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RESEARCH PAPER

Enhanced Bioavailability of L-Carnitine After Painless Intradermal Delivery *vs.* Oral Administration in Rats

Suohui Zhang • Guangjiong Qin • Yan Wu • Yunhua Gao • Yuqin Qiu • Fang Li • Bai Xu

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ABSTRACT

Purpose *In vitro* and *in vivo* permeation studies were conducted to evaluate the characteristic of percutaneous administration of high hydrophilic drug L-carnitine (LC) by Functional MicroArray (FMA) painless intradermal delivery system.

Methods *In vitro* study was designed to assess the effects of various skins, donor concentration and hydrogels from different carbomer derivatives on the release of LC in a Franz-type diffusion cell. The LC gel patches with carbomer 980 P were prepared and successfully applied to pharmacokinetic study of SD rats with and without FMA. Intravenous injection and oral administration were performed to support pharmacokinetic calculations and comparison of bioavailability.

Results Enhanced delivery of LC using FMA was achieved in skin of different species *in vitro* studies. The 750 mg LC gel patches were applied to rats over 6 h, and approximately 27% of loaded dose was transported into rat. A 2.8-fold enhancement of absolute bioavailability for LC with FMA intradermal delivery system was observed compared with oral LC administration *in vivo* study.

Conclusions Both *in vitro* and *in vivo* studies demonstrated that the FMA intradermal delivery system can enhance the delivery and bioavailability of LC.

KEY WORDS absolute bioavailability · functional microarray · hydrogel patch · microneedles · intradermal delivery system · L-carnitine

S. Zhang • G. Qin • Y. Wu • Y. Gao (✉) • Y. Qiu • F. Li • B. Xu
Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry,
Chinese Academy of Sciences,
Beijing 100190, China
e-mail: yhgao@mail.ipc.ac.cn

S. Zhang • G. Qin • Y. Wu
Graduate University Chinese Academy of Sciences,
Beijing 100049, China

B. Xu (✉)
Suzhou Natong Bionanotechnology Co. Ltd,
Jiangsu 215123, China
e-mail: bxu@nanomed-devices.com

INTRODUCTION

L-carnitine (LC) is a naturally occurring compound used by the body to transport long-chain fatty acids across inner mitochondria membrane for β-oxidation. It is a nutrient essential for energy production and fat metabolism in skeletal muscle and heart (1). More than 95% of human body's total carnitine is in myocardium and skeletal muscle (2). In recent years, LC has been extensively used as a medicine in the treatment of carnitine deficiency disorders (3,4), a variety of cardiovascular symptoms (1,5) and the prevention of drug-induced myopathies in patients with HIV infection (6). Furthermore, many researchers found that supplementation of LC has potentially beneficial effects on athlete performance (7), obesity (8), liver healthy (9), hemodialysis (10), male infertility (11) and diabetes (12).

Currently, LC is marketed throughout the world in the form of capsules, tablets, and injectables. Typical doses range from 10–50 mg/kg/day (13). However, oral administration is restricted to a slow uptake into intestinal epithelium due to the relatively high polarity of LC, which impedes its free diffusion across lipid membranes (14,15). Therefore, there is lower oral bioavailability (16). High oral doses of LC may cause body odor and stomach cramps in some patients. On the other hand, intravenous administration of LC makes a transient increase in the body carnitine

pool; it also leads to difficulty distributing into slowly equilibrating tissues such as muscle (13). Consequently, treatment with short-term therapy intravenously leads to little change in muscle LC pool (17), which inhibits the vital effect of LC on fatty acid oxidation in skeleton and cardiac muscle. In summary, the major issue of LC administration is poor bioavailability.

A traditional transdermal patch of LC was in development with a reported reduction of visible signs of cellulite on the skin (18). But there are no rigorous studies and published data on permeability and percutaneous systemic delivery of LC to our knowledge. A proper transdermal formulations would make it possible to deliver LC continuously into systemic circulation at a well-controlled therapeutic rate and avoids gastrointestinal disturbances by the oral route. The major challenges of percutaneous delivery of LC have been attributed to its high dose requirement and extremely high polarity [Log $P=-5.48$] (19). Hence, it is necessary to present a rational strategy to enhance topical permeation of LC to achieve therapeutically meaningful systemic concentration, especially in muscles. As a promising approach, microneedle treatment provides a minimally invasive method to create drug delivery paths, called microconduits, in the skin barrier for enhancing drug delivery. Clinical administration showed that 180 and 280 μm octagonal microneedles significantly reduced pain and discomfort compared to the 25-G hypodermic needle, and the repair and resealing of microconduits were apparent at 8–24 h post application (20). Previously, we have reported that the utility of a solid microneedle system was more efficient in increasing the skin permeability both *in vitro* (21–23) and in clinical application (24).

In this paper, we provide a detailed investigation of percutaneous transport of LC by using painless Functional MicroArray (FMA) with microneedles of 150 μm length. *In vitro* study evaluated the effects of different species of skins, donor concentration and gel formulations on the permeability of LC by FMA intradermal delivery system. *In vivo* study assessed the absolute bioavailability and pharmacokinetic profile of topical gel patch of LC, compared to intravenous and oral administration, to indicate the efficacy of FMA for LC percutaneous administration. The enhancement of LC bioavailability by FMA is the major discovery of this research work.

MATERIALS AND METHODS

Chemicals and Reagents

LC ((3R)-3-hydroxy-4-trimethylazaniumylbutanoate, MW 161.2 Da), carbomer 940 P (CP940), carbomer 974 P (CP974), carbomer 980 P (CP980) and carbomer 1342 P (CP1342) were all purchased from GuoRenYiKang Technology (Beijing, China). HPLC-grade methanol was obtained from Dikma Technology (Beijing, China). All solutions were prepared with ultrapure water (resistivity >18 MΩ.cm). All chemicals used were analytical or pharmaceutical grade.

Functional MicroArray System (FMA)

The Functional MicroArray system has been described previously without modification (22,23). Each FMA has 484 microneedles perpendicular to the wafer, over an area of 10×10 mm^2. Each microneedle has an octagonal pyramidal shape. They are 150 μm in height, with a base length of about 100 μm, a cone angle of 38° and a needle tip less than 100 nm. The FMA was fixed onto the supporting mechanism of an applicator to form an intradermal drug delivery system, which provided an insertion force of approximately 2 N (Fig. 1).

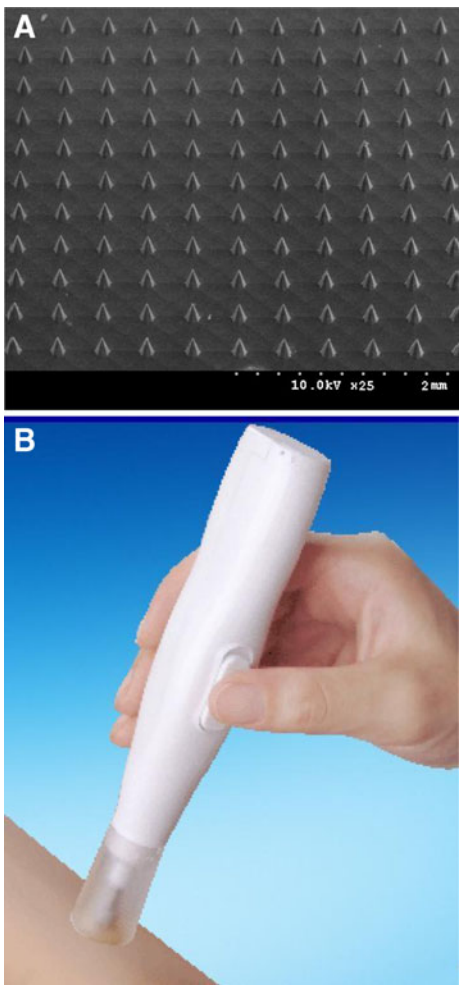

Fig. 1 The pictures of Functional MicroArray employed and the applicator used. (A) Functional MicroArray; (B) applicator.

Preparation of Carbomer Hydrogels

Hydrogels of LC were prepared using carbomer polymers, including CP940, CP974, CP980 and CP1342. Briefly, at a concentration of 0.5% (w/w), carbomers were added to ultrapure water with vigorous mixing, and the dispersion was allowed to hydrate and swell for about 2 h. Then the gels were formed by dropwise addition of triethanolamine (0.8% w/w) to neutralization. Finally, appropriate amount of LC powder was dissolved in the above gels with stirring at room temperature.

Skin Preparation *In Vitro*

Male Sprague–Dawley rats (220–250 g) were provided by Beijing Weitong Lihua Experimental Animals Ltd. Co. (Beijing China). Forty-eight hours before the test, rats were anesthetized by ether, and then the abdominal hair of each rat was shaved with an electric hair clipper. At the beginning of *in vitro* studies, animals were euthanized using carbon dioxide, and full-thickness skins were removed. All research protocols adhered to the Guide for the Care and Use of Laboratory Animals (1996).

Samples of full-thickness dermatomed human cadaver skins (≈ 800 μm) were obtained from the Burns Institute, 304th Clinical Department, The General Hospital of PLA, Trauma Center of Postgraduate Medical College and were free from overt pathology. The skin samples were kept frozen in liquid nitrogen and used within 3 months.

Porcine ear skins (adult pig) were purchased from a local slaughterhouse immediately following the animal death, and the whole skins were carefully dermatomed to a thickness of 800 μm with skin grafting knife. The excised porcine ear was wrapped in plastic film and stored in liquid nitrogen until use within 3 months.

In Vitro Permeation Study

The method of the percutaneous absorption study adheres to the Test Guideline 428 of Organization for Economic Cooperation and Development (2004). The experiment was performed with a system employing Franz-type glass diffusion cells. The temperature in the receptor chamber was maintained at physiological temperature of 37.0±0.1°C with an external, constant temperature, circulating water bath.

Skin samples were treated using FMA delivery system. The insertion force was provided by the applicator. The skins without FMA treatment were used as control. The skin was mounted on a receptor chamber (2.5 ml) with the stratum corneum side facing upward into the donor chamber with effective permeation area of 0.66 cm^2. The receptor and donor chambers were filled with PBS solution, and the receptor fluid was continuously stirred with a magnetic bar at 280 rpm to maintain homogeneity. After 1 h equilibration, the solution in the receptor chamber was replaced with fresh PBS, and 300 μl of LC solution or 300 mg of LC gel was applied on the skin in the donor chamber, which was then covered with a parafilm to avoid any evaporation process. The samples of receptor cell were withdrawn through at predetermined time intervals, and the receptor phase was immediately refreshed by equal volume of PBS buffer to keep a constant volume. The samples were analyzed by HPLC/UV. The results were expressed as the mean±S.D. (n=3–4 independent samples).

In Vivo Permeation Study

Male Sprague–Dawley rats (250 ±10 g) were equally divided into four groups (3 for each): groups A, B, C and D. Before administration, all rats were allowed to acclimatize for 1 week. One day before administration, rats were fasted overnight but allowed access to water. The hair of abdominal skin was carefully shaved by electric clippers for groups A and B. The rats in group A were pretreated by FMA with an area of 2 cm^2; group B was untreated as a control. Then hydrogel patch containing LC 750 mg was applied to each rat by an adhesive housing for 6 h. Group C and group D were control groups of oral and intravenous injection administration of 1 ml LC solution (200 mg/ml). A volume of 0.5 ml blood samples was taken before administration and at predetermined time intervals after LC administration. Plasma samples were immediately separated by centrifugation at 4000×g for 7 min and stored at −80°C until assay.

Patch Residual Assay of LC

To determine the apparent dose of LC delivered from gel patch in the *in vivo* study, patches were collected after 6 h application and stored at 4°C until assay. Each patch was transferred into a suitable flask, and ultrapure water was added. The mixture was shaken for 20 min and then centrifuged for 8 min at 10000×g. Supernatant solution was analyzed by HPLC/UV. Apparent dose of LC delivered was calculated as difference between initial and residual drug content in patch.

Assay Methods

In Vitro

The quantitative determination of LC was performed by HPLC using methanol-190 mM KH_2PO_4 water (13:87) as

mobile phase at a flow rate of 0.6 ml/min, by LC-2010A (Shimadzu, Japan). The injection volume was 20 μl. The analysis was performed in a YMC-Pack ODS-A C18 column with dimensions of 250×4.6 mm i.d., 5 μm particle size (YMC Inc., USA). The column eluant was monitored at 225 nm. The detection limit is 20 μg ml^{-1}. The inter- and intra-day variability was less than 5%.

In Vivo

Pretreatment method of samples: 120 μl of plasma was deproteinized with 600 μl of 0.6 M perchloric acid. The mixture was shaken for 30 s and allowed to stand in an ice bath for about 15 min. After being centrifuged at 4000×g for 7 min, 500 μl of supernatant was transferred to a new tube in which 300 μl of 0.5 M K_2CO_3 was added; the mixture was shaken for 30 s and incubated in an ice bath for 15 min. Precipitate of $KClO_4$ was removed by centrifugation at 4000×g for 7 min, and 200 μl of the supernatant with free LC was separated for the assay. The recovery ratio of LC in plasma was 85±2%.

Assays: The concentration of free LC in serum was determined by enzymatic assay kit with a limitation detection of 0.8 μg /ml. (BIOSENTEC France), Cat. No.066.

Calculations and Statistical Analysis

In Vitro

The cumulative amount of drug permeated per unit area *versus* time was plotted. The permeation rates of LC were calculated from the slope of linear portion of the plots. Data analysis was carried out with Microsoft Excel, Version 2000. Results were presented as the mean±S.D. (n=3–4 independent samples). Statistically significant differences were determined using the analysis of variance (ANOVA) with $P<0.05$ as a minimal level of significance.

In Vivo

Maximum plasma concentration (C_{max}) and the time to reach this peak (T_{max}) were directly identified from the pharmacokinetic curves (LC plasma concentration *versus* time). Area under the plasma concentration-time curve ($AUC_{0\to\infty}$) was calculated by using the trapezoidal rule. Absolute bioavailability (A.B.) of LC after oral and FMA administration compared to intravenous administration was calculated using the following equation:

$$\text{A.B.} = \frac{\text{AUC FMA/oral}}{\text{dose FMA/oral}} \times \frac{\text{dose iv.}}{\text{AUC iv.}} \times 100$$

RESULTS AND DISCUSSION

In Vitro Percutaneous Delivery of LC through Skins of Different Species

First, the effect of skins from different species on LC permeability was investigated. As indicated in Fig. 2, the highest cumulative amount of LC over 8 h by passive diffusion could be seen for rat skin. After pretreatment with FMA, the cumulative amount of LC was greatly enhanced for all skins, and it was similar between human and porcine ear skin.

The results of percutaneous delivery of drug are influenced by skin of different species. Human skin is considered as the best material for *in vitro* experiment, but its availability and proper storage are sufficiently challenging. Fortunately, a wide range of animal skin models have been well established as alternatives to human skin. These animal skin models include rat, mouse, rabbit, pig, guinea pig and snake skins. However, there may be variation in the drug permeability of various skins, so it is necessary to evaluate the effect of skin model used in the study of a new drug delivery method and its proper formulation.

Table I shows the permeation rates of LC across different species of skin by passive diffusion or with FMA pretreatment. The permeation rates of LC by FMA were significantly enhanced in all species of skin compared to passive diffusion. The permeation rate of LC through human skin was enhanced 59 times compared to passive diffusion and was nearly the same with porcine ear skin, but there were significant differences between human and porcine ear skin by passive diffusion ($p<0.05$). Furthermore, the permeation rate of LC through rat skin was about 13.8 times higher than that of human skin by passive diffusion. After FMA-puncture,

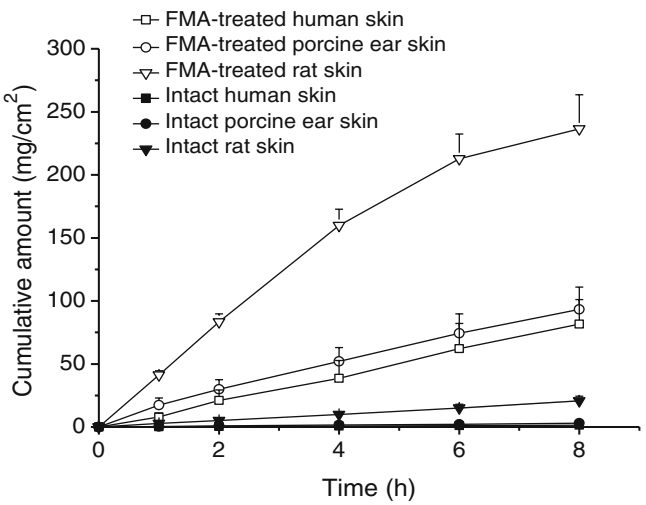

Fig. 2 The LC profiles of cumulative amount *vs.* time using skins of different species with and without FMA. Mean±S.D., $n\geq3$. Each donor concentration was 640 mg/mL.

Table I Permeation Rates and Enhancement Ratio (ER) of LC Across Different Species of Skins with and without FMA Delivery System

Skin type	Thickness	Permeation rates (mg/cm^2/h)		ER
		FMA	Passive diffusion	(%)
Human	Dermatomed≈ 800 μm	10.65±2.12	0.18±0.02	59
Porcine ear	Dermatomed≈ 800 μm	11.08±2.05	0.37±0.04	30
Rat	Full thickness	40.16±3.97	2.49±0.40	16

Enhancement ratio (ER), FMA permeation rates / passive permeation rates
Data are mean±S.D., n≥3.

the difference was only 3.7 times, significantly reduced compared to passive diffusion. The above results implied that FMA may reduce the interspecies variations in skin permeability. Previous studies have indicated that iontophoresis could reduce the interspecies differences in transdermal permeation of drugs (25,26). The results of the present study were similar to the above observations. They indicate that FMA puncture overcomes the stratum corneum, the major skin barrier. Therefore, differences among the resistance of various species of skin were decreased.

In Vitro Effect of Donor Concentration

Further experiments were carried out using porcine ear skin pretreated by FMA because the peameability of LC through porcine ear skin is close to human skin. The influence of drug concentration on the FMA intradermal transport of LC was presented in Fig. 3. The permeability of LC increased with

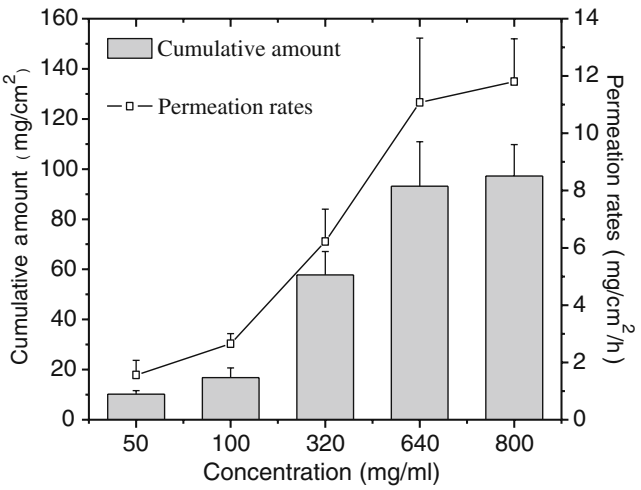

Fig. 3 The effect of different LC donor concentrations on the LC cumulative amount and permeation rates through porcine ear skin by FMA delivery system during 8 h. mean±S.D., $n=3$.

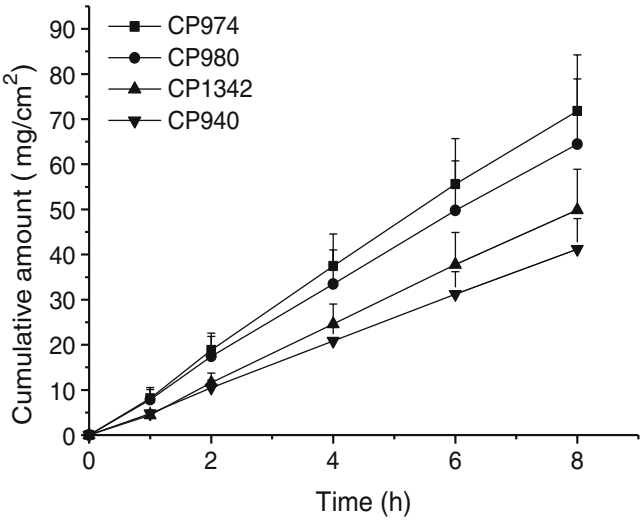

Fig. 4 Diffusion profiles of the gels with different types of carbomer polymers on the release of LC across porcine ear skin by FMA-treated. mean±S.D., $n=4$.

increasing donor concentration until 640 mg/ml. In summary, the cumulative amount and permeation rates of LC were not significantly different between 650 mg/ml (93.23±17.76 mg/cm^2 and 11.08±2.05 mg/cm^2/h) and 800 mg/ml (97.20±12.60 mg/cm^2 and 11.80±1.50 mg/cm^2/h) ($P>0.05$). The results show that there may be a certain threshold of FMA transport, since increasing donor concentration no longer resulted in increase of drug transport. This type of cut-off phenomenon has been reported in microneedle-mediated transdermal delivery of human IgG (28). This may be due to the transport pathway of the drug through skin becoming saturated at a higher concentration.

In Vitro Effect of Different Type of Carbomer Polymers

At present, numerous grades of carbomer polymer are commonly available for the transdermal hydrogel formulations because of their low irritation and high viscosity at low concentration. Different types of carbomer varying in cross-link density and molecular weight could influence

Table II Summary of Apparent Dose of LC Delivered with and without FMA Delivery System

Parameters	With FMA	Without FMA
Total amount of LC in patch (mg/rat)	750	750
The residual amount in patch (mg/rat)	550±22	764±30
Apparent dose of LC (mg/rat)	200	ND
LC delivered (%)	27%	ND

ND not determined

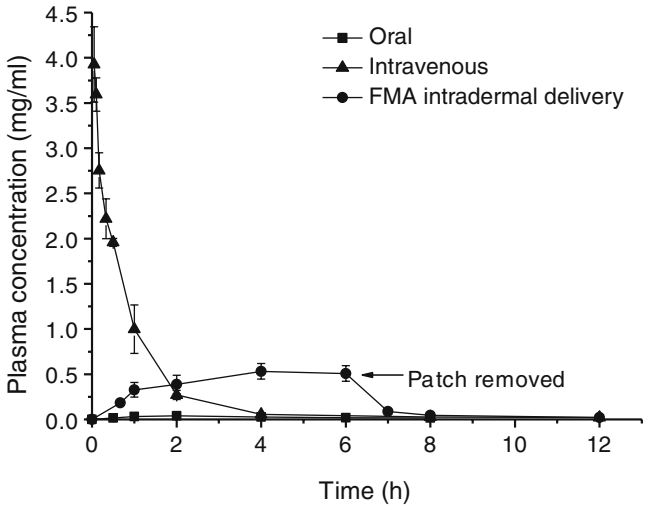

Fig. 5 Mean plasma concentration *vs.* time profiles of LC after single intravenous (200 mg/rat), oral (200 mg/rat), and FMA intradermal administration (750 mg/rat; 2 cm^2). mean±S.D., $n=3$.

the diffusion pathway and drug release (29). The influence of different carbomer polymers (e.g., CP940, CP974, CP980, CP1342) on the permeability of LC was studied across porcine ear skin. Skin was pretreated by FMA, and LC hydrogel (640 mg/g) was applied to each donor.

Fig. 4 illustrates the cumulative amount of LC penetrated from the various kinds of carbomer formulations. Total cumulative amount of LC from CP980 and CP974 was significantly higher than that from CP1342 and CP940 ($P<0.05$). Furthermore, there was no significant difference between the cumulative amount from CP974 (71.78± 12.44 mg/cm^2) and CP980 (64.43±14.48 mg/cm^2) ($P>0.05$). It was noteworthy that the viscosity of CP980 calculated by rheological synergism was significantly higher than CP974 (30). Therefore, gel containing CP 980 was elected as a suitable carrier for topical application of LC.

In Vivo Assessment of Apparent Dose of LC Delivered into Rats from Gel Patch

The residual content of drug in the patch was investigated to assess the apparent LC dose delivered by applying topical gel formulation CP980 (750 mg/rat) to rats with and without FMA pretreatment.

Table II presents the mean apparent dose of LC gel patch delivered after 6 h topical application with and without FMA pretreatment. About 27% of initial amount of LC was delivered into rats by FMA pretreatment, totaling 200 mg/rat. However, there was no significant difference between the residual and initial content by passive diffusion. These results showed that FMA intradermal delivery successfully allowed much higher apparent dose of LC delivery than traditional percutaneous delivery. It implied that FMA intradermal delivery system could solve the problem of high dose requirement for LC.

Phamacokinetics and Absolute Bioavailability of LC

Fig. 5 shows the plasma concentration-time profiles of LC after intravenous (200 mg/rat), oral (200 mg/rat) and FMA percutaneous administration (750 mg/rat). The corresponding pharmacokinetic parameters are summarized in Table III. After intravenous administration, the plasma concentration was up to 3.90±0.42 mg/mL at 3 min, but rapidly decreased to 0.27±0.05 mg/mL at 2 h. Oral administration brought low plasma levels, and the plasma concentration peaked at 2 h (0.040±0.011 mg/mL). The absolute bioavailability was 8%. Compared to oral administration, higher plasma concentrations were obtained by using FMA intradermal delivery system. At the same time, the $AUC_{0-\infty}$ was 3016 mg.h.L^{-1}, 10-fold higher than that of oral administration, and the absolute bioavailability was 22%. A maximum concentration (C_{max}) of 0.53±0.086 mg/mL was achieved at 4 h, which was about 13-fold higher than that following oral administration. Furthermore, the plasma level was maintained relatively smooth from 1–6 h in the range of 0.33 mg/mL to 0.51 mg/mL. After patch removal, plasma level of LC rapidly declined, suggesting LC percutaneous delivery into the corium and into systemic circulation. Pharmacokinetic study demonstrated that, after FMA intradermal administration, the absorption of LC was stable over the whole administration period of 6 h compared to intravenous application. It is notable that absolute bioavailability for LC FMA intradermal delivery was approximately 2.8 times higher than oral application. A variety of experiments, including human (31) and rats (14,15), have concluded that the low bioavailability of oral-delivered LC has been ascribed to poor absorption across the intestinal epithelium. Hence, it's easy to understand that the bioavailability of LC following FMA intradermal delivery would be higher than oral administration because of its direct absorption via blood

Table III Pharmacokinetic Parameters of LC Determined After Single Intravenous, Oral and FMA Intradermal Administration ($n=3$)

	Dosage	C_{max}(mg/ml)	T_{max} (h)	$AUC_{0-\infty}$ (mg.h.L^{-1})	A.B. (%)
Intravenous	200 mg/rat	–	–	3733±215	100
Oral	200 mg/rat	0.040±0.011	2	317±48	8
FMA delivery	750 mg/rat	0.53±0.086	4	3017±548	22

vessels among dermal layer. Therefore, FMA intradermal delivery administration would provide a new and effective administration strategy for enhancing bioavailability of LC and potentially improve patient compliance with additional benefits such as controlled stable release of drugs to minimize toxicity while maximizing therapeutic outcome.

CONCLUSIONS

The present work illustrated that FMA painless intradermal delivery system could be used for percutaneous administraton of LC. *In vitro* studies indicated that the LC permeability with FMA-assisted transport across different skins was significantly increased compared to passive diffusion, and permeation across human skin was about 59 times higher than passive diffusion. The pharmacokinetic study showed that FMA intradermal delivery of LC gel patch would give relatively smooth and continuous plasma levels compared to conventional dosage forms. A 2.8-fold enhancement of absolute bioavailability was obtained compared to oral administration. In summary, FMA intradermal delivery system represents a promising and beneficial method for LC administration and may be possibly extended to other high hydrophilic drugs. Further studies should be conducted to probe the tissue concentration of LC in rat tissues (e.g., muscle, heart, kidney and liver) by FMA intradermal delivery.

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