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Original Research Article

Penetration and pharmacokinetics of ferulic acid after dermal administration

Jie Bai, Chang Yang, Shou-Ying Du*, Peng-Yue Li, Bo-yu Dong, Bing Yang, Xiao-nan Chen, Ya-hua Cui

Department of TCM Pharmaceutics, School of Chinese Pharmacy, Beijing University of Chinese Medicine, No. 11 North Third Ring Road, Chaoyang District, Beijing, 100029, China

For correspondence: **Email: baijie22811@163.com, dusy@bucm.edu.cn; **Tel:** +86-010-84738615*

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Abstract

Purpose: *To study the in vitro penetration and in vivo pharmacokinetics of ferulic acid (FA), and the correlation between them after dermal administration.*

Methods: *Franz diffusion cell was used to study in vitro penetration of FA. The concentration of FA in the Franz receiver solution was assessed by high performance liquid chromatography (HPLC). Prior to in vivo pharmacokinetics experiments, probe recovery was validated with respect to influencing factors such as flow rate, FA concentration, within-day stability and reproducibility of the probes. In in vivo pharmacokinetic experiment, six male CD-1 hairless mice were used. The micro-dialysis (MD) probe was implanted in the dermis of the rat skin, and dialysates from probe outlet were quantified directly by HPLC. In in vivo studies, deconvolution methods were used to determine the relationship between in vitro and in vivo data, and the correlation coefficient of linear equations.*

Results: *There was significant effect of pH (5 ~ 8) on the penetration of FA. Increase in pH caused commensurate decrease in permeability. The C_{max} of FA was 300.74 ± 31.86 ng/mL while T_{max} was 138.00 ± 22.80 min after dermal administration of 1 mg/mL FA dissolved in phosphate buffered saline (PBS). The correlation coefficient (r) between in vitro and in vivo data was 0.9905.*

Conclusion: *Both in vivo and in vitro experiments demonstrate that FA permeates the stratum corneum of skin rapidly. The unionized form of FA shows better penetration than the ionic form. In addition, results from correlation analysis indicate that the in vitro penetration characteristics of FA can be applied to predict its in vivo pharmacokinetics.*

Keywords: *Ferulic acid, Dermal penetration, Microdialysis, Pharmacokinetics, Deconvolution methods*

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INTRODUCTION

Ferulic acid (FA) is one of the most abundant phenolic compounds in plants, especially *Ligusticum wallichii, Angelica sinensis* and *Rhizoma cimicifugae*, which are used extensively in Chinese Traditional Medicine [1]. Previous studies have reported diverse medicinal effects of FA, such as anti-inflammatory, anticancer, antioxidant, neuroprotective and cardioprotective activities [2]. The antioxidant properties of FA have been exploited in managing UV-induced

skin damages like erythema, photo-aging and photo-carcinogenesis [1,3,4]. As a promising photoprotective agent, topical administration of FA is the most direct and effective way to avoid UV damage. However, the therapeutic effect of FA is limited due to the stratum corneum which acts as skin barrier [5]. Hence, there is need to evolve a new method for enhancing the penetration of FA through skin. This should be based on studies on the skin penetration and pharmacokinetic behaviors of FA, such as absorption, distribution and metabolism [6].

In this study, Franz diffusion cell experiment and microdialysis (MD) technology were carried out to investigate the *in vitro* and *in vivo* penetration of FA. Furthermore, to learn more about the relationship between *in vitro* penetration and *in vivo* pharmacokinetics of FA, a correlation analysis between them was performed.

EXPERIMENTAL

Chemicals and reagents

Standard FA was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Phosphate buffered saline (PBS) was prepared by mixing 0.04 mol/L of NaH_2PO_4 and aqueous solution of Na_2HPO_4, using NaCl to modify the osmotic pressure. Solutions of PBS of different pH were prepared by adding different amounts of aqueous 0.1% phosphoric acid, and adjusting their pH with FE20 pH meter [Mettler-Toledo Instruments (Shanghai) Co., Ltd, China]. Solutions for dermal application (1 mg/mL) were prepared by dissolving FA in PBS of different pH. The receiver solution used in *in vitro* study, and perfusate used in *in vivo* study were pure PBS. Methanol (Fisher Scientific, New Jersey, USA) and acetic acid (Tedia Company, Inc., Fairfield, USA) were of HPLC grade. All other chemicals were of analytical-reagent grade. Water used in all experiments was passed through a Milli-Q water purification system.

Animals

Five-week-old male CD-1 hairless mice (15 - 18 g) from the Animal House of the Chinese Pharmacy Department of Beijing University of TCM were used for this study. The animals were placed singly in cages in a specific pathogen-free (SPF) room with controlled temperature (25 °C) and humidity (70 – 75 %), and were fed SPF diet. All experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals approved by the Committee of Ethics of Animal Experimentation of Beijing University of Chinese Medicine (approval no. BUCM-2-2017010101-1001).

HPLC conditions

The HPLC system used was an Agilent model 1260 series (Agilent Technologies, Germany), incorporating an automatic injector, a quaternary pump, a vacuum degasser, a thermostat column compartment and a variable ultraviolet−visible (UV−vis) detector. Separation was performed on a reverse-phase Hibar C_{18} column (250 × 4.6 mm, 5 µm) at 35 °C preceded by a security guard cartridge of the same material, both provided by Merck (Merck KGaA, Germany). The mobile phase was composed of water: acetic acid (100: 1, v: v) and methanol, with isocratic elution of 40: 60 at a flow rate of 1.0 mL/min. The volume of sample injected at a time in the injector was 15 µL, and the detection wavelength was set at 323 nm. The linear regression of FA displayed good linear relationships over the ranges of 0.016-40.8 µg/mL (for receiver solution) and 0.016-4.08 µg/mL (for dermal dialysate). The calibration curve for FA in receiver solution was $a = 92.334c – 1.7245$, with r value of 0.9999. In receiver solution, the calibration curve was $a = 92.68c – 1.4123$, with r value of 0.9999. The limit of detection (LOD) of FA was 4 ng/mL and the limit of quantification (LOQ) was 14 ng/mL, for both receiver solution and dermal dialysate.

Penetration experiment

This experiment was performed in Franz diffusion cells (Tianjin Pharmacopoeia Standard Inc., Tianjin, China), with penetration area of 1.76 cm^2 and receiver cell volume of 17 mL [7]. The skin was mounted on a receiver cell with the stratum corneum side facing upward toward the donor cell. During the experiment, receiver cells were filled with PBS (pH 7.4), which was continuously stirred at the rate of 300 rpm/min. Temperature (32 ± 0.2 °C) was maintained with an external circulating water bath. In the donor cell, 800 µL of 1 mg/mL FA solutions of different pH were (separately) applied homogeneously on the skin. At pre-set time points (20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240 min), 300 µL of receptor solution was withdrawn, and the same volume of fresh receptor was added to the receptor cell in order to maintain the sink condition. Samples were analyzed for FA by HPLC as described under HPLC conditions. Throughout the experiments, the donor compartments and sampling arms were occluded to prevent evaporation.

The cumulative amount of compound (Q_n, μg/cm^2) in the receiver cell was determined as in Eq 1 [8].

$$Q_n = \frac{VC_n + \sum_{i=1}^{n-1} C_i V_i}{S} \quad \text{..... (1)}$$

where C_n is the drug concentration in the receiver medium at each sampling time; C_i is the drug concentration in the *i*th sample; V and V_i are the volumes of the receiver solution and the sample, respectively, and S is the effective diffusion area.

Steady-state flux (J_{ss}) was calculated by linear regression interpolation of the experimental data at a steady state as shown in Eq 2.

$$J_{ss} = \frac{\Delta Q_n}{\Delta t} \quad \text{..... (2)}$$

Apparent permeability coefficient (P_{app}) was calculated according to Eq 3.

$$P_{app} = \frac{J_{ss}}{C_d} \quad \text{..... (3)}$$

where C_d is the concentration in the donor compartment (it was assumed that under the sink conditions, the concentration in the receiver compartment was negligible relative to that in the donor compartment).

Validation of *in vitro* recovery

Due to constant flow of perfusate, complete equilibration is not usually attained between the perfusate and extracellular fluid (ECF) of the surrounding tissue. This implies that only a part of the actual drug is collected in the dialysate. Hence before the animal experiment, *in vitro* recovery was validated first, and factors that influence recovery, such as flow rate, concentration, within-day stability and reproducibility were also studied.

The effect of flow rate on recovery was studied by immersing the probes in stirred and temperature-controlled (37 ^{o}C) PBS containing FA (750 ng/mL) as a dialysis medium (C_{in}). The probes were perfused with drug-free PBS at adjusted flow rates (0.5, 1, 1.5, 2, 2.5, 3 and 4 μL/min). Three replicates of micro-dialysate sample (30 μL) over at least 1 h of sampling were collected at each flow rate. The concentration of FA in micro-dialysate samples (C_{out}) was determined by HPLC. Relative recovery by gain (*RG %*) of FA was calculated using Eq 4 [9].

$$RG\ (\%) = \frac{C_{out}}{C_{in}} \times 100\% \quad \text{..... (4)}$$

The effect of drug concentration on recovery was studied by immersing the probes in stirred and temperature-controlled (37 ^{o}C) PBS containing various concentrations of FA (11.2, 54.2, 750, 2750 ng/mL) as dialysis medium (C_{in}). The probes were perfused with drug-free PBS at a flow rate of 1.5 μL/min. Three replicates of micro-dialysate samples (30 μL, C_{out}) were collected at each FA concentration. Relative recovery of FA was determined by gain and calculated as in Eq 4.

The stability of the probes was studied by placing the probe in blank PBS at room temperature while the probe was continuously perfused at a flow rate of 1.5 μL/min for 6 h with PBS containing FA (720 ng/mL). Reproducibility was studied by changing the perfusate from PBS containing FA (690 ng/mL) to analyte-free PBS, while the medium was analyte-free PBS. The probe was continuously perfused at a flow rate of 1.5 μL/min, and the recovery was examined over four cycles at in-door temperature.

Validation of *in vivo* recovery

The MD system (CMA, Stockholm, Sweden) consisted of two CMA/100 micro-injection pumps, two syringes and two CMA line probes (10 mm membrane). A hairless mouse was anaesthetized with i.p. injection of 20% urethane, with its skin temperature maintained at 37 °C during the experiment with the help of an infrared lamp. Then, a linear microdialysis probe was implanted in the dermis and perfused continuously with PBS containing 690 ng/mL of FA (C_{in}) for 6 h at a flow rate of 1.5 μL/min. Then, three replicates of microdialysate sample (30 μL, C_{out}) were collected. Lastly, relative recovery ($R_{in\ vivo}$) of FA used to correct the real concentration in dermis was calculated using Eq 5.

$$R_{in\ vivo} = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \quad \text{..... (5)}$$

***In vivo* pharmacokinetic studies**

After the implant of microdialysis probe, the probe was perfused with pure PBS for at least one hour. The tip of a 5 mL syringe was clipped off and fixed directly onto the skin above the dialysis membrane with cyanoacrylate glue to provide an administration chamber. This chamber kept the test solution in place. The connection between the chamber and the skin was also sealed to prevent leakage through the probe entrance and outlet in the skin [10]. Solutions of FA (800 μL) were deposited in the

penetration chambers, and the dialysate samples were collected in vials directly from probe outlet for 4 hours with an interval of 20 min, and at a flow rate of 1.5 µL/min. After 4 hours, FA solutions were removed from the skin surface, and the surface was gently wiped with cotton-ball. Then, another six dialysate samples were collected. At the end, the skin was excised for histological analysis of the implantation, to ensure that the probe was implanted in the dermis.

Statistical analysis

In the penetration study, SAS 9.3 (SAS Institute Inc. USA) was used for statistical analysis, and statistically significant differences were assumed at $p < 0.01$. In the *in vivo* pharmacokinetic studies, the concentration of FA in dermis was calculated by *Concentration* = $C_{dialysis}$ /$R_{in\ vivo}$, where $C_{dialysis}$ and $R_{in\ vivo}$ refer to the concentration in the dialysis and *in vivo* recovery, respectively. Pharmacokinetics curve was generated by plotting FA concentration in dialysate against time (midpoint of the sampling interval after drug administration). Pharmacokinetic data for the first 4 h were calculated by Kinetic 4.4 (Thermo Electron Corporation, USA) using non-compartmental method. The correlation between penetration data and pharmacokinetics data was calculated by deconvolution methods, where Q_n represents the *in vitro* cumulative penetration, while the area under the time-versus-concentration curve (AUC) was used to illustrate the *in vivo* absorption during a chosen time interval, with index of fraction input (R) and *Q* % reflecting the ratio of $AUC/AUC_{0\text{-}240\ min}$ and $Q_n/Q_{total,}$ respectively (where Q_{total} refers to cumulative penetration at 240 min). The linear regression equation was obtained from *Q* % and *R*.

RESULTS

Penetration experiment

The result showed that apparent permeability coefficients (P_{app}) of FA in donor solution at pH values of 5, 6 and 8, were 3.3507±0.3401, 1.4636±0.4108) and 0.3500±0.0283, respectively, after topical administration of FA solution. These results indicate that the pH of the donor solution had a significant effect on FA penetration ($p < 0.01$): P_{app} FA decreased with increase in pH.

Validation of *in vitro* recovery results

There was an exponential relationship between estimated recovery and flow rate (Figure 1). The fit equation was y=13.1961 + 99.7542 $e^{-x/1.22679}$, r = 0.9984. A flow rate of 1.5 L/min was chosen in this study, because it resulted in enough quantity of sample for analysis and met sensitivity of the analytical assay.

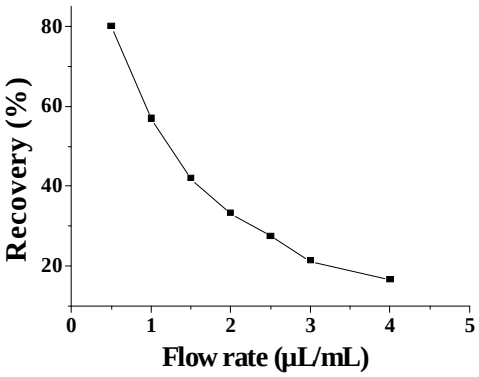

Figure 1: Effect of flow rate on *in vitro* recovery by gain from the MD probe

The results of effect of FA concentration on *in vitro* recovery are shown in Figure 2. Linear regression was calculated between dialysate and dialysis medium, and the equation was *y = 0.4395x - 3.6666, r= 0.9998*, whose slope (0.4395) was equal to the average recovery. The result indicated that concentration had no effect on recovery. Thus, the MD method can be used to determine drug concentration in dermis.

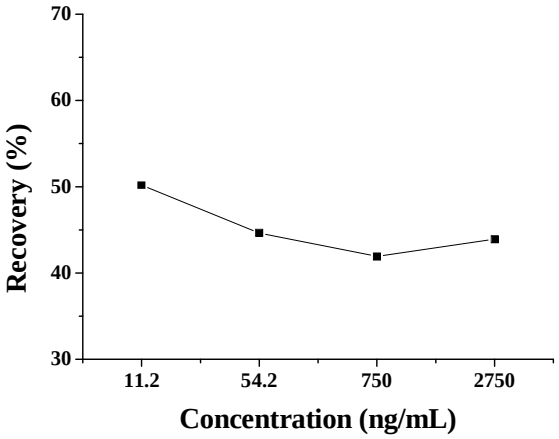

Figure 2: Effect of concentration on *in vitro* recovery by gain from the MD probe

The stability experiment showed that probe recovery was stable over 420 min with an average value of 24.82 ± 1.01 % (n = 3). The within-day reproducibility of the recovery was 24.09 ± 1.60 % (n = 3) for four cycles. It was also observed that the FA concentration in the dialysate reached steady state within 80 min after each change in perfusate.

In vivo pharmacokinetics

The *in vivo* recovery ($R_{in\ vivo}$) determined before pharmacokinetic study was 16.50 ± 1.92 %, and it was stable for 4 h (Figure 4). The mean dialysate concentration–time profiles are illustrated in Figure 5. It was revealed that FA penetrated slowly through skin and reached C_{max} of 300.74 ± 31.86 ng/mL in 138 ± 22.8 min. The area under concentration-time curve (AUC), $t_{1/2}$ and MRT (mean residence time) of FA after administration were 44051.74 ± 6787.67 μg/mL·min, 168.78 ± 57.56 min and 301.87 ± 64.50 min, respectively.

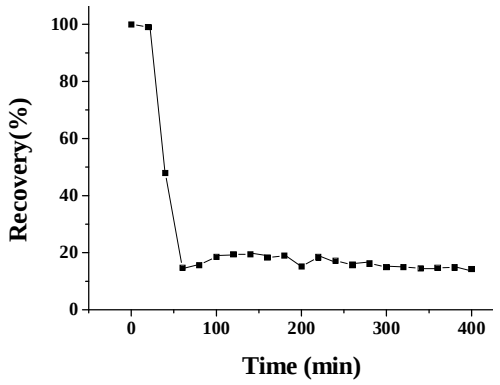

Figure 4: Within-day stability of *in vivo r*ecovery of MD probe (*n*=3)

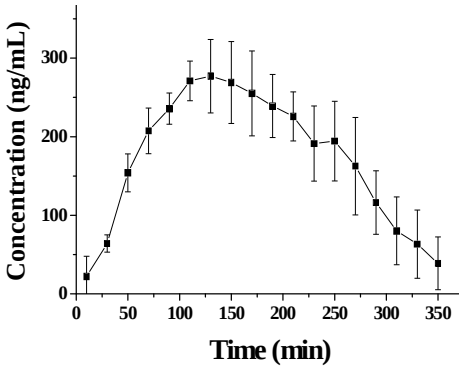

Figure 5: Dermal concentration-time curve of FA in hairless male rats after dermal administration of FA solution at a concentration of 1 mg/mL in PBS (mean ± SD, *n* = 6)

Correlation between FA penetration and pharmacokinetics

The result of correlation between *in vitro* and *in vivo* penetrations of FA after dermal application showed that the linear regression between *Q* and *R* was *Q (%) = 89.334 R + 11.842,* with *r* value of 0.9905, where *Q* % refers to the ratio Q_n/Q_{total} (Q_{total} is cumulative penetration at 240 min) and *R* is the ratio of $AUC/AUC_{0\text{-}240min.}$ When df was 10, the critical value $r_{10,\ 0.01}$ was 0.7079. The value of *r* obtained from *in vitro* and *in vivo* correlation was higher than $r_{10,\ 0.01}$, which indicates a good correlation between the percutaneous absorption of FA *in vivo* and its release *in vitro*. The slope of the equation demonstrated the difference between the two methods.

DISCUSSION

The dissociation constant of FA is 4.58 [11]. At pH values of 5, 6 and 8, the proportion of FA in the un-ionized form was 27.55, 3.66 and 0.038 %, respectively. This means that the un-ionized form of FA decreased with increase in the pH of the medium since P_{app} was only 0.3500 ± 0.0283 in PBS at pH 8, compared with P_{app} values of 3.3507 ± 0.3401 at pH 5, and 1.4636±0.4108 at pH 6. The obvious relationship between FA dissociation and P_{app} demonstrates that ferulic acid in un-ionized form is more likely to penetrate the skin than the ionized form.

Some studies have reported the plasma pharmacokinetics of FA after oral administration [12,13]. A knowledge of the dermal pharmacokinetics of FA would be helpful in achieving more insight into the therapeutic benefits of its antioxidant activity to UV-induced dermal damage. Drugs permeate the skin through three routes: appendage (including eccrine glands and hair follicles), intercellular and intracellular pathways [14]. For ionic compounds, the route of appendages plays a crucial role in the drug delivery through the skin, but the appendage covers only a minor fraction of the skin. This limits the amount of drug that can permeate the skin by the appendage route. The present study detected FA in the dermis from the outset of administration, most likely due to the permeation of ionized form of FA. After the initial permeation procedure, the other two routes began to play a predominate role, and then FA in the un-ionized form slowly permeated the skin and reached a C_{max} of 300.74 ± 31.86 ng/mL in 138 ± 22.8 min. When the penetration of FA was studied at pH 8, it was not detected in the dermis. Since the absolute un-ionized form of FA in this donor solution was very small, the predominant route did not work efficiently. This is consistent with the assumption that FA un-ionized forms permeates the skin more easily. After the removal of FA, an obvious concentration decrease was found in the pharmacokinetics curve, which indicated that the amount of FA deposited in skin was very small. The experiment lasted for 6 h, because the FA concentration was below the LQD at 6 h, which corresponded to mean residence time (MRT) of 301.87 ± 64.50 min.

CONCLUSION

Ferulic acid (FA) permeates the rat skin rapidly, which may be responsible for its powerful photo-protective effect. Since pH has a significant effect on FA penetration, it is advisable to ensure that FA exists in the un-ionized form in the development of future formulations, to ensure stronger antioxidant activity. The pharmacokinetics of FA after dermal administration can be predicted via a simple Franz cell deconvolution method. This is the first time that deconvolution method has been applied to a dermal pharmacokinetics study. Further studies are needed to determine whether this method can also be applied to other compounds.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dr Shou-Ying Du conceived and designed the study. All the experiments were carried out by Dr Jie Bai; Mrs Chang Yang helped with the organization and modification of the manuscript. The references of this study were provided by Dr Peng-Yue Li. Data were organized and analyzed by Mrs Bo-Yu Dong and Mrs Bing Yang. The in vitro penetration experiments and in vivo pharmacokinetic studies were performed with the assistance of Mrs Xiao-nan Chen and Mrs Ya-hua Cui. All authors read and approved the manuscript for publication.

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