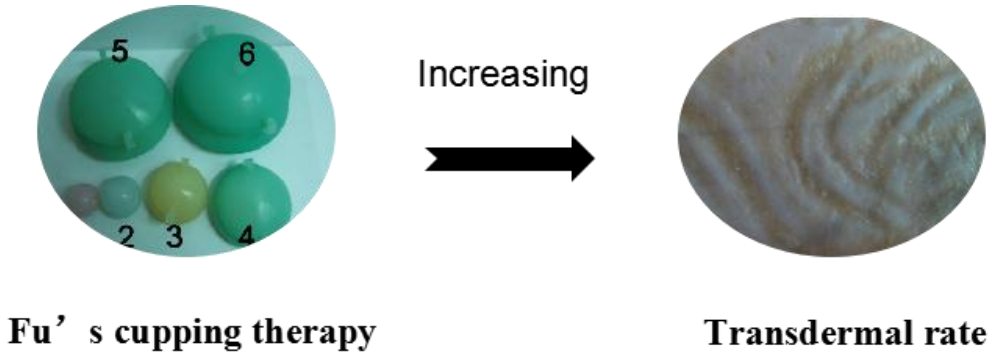
Highlights

The combination of cupping therapy and transdermal drug delivery can improve the transdermal rate of the drug.

Editor's Summary

Traditional cuping therapy helps to the skin absorption. As a new physical transdermal drug delivery technology, Fu's cupping therapy combines traditional cuping therapy and drug therapy, which is worthy of further research.



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Abstract

In this study, six kinetics models of indomethacin hydrophilic gel patch transdermal *in vitro* release was established, including zero-level, first-order, Higuchi-level, Ritger-Peppas, Weibull and Hixcon-Crowell dynamic equations. The chemical permeation enhancers, including 3% and 5% Azone, and iontophoresis were used as the control. Transdermal diffusion tests were performed *in vitro* and indomethacin was quantified by high performance liquid chromatography system. The transdermal parameter of the Higuchi and Weibull dynamic equations, indicated that Fu's cupping therapy (FCT) could significantly improve Higuchi and Weibull kinetic parameters in vitro transdermal, increased transdermal rate and permeability coefficient, reduced lagging time. Additionally, statistical analysis speculated the skin barrier function could be restored after 46 h treatment. Hence, as a new physical transdermal drug delivery technology, transdermal permeation effects produced by FCT are obvious, which has the characteristics of traditional Chinese medicine and has important clinical application value.

Keywords: Indomethacin, Diffusion kinetics models, Fu's cupping therapy, Transdermal permeation technology, Chemical penetration enhancers, Traditional Chinese medicine

摘要

本研究建立吲哚美辛亲水凝胶贴剂的体外经皮渗透的 6 种动力学模型：零级、一级、Higuchi 级、Ritger-Peppas 级、Weibull 级与 Hixcon-Crowell 级动力方程 Q1/3 = kt + b，评价付罐疗法（FCT）体外经皮促渗作用与特点。3%氮酮和 5%氮酮化学促渗剂与离子导入为参比，用 HPLC-工作曲线对吲哚美辛（IM）定量，进行体外透皮扩散，结果进行回归建模。结果表明：FCT 促渗处理组能明显提高吲哚美辛的透皮效果，改善体外透皮的 Higuchi 级与 Weibull 级动力学参数，提高透皮速率与渗透系数，减低时滞。FCT 作为新的物理经皮给药促渗新技术，效果明显，具有中医特色和重要的临床应用价值。

关键词：吲哚美辛；扩散动力学模型；付罐经皮物理促渗技术；化学促渗剂；中医药

Abbreviations: TCM, Traditional Chinese medicine; FCT, Fu's cupping therapy; CPEs, Chemical permeation enhancers; IM, Indomethacin; ER, Enhancing rate; HPLC, High performance liquid chromatography system; PBS, Phosphate buffered saline; RSD, Relative standard deviation; FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group.

Competing interests: The authors report no conflicts of interest in this work.

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Background

The earliest record of cupping therapy appeared in the *Wushier Bing Fang* published in Xihan Dynasty of China (202 B.C. - 8 A.D.). During the Tang Dynasty of China, there was a detailed record of the use of "Corner canister" (made of horns) for disease treatment. Cupping therapy is administration of the drug after cupping, because the pores of the skin open as well as the magnetic field effect is conducive to the transdermal absorption of the drug, so that the drug directly reaches the affected part. The pain and the side effect caused by oral or intravenous or intramuscular administration to the liver and kidney can be avoided by cupping therapy. Besides, it has the ideal effect with a small amount of medicine, and has the effect of acupoint injection, and has no pain in the skin [1]. Therefore, the canister is the original form of percutaneous administration of cupping, and transdermal administration after cupping is an important clinical application of traditional Chinese medicine (TCM). The mechanical and physical stimulation effect on the human skin by cupping therapy is mainly the skin epidermal layer fissure, and the percutaneous absorption rate of the drugs and the clinical curative effect can be improved.

In recent years, it has been found that the skin treated by cupping exhibited significant effects on percutaneous penetration of tetramethylpyrazine, verapamil, and tetrahydropalmatine [2 - 5] and can significantly improve the effects of these drugs against myocardial ischemia and local inflammation. With the development of cupping therapy and the discovery of new methods, as well as the in-depth study of the mechanism of action, cupping has a good therapeutic effect for several diseases, which is considered superior to other conventional Chinese and Western medicines. According to reports [3 - 8], cupping has been widely used in the treatment of the following diseases: pertussis, exogenous cough, asthma, cold, pneumothorax, stroke sequelae, mouth oblique, snoring, hypertension, rheumatoid arthritis, diarrhea, hemorrhoids and so on.

Fu's cupping therapy (FCT) belongs to a new type of cupping method. It is made of soft silicon and combined with a variety of cupping methods. More different effects can be achieved through the regulation of the microenvironment in the cup than that of the traditional cupping therapy. Similarly, the study found that by regulating the temperature and negative pressure of the drug solution in the tank, it has similar effects of warming, drug application, cupping, and transdermal administration [2 - 5]. In our previous studies, we have developed high-, medium-, and low-concentration of chemical permeation enhancers (CPEs) for the penetration-inducing system and physical penetration, which is iontophoresis [9 - 11]. Based on this, the present study used CPEs and iontophoresis as control. The effect of FCT on the kinetic parameters of percutaneous absorption and penetration enhancement of indomethacin patch were studied by using the six *in vitro* drug permeation and diffusion kinetic models. This study was aimed to evaluate the effect and characteristics of FCT penetration on the transdermal absorption of the model drugs, and speculate the effect of FCT on the skin keratin barrier (obstructing the percutaneous absorption of drugs), and evaluate the safety of FCT on the skin, and offer the reference basis for the clinical application of FCT in promoting drug absorption, and provide the current scientific basis for the effect of cupping therapy on skin.

Materials and methods

Materials

The reference substance of Indomethacin (purity about 99.9%), was purchased from National Institutes for Food and Drug Control (batch number:100258-200904); Indomethacin hydrophilic gel patch was provided by the preparation laboratory of Guiyang College of Traditional Chinese Medicine; Acetonitrile and chromatographic grade was obtained from Tianjin Kemiou Chemical Reagent Co., Ltd.; Green tea anti-allergic hair removal cream was purchased from Guangzhou Youxi Cosmetics Co., Ltd.; The purified water was obtained from Wahaha Group Co., Ltd..

Animals

Male and female Sprague Dawley rats with body weight from 260 to 300g were provided by the Experimental Animal Center of the Third Military Medical University of the People's Liberation Army, certificate number: SCXK - (Army) 2012-0011. The animals were housed at 25 ± 1 ℃ with access to food and water ad libitum in a specific pathogen-free environment. All animal experiments were carried out in accordance with protocols evaluated and approved by the ethics committee of Guiyang College of Traditional Chinese Medicine.

Instruments

Agilent 1260 High Performance Liquid Chromatograph (Agilent, USA); TK-12B Transdermal Diffusion Tester (Shanghai Yukai Technology Trade Co., Ltd.); AE240 Electronic Balance (METTLER); JA2003 Analytical Balance (Shanghai Liangping Instrument Co., Ltd.); TGL-16C centrifuge (Shanghai Anting Scientific Instrument Factory); HYS-339 digital meridian therapeutic instrument (ion importer, Shenzhen Haoyisheng Electronic Technology Co., Ltd.). Scanning Electron Microscope (EM-30Plus, Korea COXEM Company)

HPLC determination

The concentration of indomethacin (IM) was determined by the High Performance Liquid Chromatography System (HPLC) with the VWD detector (Agilent, USA) and a Diamonsil C18 column (250 × 4.6 mm, 5 μm) maintained at 35 ℃. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid water (60 : 40), was pumped at a flow rate of 1.0 mL/min and monitored at a wavelength of 228 nm.

Reference solution preparation. The appropriate amount of IM reference substance was precisely weighed,

and dissolved in pH7.3 PBS buffered saline solution, and then the concentration of IM 236.80 μg/mL was obtained. The solution was diluted to different concentrations as needed with the PBS solution.

Preparation of test samples. From each group of IM hydrophilic gel patch, 2mL *in vitro* transdermal receptor solution was taken, and then filtered with 0.45 μm membrane, and 10 μL of the sample was injected for HPLC analysis.

Specificity. The blank gel patch transdermal receiving solution was filtered through 0.45 μm membrane after centrifugation and used as a negative sample. The above test sample, control substance, and negative sample respectively were analyzed by the above chromatographic conditions. As shown in Figure 1, there was no other interfering peak at the retention time of IM, this showed that the method is specific..

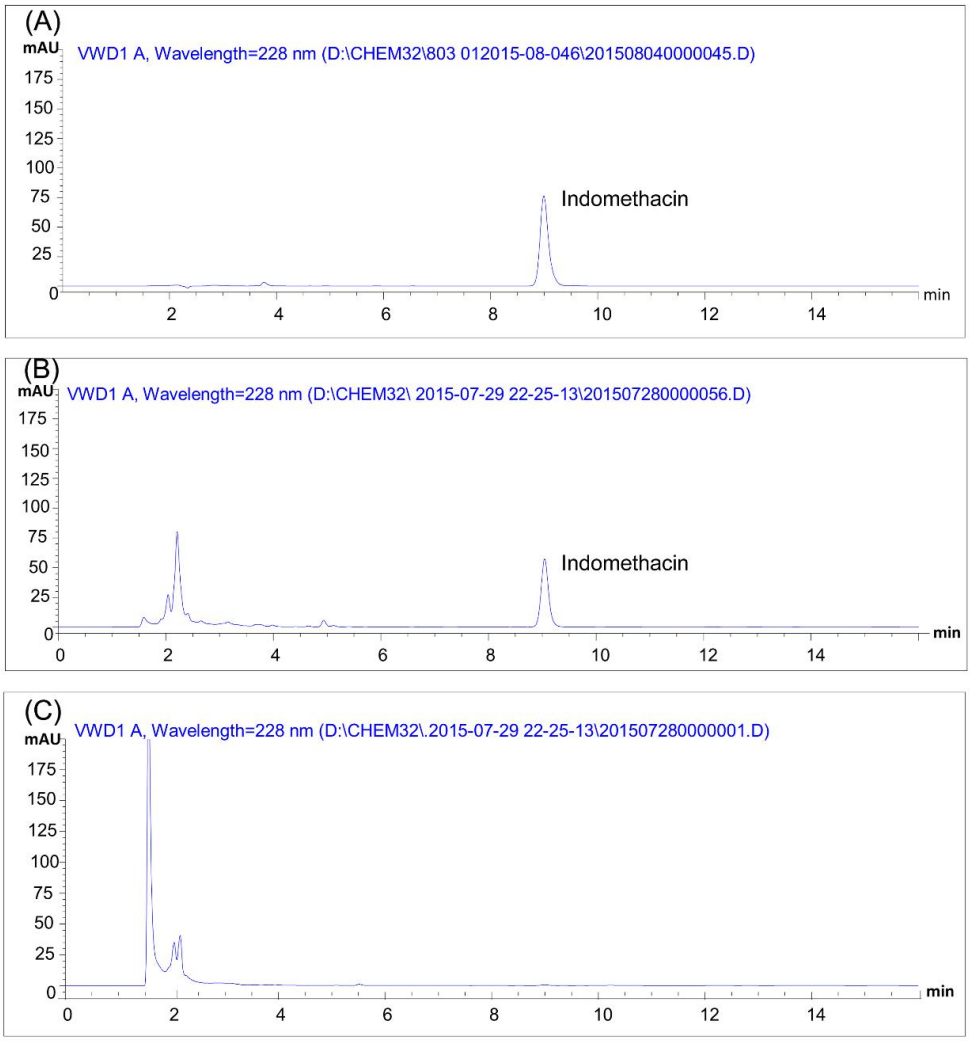

Figure 1 Quantitative HPLC chromatogram specificity investigation

A: Chromatograms of control substances of indomethacin; B: Chromatograms of test samples; C: Chromatograms of the negative samples. HPLC, High Performance Liquid Chromatography.

Standard curve. The IM standard PBS solution was diluted to 0.47 μg/mL, 3.32 μg/mL, 14.21 μg/mL, 33.15 μg/mL, 47.36 μg/mL, 71.04 μg/mL and analyzed by the HPLC. The peak area was taken as the abscissa (X) and the concentration (μg/mL) was as the ordinate (Y) to plot the standard curve. The linear regression equation was Y = 0.031X - 0.114 (r = 1). It showed that the method was linear in concentration range of 0.47 to 71.04 μg/mL.

Precision. The IM standard solution was analyzed 6 times and the relative standard deviation (RSD) was calculated. The result was found 0.64%, suggesting a good precision under the chromatographic conditions.

Stability. The same sample was analyzed at 0 h, 6 h, 10 h, 16 h, 20 h, and 24 h under the chromatographic conditions and the RSD was found to be 0.21%, which indicating the acceptable stability of the sample for 24 h.

Repeatability. Six samples of the same concentration from the same batch of transdermal receptor solution were prepared and analyzed, and the RSD was found to be 0.13%, indicating a good repeatability.

Recovery. Six samples of known concentration of transdermal receptor were taken and IM reference solution was added and analyzed, and then the recovery rate was calculated. As shown in Table 1, the recovery rate was 101.2 % ± 1.56.

Table 1 Sample recovery test

Number	Original content (μg)	Amount added (μg)	Total amount (μg)	Recovery rate (%)	Mean (%)	SD
YP1	28.41	28.42	57.74	103.2	101.2%	1.56
YP2	28.45	28.42	56.63	99.2		
YP3	28.50	28.42	56.75	99.4		
YP4	28.48	28.42	57.5	102.1		
YP5	28.44	28.42	57.31	101.6		
YP6	28.41	28.42	57.22	101.4		

***In vitro* transdermal diffusion**

***In vitro* skin and patch preparation.** The rats were selected according to the requirements and the skin was isolated after abdominal injection of anesthesia [4 - 7], and then stored in the refrigerator and used up within one month. According to the prescription and preparation process of the matrix [5], the IM gel patch without penetration enhancer and with different penetration enhancers were prepared for which the drug loading was 12.5 mg.

***In vitro* transdermal experiment.** The transdermal experiment was conducted by the existing methods with modification [5 - 12]. Briefly, the isolated skin of the animals was divided into 8 groups (n = 6): blank penetration group, 3 groups of CPEs (3% Azone, 5% Azone, 3% Azone-5% Mint oil), iontophoresis physical penetration reference group (the mode parameter: acupuncture mode, low frequency, strength 5, 15 min), and 3 groups of FCT (high, medium and low). Franz transdermal diffusion device was used to conduct the experiment. The receptor compartment volume (V) was 7.0 mL, contact area (A) was 2.92 cm^2, human simulated buffer pH = 7.3 PBS solution was used as the receiving solution. The stratum corneum of the skin was closely adhered to the patch sheet with the area of 3.14 cm^2 and fixed on the diffusion cell to ensure the absence of air bubbles, and the top was sealed with a plastic film. The water bath thermostat was maintained to 37 °C ± 1 °C, electromagnetic constant speed stirring was set as 320 r/min, all the solution was taken out at 3 h, 6 h, 9 h, 12 h, 16 h, 24 h, 30 h, 36 h, and then the same amount of the blank receiving solution was replaced. The samples were analyzed by HPLC to determine the concentration of IM and then the cumulative penetration amount and the per unit area cumulative amount Q (μg/cm^2) at each time point were calculated.

$$Q = \frac{\sum_{i=1}^{n} Ci \times Vi}{A}$$

Where, Q is the per unit area cumulative amount, A is the contact area of the skin, Ci is the concentration of the IM and "Vi" is the volume of the receiving solution withdrawn.

Iontophoresis. The conditions used in iontophoresis was: electrode orientation (+, −), processing mode, current intensity (3, 5, 10, 15), frequency (low, medium, high), processing time (5 min, 10 min, 15 min), combined with clinical use conditions and *in vivo* tests [3], the conditions determined: acupuncture mode, low frequency, intensity 5, time was 15 min. The IM patch was connected to the negative electrode, and the intermediate frequency was used in the human bodies, and the rest remain unchanged, this was a low strength treatment condition commonly used for iontophoresis. Since the transdermal rate of the drug attached to the negative electrode was relatively high and adhered to the patch, and then the patch was adhered to the surface of the skin.

FCT's percutaneous penetration. The main influencing factors of cupping [2 - 9] are cupping method, pressure and time. In this study, different sizes of cups were used to control the pressure of cups [9]. The cups of No. 2, 3, 4 (Figure 2A) were used to regulate the cupping pressure and adjust the strength of the human body stimulation, a certain volume (V) of internal air was drawn out via the cupping reserved mouth (Figure 2B) to form a quantitative negative pressure environment. Each group of cupping was operated according to clinical requirements [2 - 9]. The method of "retaining cup" is to leave the cup in the extracted part or to follow the meridian to retain the cup for 5 to 15 min, preferably 7 min. It has warming effect, so it is also called for the "warm cupping method". The "shake cupping" method is also known as the spin cupping method. Firstly, the cup was sucked on the skin and then the canister was shaken rhythmically. During shaking, the attention should be paid to softness of the force, the speed should not be too fast, the angle of shaking should be appropriate, and the operation should be used once per 2 seconds. The "movable cupping", also known as the sliding cup method, the "scraping cup" is mostly applied to the parts with large lesions and thick muscles. Firstly, a layer of massage oil and petrolatum (or glycerin) was applied to the area, and the cup body was tightened on the skin with left hand to make it tight. The cup body was pulled by the right hand to slide in a certain direction and walked along the meridian with the degree of skin flushing. Different types of cups and cupping methods were used to establish a high-, medium-, and low- intensity of permeability system [9]. The cups of No. 2 was used and shaken cupping for 6 minutes in FCT low-intensity group. The cups of No. 3 was used and retained cupping for 11 minutes in FCT medium-intensity group. The cups of No. 4 was used and moved cupping for 15 minutes in FCT high-intensity group.

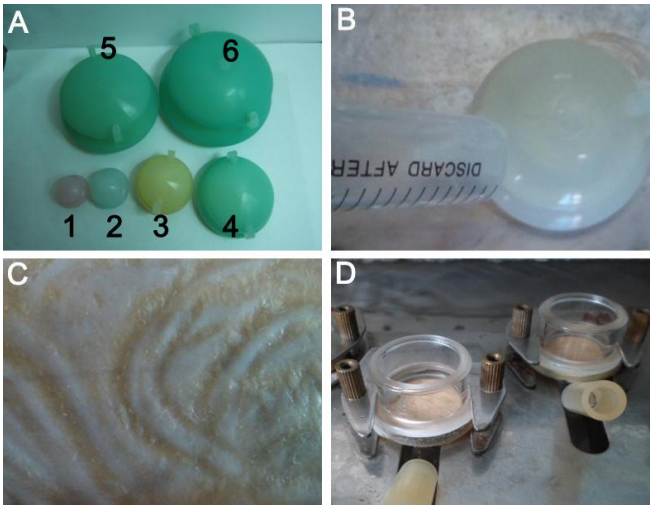

Figure 2 Treatment of percutaneous permeation by cups

A. Cups with different sizes; B. Negative pressure generated by emptying of cup; C. Rat skin treated by cupping; D. Franz transdermal diffusion device. This images (A, B, C, D) were obtained from [9].

Table 2 Equations of diffusion kinetics of each group

Group	Zero-order	First-order	Higuchi	Ritger-Peppas	Hixcon-Crowell	Weibull
3% Azone	Y = 0.0106x + 0.0373, R^2 = 0.9574	Y = -0.0138x - 0.0221, R^2 = 0.979	Y = 0.0432x - 0.0135, R^2 = 0.9945	Y = -0.1917x + 0.2339, R^2 = 0.9606	Y = -0.0035x + 0.3209, R^2 = 0.9574	Y = 0.6648x - 7.8272, R^2 = 0.9973
5% Azone	Y = 0.0134x + 0.0322, R^2 = 0.9751	Y = -0.0189x - 0.0016, R^2 = 0.994	Y = 0.1075x - 0.1568, R^2 = 0.9985	Y = -0.2558x + 0.3327,R^2 = 0.9307	Y = -0.0045x + 0.3226, R^2 = 0.9751	Y = 0.726x - 7.8423, R^2 = 0.9983
3% Azone and 5% mint oil	Y = 0.002x - 0.0008, R^2 = 0.9944	Y = -0.0021x + 0.0013, R^2 = 0.9956	Y = 0.0161x - 0.0285, R^2 = 0.9905	Y = -0.0284x + 0.0381,R^2 = 0.9212	Y = -0.0007x + 0.3336, R^2 = 0.9944	Y = 0.9291x - 10.51, R^2 = 0.9988
Iontophoresis	Y = 0.0108x - 0.0271, R^2 = 0.9945	Y = -0.0133x + 0.0464, R^2 = 0.9912	Y = 0.0839x - 0.1694, R^2 = 0.9614	Y = -0.1714x + 0.259, R^2 = 0.838	Y = -0.0036x + 0.3424, R^2 = 0.9945	Y = 1.2193x - 9.951, R^2 = 0.9987
Blank	Y = 0.0079x - 0.0315, R^2 = 0.9919	Y = -0.009x + 0.0412, R^2 = 0.9844	Y = 0.0606x - 0.1332, R^2 = 0.9422	Y = -0.1146x + 0.1813, R^2 = 0.8099	Y = -0.0026x + 0.3438, R^2 = 0.9919	Y = 1.3752x - 10.831, R^2 = 0.9995
FCTL	Y = 0.0108x - 0.0285, R^2 = 0.9978	Y = -0.0133x + 0.0476, R^2 = 0.9943	Y = 0.0839x - 0.171, R^2 = 0.967	Y = -0.1712x + 0.2606, R^2 = 0.8443	Y = -0.0036x + 0.3428, R^2 = 0.9978	Y = 1.4174x - 10.975, R^2 = 0.9996
FCTM	Y = 0.0136x + 0.0093, R^2 = 0.987	Y = -0.0188x + 0.0235, R^2 = 0.9977	Y = 0.1082x - 0.179, R^2 = 0.9936	Y = -0.2505x + 0.3454, R^2 = 0.9033	Y = -0.0045x + 0.3302, R^2 = 0.987	Y = 0.891x - 8.4435, R^2 = 0.9973
FCTH	Y = 0.0207x + 0.088, R^2 = 0.9503	Y = -0.0322x - 0.0476, R^2 = 0.9831	Y = 0.1415x - 0.1321, R^2 = 0.9908	Y = -0.324x + 0.3067, R^2 = 0.9506	Y = -0.0069x + 0.304, R^2 = 0.9503	Y = 0.6344x - 7.1397 R^2 = 0.9924

FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's cupping therapy.

Kinetic model establishment

***In vitro* transdermal kinetic model.** After the IM cumulative permeation amount "Q" (μg) at each time point was obtained, the cumulative permeation percentage "Q%" was calculated from the ratio of the cumulative permeate to the total amount, and "1-Q%" was the percentage of drug content in the patch matrix at different time point. The "Q% - t" curve of patch in vitro transdermal diffusion was obtained [9].

Six *in vitro* transdermal diffusion kinetic models [10 - 17] were selected as the evaluation methods. In the equation, "Q" and t represents the cumulative permeation per unit area (μg/cm^2), and different time points (h), respectively. Details as follows:

Zero-order dynamic equation is Q = kt + b;

First-order dynamic equation is ln Q = kt + b;

Higuchi plane- diffusion equation is Q = b + kt$^{1/2}$;

Retger-Peppas dynamic equation is ln Q = b + klnt;

Weibull dynamic equation is ln [1/(1-Q%) =P, lnP = K ln $^{(t - \tau)}$ + b;

Hixcon-Crowell dynamic equation is Q%$^{1/3}$ = kt + b.

When Q was adjusted to Q% = M_i/M_∞, it means the cumulative permeation percentage Q%, calculated according to the formula, and the kinetic model is analyzed. Among them, 1/2, 1/3, 2/3 and n are all indices in the equation. As follows: zero-order dynamic equation is Q% = Kt + b. First-order dynamic equation is ln (1-Q %) = -Kt + b. Higuchi plane-diffusion equation: Q% = Kt$^{1/2}$ + b. Retger-peppas power Equation is ln (Q%) = b + Klnt. Hixcon-Crowell dynamic equation is (1 - Q%) 1/3 = -Kt + b. Weibull dynamic equation is lnP = Kln (t - τ) + b, ln [1/(1 - Q%)] = P.

Regression modeling. According to diffusion dynamics model and regression principle, modeling of each group was performed using Excel software: zero-order equation uses Q to t for linear regression, the first-order equation uses ln (1-Q%) to t for linear regression, the Higuchi plane diffusion equation uses Q% to t$^{1/2}$ for linearly regress, retger-peppas dynamic equation uses ln (Q%) to lnt for linear regression, hixcon-Crowell's dynamic equation uses (1 - Q%) $^{1/3}$ to t for linear regression, the Weibull dynamic equation uses ln[1/(1 - Q%)] to ln(t - τ) for linear regression. Diffusion dynamics model equations and determination coefficients R (Table 2). The results showed that the Higuchi and Weibull dynamic equation regression models are better, and coefficient R ≥ 0.99, the kinetic model parameters obtained are available for analysis between groups.

Transdermal parameter. According to the model, the Higuchi and Weibull dynamic equations were used to analyze the diffusion kinetic parameters of each group. The slope of the Higuchi equation is the transdermal rate Js (μg·cm-2/h), the intercept is time lag (TL, h). According to the formula Js = P × C_0, the percutaneous permeability coefficient P of each group (cm/h) was calculated. C_0 represents the drug concentration in the skin contact medium (patch). At the same time, the parameters of Weibull were calculated: the shape parameter m represents the slope of the fitted curve, lnt β represents the intercept of the linear fit, β represents the scale parameter and Td represents the time that 63.2% of the drug dissolution cumulatively through the medium, position parameter τ indicates the lag time of the drug. The Weibull dynamic equation parameters of each group have theoretical reference values. The results are shown in Table 3 and Table 4.

Results

Comparative analysis

According to the analysis of modeling results, we have analyzed the Higuchi dynamic model parameters (Table 3). According to previous studies [8, 9], in vitro results indicate the effect of FCTL and FCTL M group promoting penetration is remarkable, and is higher than that of the CPEs and the iontophoresis group, so FCT has a percutaneous penetration effect for IM. At the same time, the ER was markedly improved at 24 h and 36 h, respectively, as compared with the blank group, which was slightly higher than CPEs and iontophoresis. However, the decrease in ER after a period of time might be related to the recovery of skin barrier function with time. Later studies need to evaluate the recoverability of skin barrier function after FCT penetration.

In order to further explore the characteristics of percutaneous penetration in vitro, the transdermal parameter of the Higuchi and Weibull dynamic equations. As shown in Table 3, the transdermal rate Js, the permeability coefficient P and the time-delay TL are negatively correlated, and the transdermal rate Js is positively correlated with the permeability coefficient P. The trend of change between the three is basically consistent and can be used for comparative analysis between groups. In Table 3, Higuchi kinetic parameters: compared with the blank group, the single chemical penetration enhancer group, the iontophoresis group, and the FCT group all reduced the drug transdermal retention time T to varying degrees, and improved the transdermal rate of the drug and the permeability coefficient of the skin. It shows that FCT and CPEs can weaken the barrier function of the skin. At the same time, the transdermal rate and skin permeability coefficient after FCT treatment were significantly higher than other groups, indicating that FCT has a greater effect on skin barrier and stimulation. However, the 3% Azone - 5% Mint oil system reduced the IM transdermal rate by only 14.1% and the 9h ER was 0.41 times as compared with the blank group, which was shown to inhibit IM transdermal action. This could be due to the presence of drug crystals which is consistent with the literature reports [10 - 11].

In the Weibull dynamic equation, the results showed that FCT can significantly reduce the fitted intercept "τ, Td", scale parameter β (equivalent to TL), increase the slope of the curve m, indicating that FCT can weaken the skin barrier and improve the transdermal absorption of drugs. The transdermal sampling point of the FCT high-intensity group (FCTH) group was only carried out for 24 h, so it was impossible to analyze.

Table 3 Transdermal kinetic parameters of Higuchi equation analysis (n = 6)

Group	T_{lag}/h	J_s (μg·cm^{-2}/h)	P (cm/h)
Blank	4.831	0.061	8.22E-05
3%Azone	0.175	0.043	5.04E-04
5%Azone	2.128	0.108	1.95E-03
3% Azone and 5% mint oil	3.134	0.016	4.15E-04
Iontophoresis	4.077	0.084	8.78E-04
FCTL	4.154	0.084	1.18E-04
FCTM	2.737	0.108	1.16E-03
FCTH	0.872	0.142	1.50E-03

FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's cupping therapy.

Table 4 Transdermal kinetic parameters of Weibull equation analysis (n = 6)

Group	τ	m	Lnt β	β	1/m	Td
Blank	0.820	1.375	-10.831	50576.824	0.727	2622.917
3%Azone	2.389	0.665	-7.827	2508.003	1.504	129846.797
5%Azone	2.107	0.726	-7.842	2546.028	1.377	49119.454
3% Azone and 5% mint oil	1.644	0.929	-10.510	36679.736	1.076	81757.950
Iontophoresis	0.354	1.219	-9.951	20973.920	0.820	3492.855
FCTL	0.598	1.248	-10.027	22625.420	0.801	3071.968
FCTM	1.418	0.891	-8.444	4644.995	1.122	13034.534

FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's cupping therapy.

Statistical analysis

SPSS 21.0 was used for single-factor and multi-group LSD analysis. $P \leq 0.05$ indicated that the difference was statistically significant. The blank group was used as a reference, and the ER-t curve changes for each group was plotted. After the permeation treatment, the skin barrier function is changed, and the transdermal permeability of the drug is improved. As the skin barrier function is restored, the transdermal performance of the drug is lowered. Based on this point, compared with the blank group, the ER-t curve was analyzed, and the ER of each group at each time point was statistically analyzed, and the time point t with no statistical difference was found as the critical point for evaluating the initial recovery of the skin barrier (Tables 5 - 8).

Since there is no intra-group error in the blank group, the reference point of 5% Azone and iontophoresis were used to evaluate the critical point of the iontophoresis group, FCTL, and FCTM. As shown in Figure 3, there was no significant difference between the FCTL and the reference group after 16h. Compared with the blank group, the critical time point of the 5% Azone group exceeded 30 h, and there was no statistical difference, indicating that there was no difference in the skin barrier function between the 5% Azone group and the blank skin. Based on comprehensive inference, it was initially considered that the skin barrier function of the FCTL group was initially restored after 46 hours, and there was no statistical difference compared with normal skin. Among them, within the 36 h experimental time, the FCTM and FCTH group did not show any statistical difference. If further investigation is needed, the time of *in vitro* study should be prolonged.

Table 5 Multiple comparison of enhancing rate between groups at 12 h

Groups		Mean difference	Standard error	*P*	95% Confidence interval	
I	J	(I-J)			Lower limit	Upper limit
5% Azone *VS*	Blank	1.2537	0.362	0.002	0.515	1.993
	Iontophoresis	0.6680	0.362	0.075	-0.071	1.407
	FCTL	0.7640	0.362	0.0450	-0.175	1.302
	FCTM	-0.9285	0.362	0.016	-1.667	-0.190
	FCTH	-4.4213	0.362	< 0.001	-5.160	-3.682
Iontophoresis *VS*	Blank	0.6900	0.362	0.066	-0.049	1.429
	5% Azone	-0.5640	0.362	0.130	-1.302	0.175
	FCTL	-0.1040	0.362	0.775	-0.843	0.635
	FCTM	-1.5963	0.362	< 0.001	-2.335	-0.857
	FCTH	-5.0892	0.362	< 0.001	-5.828	-4.350

FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's cupping therapy.

Table 6 Multiple comparisons of enhancing rate between groups at 16 h

Groups		Mean difference (I-J)	Standard error	Significant	95% Confidence interval	
I	J				Lower limit	Upper limit
5% Azone *VS*	Blank	0.9013*	0.266	0.002	0.359	1.444
	Iontophoresis	0.4070	0.266	0.136	-0.135	0.949
	FCTL	0.2800	0.266	0.300	-0.262	0.822
	FCTM	-0.9360*	0.266	0.001	-1.478	-0.394
	FCTH	-3.3185*	0.266	< 0.001	-3.861	-2.776
Iontophoresis *VS*	Iontophoresis	0.4940	0.266	0.072	-0.048	1.037
	5% Azone	-0.4070	0.266	0.136	-0.949	0.135
	FCTL	-0.1270	0.266	0.636	-0.669	0.415
	FCTM	-1.3430*	0.266	< 0.001	-1.885	-0.801
	FCTH	-3.7255*	0.266	< 0.001	-4.268	-3.183

FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's cupping therapy.

Table 7 Multiple comparisons of enhancing rate between groups of at 24 h

Groups		Mean difference	Standard	Significant	95% Confidence interval	
I	J	(I-J)	error		Lower limit	Upper limit
5% Azone *VS*	Blank	0.4755	0.174	0.010	0.121	0.831
	Iontophoresis	-0.0800	0.174	0.651	-0.435	0.276
	FCTL	-0.0980	0.174	0.577	-0.453	0.257
	FCTM	-0.8697	0.174	< 0.001	-1.225	-0.515
	FCTH	-2.1773	0.174	< 0.001	-2.532	-1.822
Iontophoresis *VS*	Blank	0.5550	0.174	0.003	0.200	0.910
	5%Azone	0.0800	0.174	0.651	-0.276	0.435
	FCTL	-0.0190	0.174	0.916	-0.374	0.337
	FCTM	-0.7902	0.174	< 0.001	-1.145	-0.435
	FCTH	-2.0978	0.174	< 0.001	-2.453	-1.743

FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's cupping therapy.

Table 8 Multiple comparisons of enhancing rate between groups at 30 h

Groups		Mean difference	Standard	Significant	95% Confidence interval	
I	J	(I-J)	error		Lower limit	Upper limit
5% Azone *VS*	Blank	0.2370	0.140	0.103	-0.051	0.525
	Iontophoresis	-0.1970	0.140	0.173	-0.485	0.092
	FCTL	-0.2090	0.140	0.149	-0.497	0.080
	FCTM	-0.76967	0.140	< 0.001	-1.058	-0.481
Iontophoresis *VS*	Blank	0.43350	0.140	0.005	0.145	0.722
	5%Azone	0.1970	0.140	0.173	-0.092	0.485
	FCTL	-0.0120	0.140	0.932	-0.300	0.276
	FCTM	-0.5732	0.140	< 0.001	-0.861	-0.285

FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's cupping therapy.

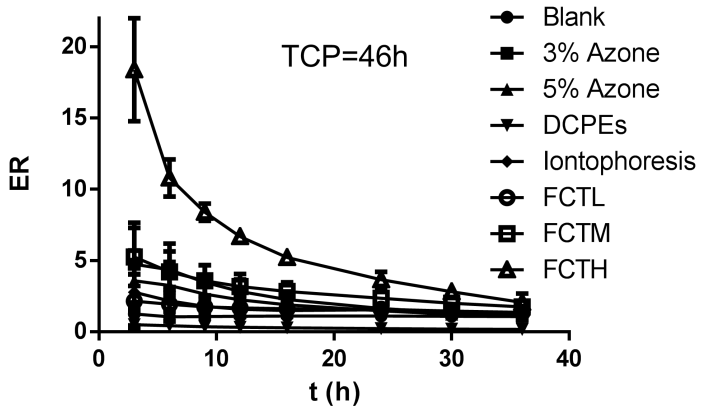

Figure 3 ER-t curve and time of critical point (TCP) of each groups

Blank, the blank (control) group; 3% Azone, the 3% Azone group; 5% Azone, the 5% Azone group; DCPEs, the diplex chemical penetration enhancers group including 3% Azone and 5% mint oil; Iontophoresis, the iontophoresis group; FCTL, FCT low-intensity group; FCTM, FCT middle-intensity group; FCTH, FCT high-intensity group; FCT, Fu's Cupping Therapy. ER means permeability-increasing intensity; TCP means critical time point for recovery of skin barrier function.

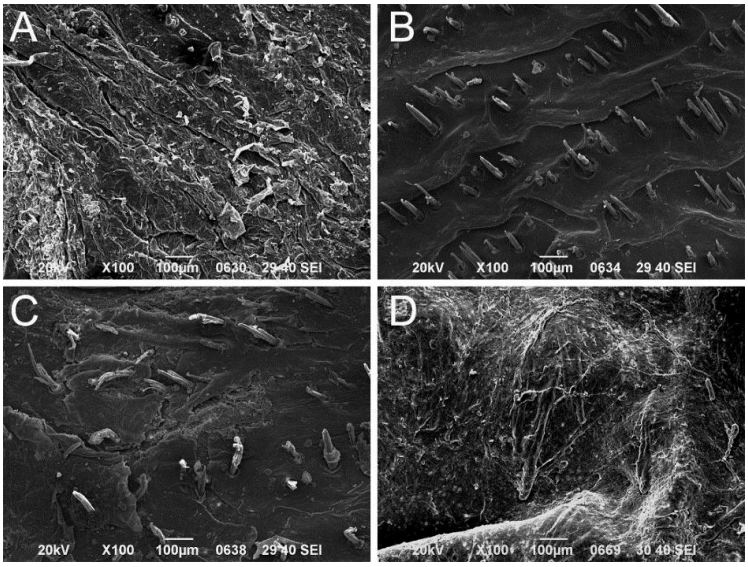

Figure 4 SEM image of the effect of FCT treatment on animal skin microstructure

A, Blank group; B, FCT low-intensity group; C, FCT middle-intensity group; D, FCT high-intensity group; FCT, Fu's cupping therapy.

The vascular structure below the epidermis

The microscopic observation revealed that FCTH group exhibited multiple skin ruptures within 24 h, and therefore, transdermal release study could not be performed. As indicated by the yellow arrow, the stratum corneum of FCTH group was almost completely destroyed after Fu's cupping process compared with other groups (Figure 4). It suggests that FCT has a strong destructive effect on the stratum corneum of the skin in the animal skin modell, and greatly reducing or even causing the barrier function to be lost, resulting in a dramatic decrease in the mechanical support, protection and resistance to external substances. However, in fact, in the clinical practice of TCM, the middle and high FCT are commonly used for the treatment of human skin meridians, which may be related to the better tolerance of human skin.

Discussions

In this study, the *in vitro* transdermal method was used to preliminarily evaluate the penetration-promoting effect of different cupping methods and the penetration-promoting system of FCT. The effects of FCT on rat skin and the kinetic characteristics of drug transdermal permeation were analyzed. Firstly, based on six diffusion kinetic models, 3% Azone, 5% Azone CPE and iontophoresis were used as reference [15 - 27] to evaluate and compare the effects and characteristics of FCT on percutaneous penetration. The results demonstrated that FCT exhibited a significant percutaneous permeation promoting effect on IM. The permeation enhancing effect of FCTL were comparable to that of iontophoresis and CPEs, while the effect of FCTM and FCTH was significantly higher than that of iontophoresis and CPEs. Weibull and Hguchi dynamics model parameters showed that compared with the blank group, the single CPE group, the iontophoresis group and the FCT group all reduced the drug transdermal retention time TL, intercept lnt β and Td to some extent, improved the percutaneous penetration rate, skin permeability coefficient and shape parameter m, which indicated that FCT and CPE could weaken the barrier effect of the skin and improve the transdermal absorption of the drug by skin. Meanwhile, the transdermal rate and skin permeability coefficient after FCT treatment were significantly higher than those of other groups, indicating that FCT had a greater effect on skin barrier.

However, during the course of the experiment, we found that the FCTH group exhibited multiple skin ruptures within 24 h. In addition, the stratum corneum of FCTH group was almost completely destroyed after Fu's cupping process. However, in the clinical practice of TCM, the middle and high FCT are commonly used for the treatment of human skin meridians. The treatment of intensive cupping may also have the therapeutic purpose that requires more stimulating cupping to treat the body. Therefore, this study used three different levels of cupping therapy in high, medium and low level to comprehensively evaluate the percutaneous penetration activity.

At the same time, for the processing method of Fu's cupping, the previous study used different sizes of cups form negative pressure by exhausting the internal air. First, the value of the individual cupping treatment pressures was not precisely quantified, and used different cupping regulation is more in line with the characteristics of clinical use of TCM. The experimental results have more reference value for TCM clinical practice. Second, it can improve the controllability and repeatability of cupping pressure. Third, the influencing factors for percutaneous penetration of FCT were investigated in our preliminary study. Combining the theory of TCM with cupping therapy [23-26], in the use of negative pressure to attract, iron the skin, pull the shallow muscles, stimulate the meridians, acupoints, pass the sense of transmission, thereby adjusting the blood and Yin and Yang, dredge the meridians, to achieve the role of rickets fitness using modern pharmacy theory to give controlled transdermal drugs, The drug can be controlled, sustained release and targeted, and the therapeutic effect of the drug can be exerted in a timely and effective manner.

Conclusion

The combination of cupping therapy and transdermal drug delivery can improve the transdermal rate of the drug, which is the modernization of traditional cupping, and provides a new idea and method for percutaneous penetration. As a new physical percutaneous penetration technique, FCT has obvious effects and has the characteristics of TCM [2, 9].

However, there are certain problems at the same time [28 - 31], such as different treatment methods for cupping have different effects on skin damage which leads to different effects of percutaneous penetration. Therefore, the investigation of the percutaneous dynamic parameters of cupping therapy will improve the clinical accuracy of the combination of cupping and transdermal administration, and provide scientific data and reference for clinical application and further study.

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