Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 352 (2008) 92–103
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Effect of *O*-acylmenthol on transdermal delivery of drugs with different lipophilicity

Ligang Zhao, Liang Fang *, Yongnan Xu, Yanyan Zhao, Zhonggui He

Department of Pharmaceutical Sciences, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang, Liaoning 110016, China

Received 25 August 2007; received in revised form 9 October 2007; accepted 17 October 2007
Available online 22 October 2007

Abstract

To develop more effective compounds as enhancers, *O*-acylmenthol derivatives which were expected to be enzymatically hydrolyzed into nontoxic metabolites by esterases in the living epidermis were synthesized from *l*-menthol and pharmaceutical excipient acids (lactic acid, cinnamic acid, salicylic acid and oleic acid) in this study. Their promoting activity on the percutaneous absorption of five model drugs, 5-fluorouracil (5-FU), isosorbide dinitrate (ISDN), lidocaine (LD), ketoprofen (KP), and indomethacin (IM), which were selected based on their lipophilicity represented by $\log K_{O/W}$, were tested *in vitro* across full thickness rat skin with each of the evaluated drugs in saturated donor solution. 2-Isopropyl-5-methylcyclohexyl 2-hydroxypanoate (M-LA) provided the highest increase of accumulation of 5-FU (3.74-fold) and LD (4.19-fold) in the receptor phase while 2-isopropyl-5-methylcyclohexyl cinnamate (M-CA) was ineffective for most of the drugs; Both 2-isopropyl-5-methylcyclohexyl 2-hydroxybenzoate (M-SA) and (E)-2-isopropyl-5-methylcyclohexyl octadec-9-enoate (M-OA) had better promoting effects on the drugs with low water-solubility. The four *O*-acylmenthol enhancers produced parabolic relationship between the lipophilicity ($\log K_{O/W}$) of the model drugs (5-FU, ISDN, KP, IM) and their enhancement ratio of the permeation coefficient (ER_P), indicating that the lipophilicity of the penetrants has significant effect on the permeation results, $r = 0.989$ ($P = 0.144$) for M-LA, $r = 0.965$ ($P = 0.216$) for M-CA, $r = 0.786$ ($P = 0.630$) for M-SA, and $r = 0.996$ ($P = 0.088$) for M-OA.

Keywords: *O*-Acylmenthol derivatives; Pharmaceutical excipient acids; Percutaneous absorption; Drug lipophilicity

1. Introduction

The dermal (topical) or transdermal administration of drugs for the treatment of local or systemic conditions overcomes several important limitations associated with more conventional forms of drug delivery (e.g. oral, injection), such as gastro-intestinal irritation/hepatic first-pass metabolism and inadvertent systemic drug absorption (Nanayakkara et al., 2005). However, the stratum corneum (SC), the outermost layer, is the principal rate-limiting barrier to percutaneous delivery. The horny layer is composed of keratin-rich cells embedded in a multiple lipid bilayer which mainly consists of ceramides, cholesterol and free fatty acids (Pilgram et al., 1998). It is widely accepted that the intercellular lipid domain is the main pathway by which most drugs pass through the SC (Moghimi et al., 1996). A popular technique to overcome skin impermeability is the use of penetration enhancers which reduce reversibly the permeability barrier of the SC. Over the last three decades much research has concentrated on studying the enhancing ability of a wide range of substances and their mechanisms of action (Williams and Barry, 2004). Among these, a variety of terpenes, especially *l*-menthol, have been investigated as enhancers for drugs such as fluorouracil (Cornwell and Barry, 1993), tamoxifen (Gao and Singh, 1998), zidovudine (Thomas et al., 2004), morphine hydrochloride (Morimoto et al., 2002) and several cardiovascular agents (Kobayashi et al., 1993). Recently, some researchers have synthesized a series of *l*-menthol derivatives and evaluated their promoting activity on the percutaneous absorption of ketoprofen from hydrogel in rats both *in vitro* and *in vivo* (Negishi et al., 1995; Nakamura et al., 1996; Takanashi et al., 1999; Obata et al., 2001). Unfortunately, the only synthetic *l*-menthol derivative that has exhibited both high enhancing potency and low toxicity is *O*-ethylmenthol (MET). However, none of the *l*-menthol derivatives has been used in clinical situations. In our previous study, a series of *O*-acylmenthol derivatives was synthesized as candidates for percutaneous absorption enhancers by *l*-menthol

* Corresponding author. Tel.: +86 24 23986330; fax: +86 24 23986330.
E-mail address: fangliang2003@yahoo.com (L. Fang).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.10.017

and saturated fatty acids, and their promoting activities were evaluated using model drugs with different lipophilicity (Zhao et al., 2007).

In this study, *l*-menthol was selected again as a lead compound for the synthesis of new types of *O*-acylmenthol derivatives as candidates for percutaneous absorption enhancers with some pharmaceutical excipient acids, including lactic acid (LA), cinnamic acid (CA), salicylic acid (SA) and oleic acid (OA). LA has been reported to exhibit enhancement of ibuprofen lysine (Sebastiani et al., 2005) and 5-FU (Copoví et al., 2006), and some researchers have also reported that cinnamic acid can pass through hairless rat skin (Zhu et al., 2003) and human skin easily (Bronaugh et al., 1985) and can promote the percutaneous absorption of ligustrazine hydrochloride (Zhang et al., 2007). SA, representing a pseudo β-hydroxy acid, is widely used as a peeling or keratolytic agent to treat calluses, keratosis or warts (Loden, 2000), and its dermatopharmacological effect may be related to its impact on the SC structure affecting intercorneocyte cohesion and desquamation. However, few reports have described its use as a penetration enhancer to our knowledge. OA is a well-known percutaneous penetration enhancer and mechanism of action has been studied by a variety of techniques such as infrared spectroscopy (Francoeur et al., 1990) and permeation investigations (Barry and Bennett, 1987). Accordingly, *O*-acylmenthol derivatives can be considered as a chemical combination of *l*-menthol and pharmaceutical excipient acids, both of which are known to be potent percutaneous absorption enhancers. More over, as esterases are present in the human and animal epidermis (Montagna, 1955), the ester linkage offers the possibility of degradation by skin esterases. The similar researches have been done by some other researchers (Vávrová et al., 2005a; Hrabálek et al., 2006) who found the compound could be hydrolyzed into nontoxic metabolites *in vitro* using porcine esterase. Examination of the effects of these moieties on the action of penetration enhancers, therefore, should give useful information to help with the development of absorption enhancers.

To investigate the effectiveness of these enhancers on penetrants having different physicochemical properties, the enhancement of the skin penetration of five drugs with a wide range of *n*-octanol/water partition coefficients by *O*-acylmenthol enhancers was examined in this report. The purpose of the present study was to investigate if a correlation exists between the efficacy of the *O*-acylmenthol derivatives with different lipophilicity and the log $K_{O/W}$ of the model drugs (5-FU, ISDN, LD, KP, IM, the physicochemical parameters of these five model drug are presented in Table 1).

2. Materials and methods

2.1. Materials

5-FU, ISDN, LD, KP, IM were supplied by *Fangge Pharmaceutical* Co., Ltd. (Zhejiang China), *Huangshan Zhonghuang Pharmaceutical* Co., Ltd. (Anhui China), *Jianglong Pharmaceutical* Co., Ltd. (Shanxi China), *Zhongdan Pharmaceutical* Co., Ltd. (Jiangshu China), *Dahongyin Pharmaceutical* Co., Ltd. (Ningbo China), respectively. Acetaniline, propylparaben and butoben were purchased from *Beijing Xingjin Chemical* Plant (Beijing China); isopropyl myristate (IPM), lactic acid, cinnamic acid, salicylic acid, oleic acid, tetrahydrofuran (THF), *para*-toluenesulfonic acid (PTS), *l*-menthol and *N*,*N*′-Dicyclohexylcarbodimide (DCC) were supplied by *China National Medicines* Co., Ltd. (Shanghai, China); Methanol and acetonitrile of HPLC grade were obtained from the *Yuwang Pharmaceutical* Co., Ltd. (Shandong, China). All other chemicals were of the highest reagent grade available.

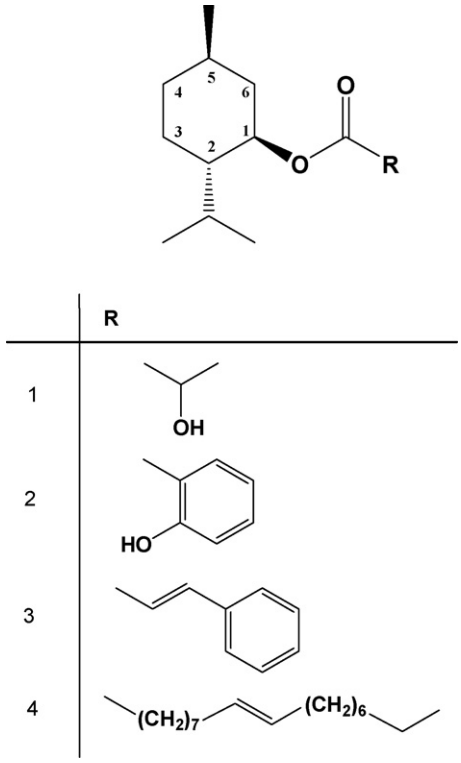

Fig. 1. The chemical structure of *O*-acylmenthol derivatives used as percutaneous penetration enhancers.

2.2. Synthesis of O-acylmenthol derivatives

The chemical structures of the synthetic *O*-acylmehthol derivatives are shown in Fig. 1. The reaction sequences for the preparation of 2-isopropyl-5-methylcyclohexyl cinnamate (M-CA) and (E)-2-isopropyl-5-methylcyclohexyl octadec-9-enoate (M-OA) are presented as follows: 0.25 mol CA or OA was dissolved in THF (90 ml), and 0.2 mol dichloro sulfoxide was added, heated to 80 °C. The reaction mixture was kept for 4 h at this temperature, than added 0.15 mol *l*-menthol dissolved in 30 ml THF, the reaction mixture was still kept at 80 °C for 3 h. The solvent of the mixture was removed under reduced pressure and cooled to ambient temperature, then adjusted the pH to ∼7 with 10% NaOH, extracted three times with diethyl ether, the diethyl ether layer was separated, washed with brine, after that it was dried over anhydrous $MgSO_4$, filtered, and the solvent was removed under reduced pressure.

Table 1
Physicochemical properties of 5-FU, ISDN, LD, KP and IM

Parameters	Drugs				
	5-FU	ISDN	LD	KP	IM
Melting point[a] (°C)	282–283	70	68–69	94	160
MW (g mol^{-1})	130.08	236.14	234.3	254.28	357.8
log $K_{O/W}$[b] at 32 °C	−0.95[c]	1.34[d]	2.56[e]	3.11[f]	3.80[g]
Solubility in water at 32 °C (mg/ml)	12.41[d]	0.52[d]	6.81[h]	0.18[i]	0.07[j]
pKa[k]	12.0	–	12.9	3.88	4.5

[a] From the Merk index.
[b] $K_{o/w}$ is *n*-octanol/water partition coefficient.
[c] Kirschbaum (1980).
[d] Li et al. (2002).
[e] Strichartz (1990).
[f] Morimoto (1992).
[g] Deppeler (1981).
[h] Brodin et al. (1984).
[i] Alvalez et al. (1999).
[j] HO et al. (1998).
[k] Calculated using MARVIN software.

The reaction sequences for the preparation of 2-isopropyl-5-methylcyclohexyl 2-hydroxybenzoate (M-SA) are outlined as follows: 0.10 mol *l*-menthol was dissolved in THF (100 ml), and 0.2 mol SA and 0.1 mol DCC were added, heated to 80 °C. The reaction mixture was kept for 24 h at this temperature. The mixture was cooled to ambient temperature and then alkalified by 10% $NaHCO_3$ and extracted three times with diethyl ether. The combined organic layers were washed with brine and the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate 15:1 as an eluent.

The reaction sequences for the preparation of 2-isopropyl-5-methylcyclohexyl 2-hydroxypanoate (M-LA) are outlined as follows: 0.20 mol LA was slowly dropped to dry toluene at 60 °C, and stirred at the same temperature for 4 h to dehydrolysis. Toluene was evaporated in vacuum and the residue was added to a 40 ml THF solution of *l*-menthol (containing 0.1 mol *l*-menthol), then added 0.1 mol PTS, the reaction mixture was kept at 80 °C for 24 h. The mixture was cooled to ambient temperature and then alkalified by 10% NaOH and extracted three times with diethyl ether. The combined organic layers were washed with brine and the residue was subjected to column chromatography (petroleum ether/ethyl acetate 15:1) to yield M-LA.

Physicochemical parameters of the synthetic enhancers are shown in Table 2, and the purity of each compound was over 98% as shown by gas chromatography (GC-14C, Shimadzu, Japan). The stabilities of *O*-acylmenthol derivatives were also investigated by gas chromatography, the results indicated these compounds were stable at ambient temperature within two years. The structures of the compounds were confirmed by NMR (ARX-300, Bruker, Switzerland) and HPLC-MS (ZQ-2000, Waters, USA). The ^{1}H NMR and MS data are as follows:

2.2.1. M-LA. ^{1}H NMR (CDCl3), δ

0.78(3H, d, J = 7.0 Hz, Me-2-isopropyl), 0.85(3H, d, J = 2.2 Hz, Me-2-isopropyl), 0.91(3H, d, J = 1.7 Hz, Me-5), 0.96–1.05(3H, *m*), 1.33–1.45(2H, m), 1.73–1.83(2H, m), 1.91–1.94(2H, m), 1.96(3H, d, Me-ester), 3.43(1H, sex, J = 4.3 Hz, 10.4 Hz, H-1), 3.73(1H, q, J = 7.0 Hz); ESI-MS *m/z*: 229.3 [M$^+$].

2.2.2. M-CA. ^{1}H NMR ($CDCl_3$), δ

0.77(3H, d, J = 7.0 Hz, Me-2-isopropyl), 0.91(3H, d, J = 3.0 Hz, Me-2-isopropyl), 0.93(3H, d, J = 2.4 Hz, Me-5), 1.03–1.20(3H, m), 1.26(1H, s), 1.51–1.63(2H, m), 1.72–1.77(2H, m), 2.08–2.15(1H, m), 4.83(1H, sex, J = 4.4 Hz, 10.8 Hz, H-1), 6.44(1H, d, J = 16 Hz), 7.38(3H, m), 7.52–7.55(2H, m), 7.67(1H, d, J = 16 Hz); ESI-MS *m/z*: 287.4 [M$^+$].

2.2.3. M-SA. ^{1}H NMR ($CDCl_3$), δ

0.80(3H, d, J = 7.0 Hz, Me-2-isopropyl), 0.91–0.95(7H, m), 1.07–1.20(2H, m), 1.26(1H, s), 1.51–1.61(2H, m), 1.72–1.77(2H, m), 1.91–1.97(1H, m), 2.08–2.15(1H, m), 5.16(1H, sex, J = 4.4 Hz, 10.9 Hz, H-1), 6.87(1H, t, J = 7.3 Hz, phen-4), 6.98(1H, d, J = 9.2Hz, phen-3), 7.44(1H, qui, J = 1.6 Hz, 7.8 Hz, phen-5), 7.84(1H, q, J = 1.6 Hz, 7.8 Hz, phen-6); ESI-MS *m/z*: 277.4 [M$^+$].

Table 2
Physicochemical properties of IPM and *O*-acylmenthol derivatives

Enhancers	Parameters			
	log $K_{O/W}$[a]	MW (g mol^{-1})	SP[b] (J cm^{-3})$^{1/2}$	PLB[c]
IPM	5.61	270.45	16.76	32.82
Menthol	2.78	156.28	20.54	19.05
M-LA	2.89	228.33	20.79	25.33
M-CA	5.28	286.40	17.97	34.16
M-SA	5.60	276.37	22.08	31.20
M-OA	9.22	420.71	16.73	52.15

[a] Calculated using MARVIN software.
[b] Solubilty parameter, calculated by approaches of Hoftyzer/Van Krevelen (Krevelen and Krevelen, 1990).
[c] Polarizability, calculated using MARVIN software.

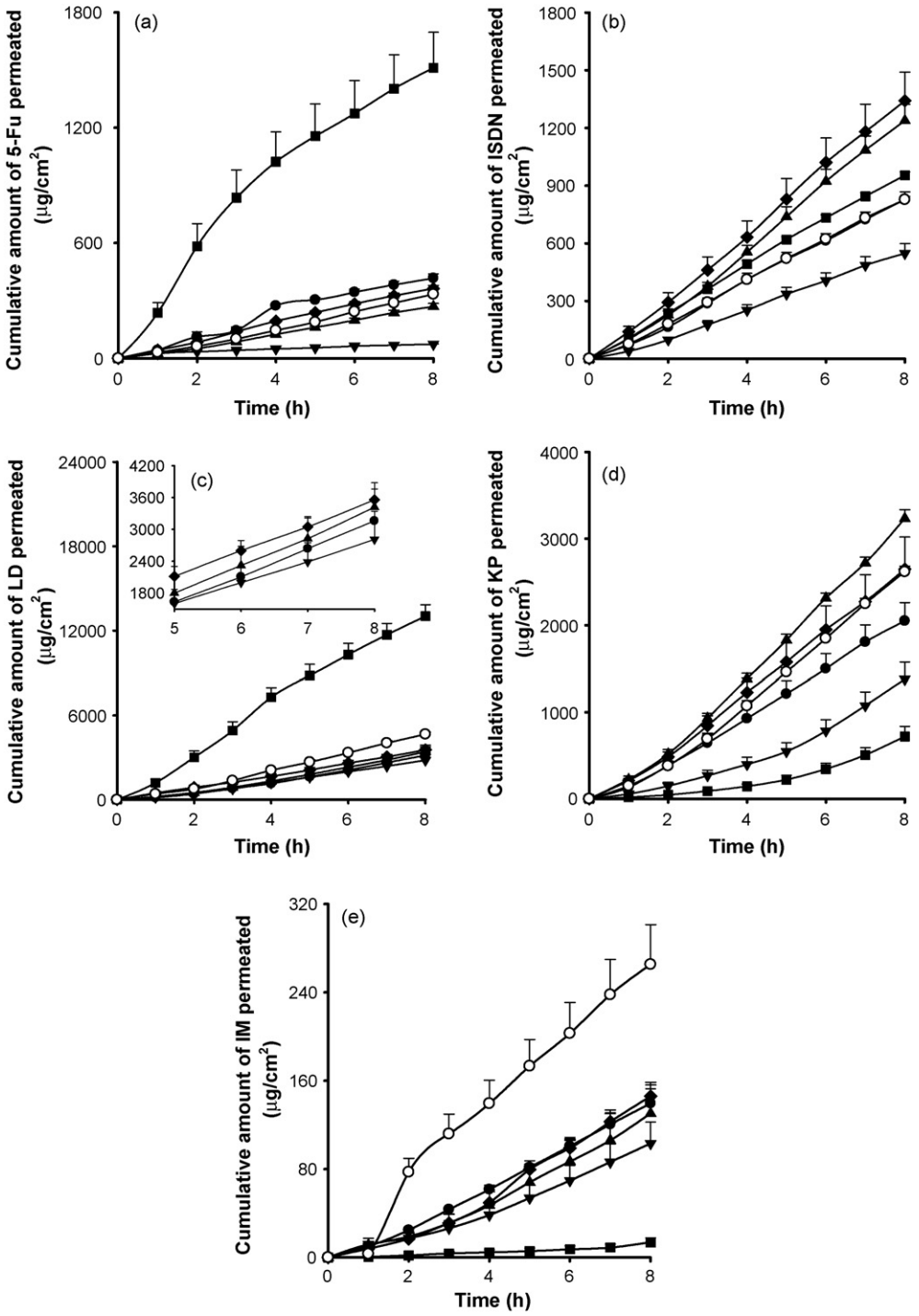

Fig. 2. Permeation profiles of drugs through rat skin (average ± S.E., $n = 4$). Profile (a) is 5-FU; profile (b) is ISDN; profile (c) is LD; profile (d) is KP; profile (e) is IM. Key: (●) is control; (○) is *l*-menthol; (■) is M-LA; (▼) is M-CA; (▲) is M-SA; (♦) is M-OA.

2.2.4. M-OA. ^1H NMR ($CDCl_3$), δ

0.75(3H, d, $J = 6.9$ Hz,Me-2-isopropyl), 0.88(3H, d, $J = 2.3$ Hz, Me-2-isopropyl), 0.91(3H, d, $J = 1.7$ Hz, Me-5), 0.80–1.11(7H, m), 1.19–1.48(19H, m), 1.59–2.05(11H, m), 2.27(2H, t, $J = 7.4$ Hz), 4.68(1H, sex, $J = 4.2$ Hz, 10.8 Hz, H-1), 5.34(2H, t, $J = 5.4$ Hz, delta); ESI-MS *m/z*: 421.7 [M^+].

2.3. Drug analysis

The HPLC system for analyzing drug concentrations was equipped with an L-2420 variable-wavelength ultraviolet absorbance detector and an L-2130 pump (Hitachi High-Technologies Corporation, Tokyo, Japan). Samples were introduced 20 μl into a Rheodyne Model 7725 loop injector

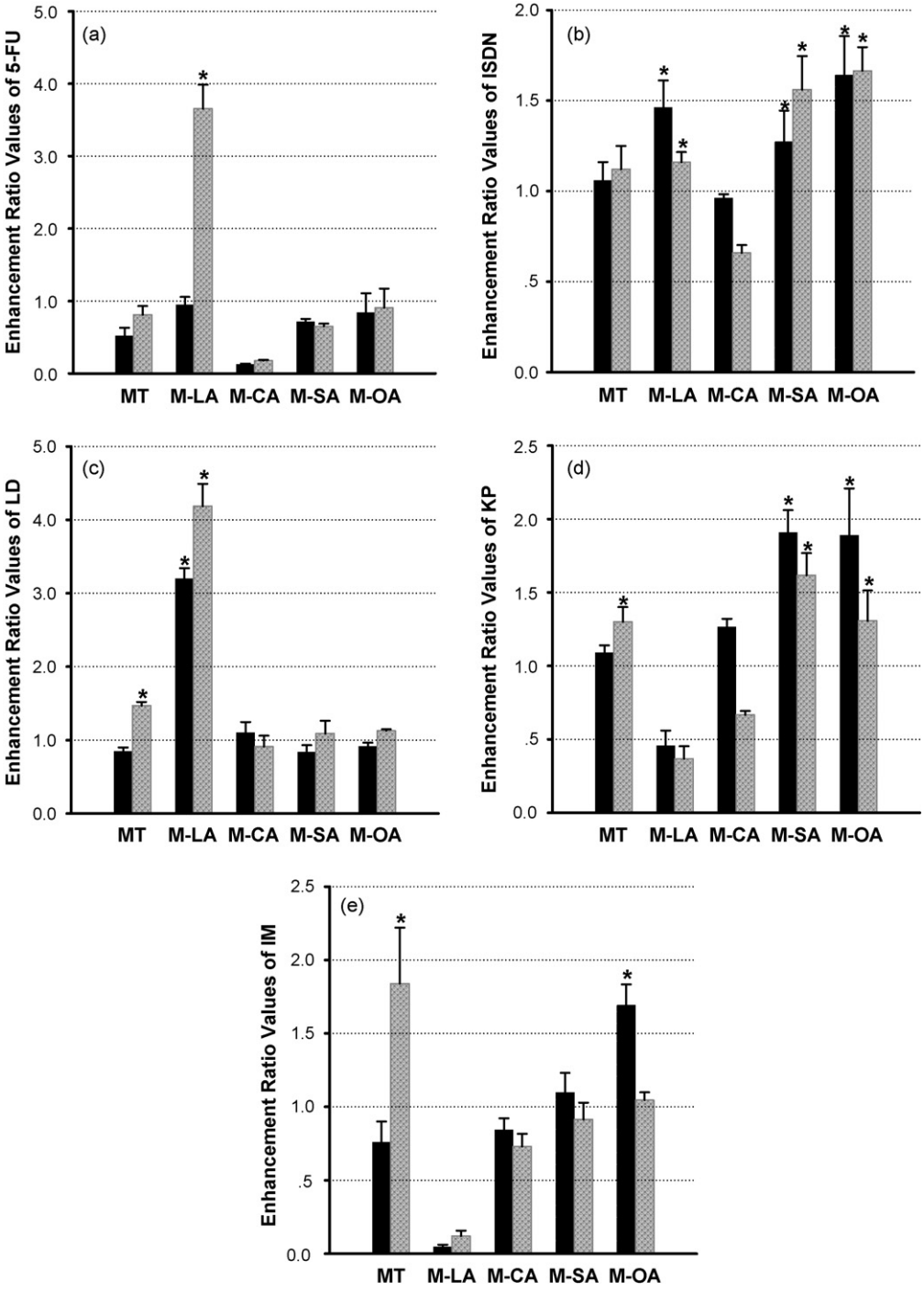

Fig. 3. Enhancement ratio (average ± S.E.; $n = 4$) of drugs through rat skin, the ER_P is the enhancement ratio for the parameter P (cm/h). ER_{Q8} is the enhancement ratio for the accumulative amount of drug ($\mu g/cm^2$) at 8 h, MT is abbreviation of *l*-menthol. Profile (a) is 5-FU; profile (b) is ISDN; profile (c) is LD; profile (d) is KP; profile (e) is IM. Key: ( ) is ER_P; ( ) is ER_{Q8}. *Mean value is significantly different from control group ($P < 0.05$).

equipped with a 20 μl loop. A reversed phase stainless-steel column (20 cm × 4.6 mm) was packed with Diamonsil C-18 (5 μm particle size; Dikma Technologies, Beijing, China). The HPLC conditions were as follows: the mobile phase for 5-FU consisted of acetonitrile and 0.0364 M/L monobasic potassium phosphate in distilled water (1:99, v/v), the wavelength was set at 265 nm (Zheng et al., 2007), the retention time of 5-FU was 5.5 min, an external calibration method was used. The mobile phase for ISDN consisted of methanol and 0.1% acetic acid in distilled water (50:50, v/v), the wavelength was set at 230 nm, and acetanilide was used as an internal standard, the retention times for ISDN and internal standard were 9.5 and 5.4 min, respectively. The mobile phase for LD consisted of methanol, distilled water, acetic acid and triethylamine (62:38:0.3:0.6, v/v), with detection at 230 nm, and acetanilide was used as an internal standard, the retention times for LD and internal standard were 6.7 and 3.8 min, respectively. The mobile phase for KP consisted of methanol and 0.5% acetic acid in distilled water

(3:1, v/v), the pH was adjusted to 6.0 with triethylamine, with detection at 260 nm, and butoben was employed as the internal standard, the retention times for KP and internal standard were 7.6 and 4.3 min, respectively. The mobile phase for IM was the same as KP, the wavelength was set at 266 nm, and propylparaben was used as the internal standard, the retention times for IM and internal standard were 7.1 and 4.7 min, respectively. The flow rate under the above five conditions was 1.0 ml/min. The lower detection limits were 0.4, 1.0, 0.2, 0.2 and 0.2 μg/ml for 5-FU, ISDN, LD, KP and IM, respectively. The reproductibility, calculated as the RSD of successive injections of six solutions carried out on five different days, was 3.0, 2.3, 3.7, 4.1 and 4.5% for 5-FU, ISDN, LD, KP and IM, respectively.

2.4. Determination of drug solubility

To determine the saturation solubility of the five drugs in IPM, with and without enhancers, excess drug was added to known volumes of vehicle, vortexed for 2 min followed by sonication for 10 min to dissolve the drug and then equilibration at $32 \pm 0.5\,^{\circ}C$ for more than 48 h. Finally, the solutions were centrifuged at 16,000 rpm for 10 min and aliquots of the supernatant saturated solution were diluted and analyzed by HPLC. The experiments were performed in triplicate.

2.5. Calculation of drug parameters and O-acylmenthol parameters

Five model drugs were selected based on their lipophilicity represented as $\log K_{O/W}$. The physicochemical parameters of the drugs and *O*-acylmenthol derivatives were obtained from the literature or calculated using MARVIN software; the Hansen solubility parameters of the compounds were calculated from the chemical structures using the approaches of Hoftyzer/Van Krevelen (Krevelen and Krevelen, 1990). The calculation of the solubility parameter was based on the average molecular weight. The units of the solubility parameters are $(J\,cm^{-3})^{1/2}$ (Hancock et al., 1997).

2.6. Permeation experiments

2.6.1. Preparation of donor solutions

Donor solutions of the different drugs were obtained by equilibration of excess amounts of solute in IPM, with and without selected concentration enhancers, then vortexed for 2 min followed by sonication for 10 min to dissolve the drug. The molar concentration of the enhancer in this study was based on the concentration of *l*-menthol which was screened in our previous report (Zhao et al., 2007) (0.32 mmol in 1 g IPM for 5-FU, ISDN and KP; 0.64 mmol in 1 g IPM for LD and IM) and an excess amount of solute was present throughout the experiments.

2.6.2. Skin preparation

Male Wistar rats weighing 180–220 g (6–8 weeks old) used in all experiments were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang China). The experiments were performed in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. The rats were anesthetized with urethane (20%, w/v, i.p.) and the abdomen was carefully shaved with a razor after removal of hair by electric clippers (model 900, TGC, Japan). Full thickness skin (i.e. epidermis with SC and dermis) was excised from the shaved abdominal site. The integrity of the skin was carefully ascertained by microscope observation, any skin which had low uniformity was rejected. After removing the fat and sub-dermal tissue, the skin was kept frozen at $-20\,^{\circ}C$ and used within one week. Before starting the experiments, the skin was allowed to reach room temperature for at least 10 h.

2.6.3. Permeation experiments

Skin permeation experiments were performed according to the method of Fang et al. (Fang et al., 2002). A diffusion cell consisting of two half-cells with a water jacket connected to a water bath at $32\,^{\circ}C$ was used. Each half-cell had a volume of 2.5 ml and an effective area of 0.95 cm^2. The dermis side of the skin was in contact with the receiver compartment and the SC with the donor compartment. The donor compartment was filled with the drug suspension and the receiver compartment with pH 7.4 PBS. During all the experiments, excess drug was maintained in the donor compartment. Both donor and receiver compartments were stirred with a star-head bar driven by a constant speed synchronous motor at 600 rpm. At predetermined time intervals, 2.0 ml of receptor solution was withdrawn from each receiver compartment for analysis and replaced with the same volume of fresh solution to maintain sink conditions. The drug concentration was determined by reversed phase HPLC with reference to a calibration curve.

2.7. Data analysis

The cumulative amount of drugs permeating through the skin was plotted as a function of time. The skin flux was determined from Fick's law of diffusion:

$$J_s = dQ_r/A dt$$

where J_s is the steady-state skin flux in $\mu g/cm^2/h$, dQ_r is the change in quantity of the drug passing through the skin into the receptor compartment in μg, A is the active diffusion area in cm^2 and dt is the change in time. The cumulative amount of drugs permeating through the skin at 8 h (Q_8) was calculated from the drug concentration in the receiver compartments. The flux was calculated from the slope of the linear portion of the profiles. The lag-time was determined by extrapolating the linear portion of the curve to the abscissa (Niazy, 1996). The permeability coefficient (P) was calculated as (Scheuplein, 1978).

$$P = J_s/C_s$$

where C_s is the saturated solubility of drugs in donor solutions.

To evaluate the promoting activity of each enhancer, enhancement ratios (ER) were calculated as skin parameters (P and Q_8)

Table 3
Permeation parameters of 5-FU, ISDN and KP through rat abdominal skin. The receiver phase used an identical pH 7.4 PBS and donor phases consisted of IPM; equivalent molar *O*-acylmenthol derivatives with *l*-menthol in IPM (0.32:1) (mmol/g)

Enhancer	5-FU				ISDN				KP			
	Solubility (μg/ml)	Flux (μg/cm^2/h)	T_{lag} (h)	P (cm/h)	Solubility (μg/ml)	Flux (μg/cm^2/h)	T_{lag} (h)	P (cm/h) $\times 10^3$	Solubility (μg/ml)	Flux (μg/cm^2/h)	T_{lag} (h)	P (cm/h) $\times 10^3$
Control	13.14	52.25 ± 3.61	0.10	3.98 ± 0.27	32625.49	107.87 ± 4.50	0.04	3.31 ± 0.14	15540.06	284.12 ± 23.68	0.72	18.28 ± 1.52
MT	23.43	47.65 ± 7.65	0.76	2.03 ± 0.33	33353.27	120.99 ± 13.04	0.44	3.63 ± 0.39	19488.89	387.62 ± 6.68*	1.23	19.89 ± 0.34*
M-LA	40.19	148.99 ± 16.77*	0	3.71 ± 0.42	23709.60	116.90 ± 2.73	0.03	4.93 ± 0.12*	17859.76	143.62 ± 23.58	3.34	8.04 ± 1.32
M-CA	12.98	6.56 ± 0.92	0	0.51 ± 0.07	21130.38	74.87 ± 6.43	0.66	3.54 ± 0.30	10705.05	249.42 ± 32.04	2.65	23.30 ± 2.99
M-SA	13.01	36.95 ± 2.68	0.64	2.84 ± 0.21	40243.77	172.28 ± 13.09*	0.71	4.28 ± 0.33*	12958.61	459.06 ± 10.648*	1.00	35.42 ± 0.82*
M-OA	14.38	46.56 ± 13.45	0	3.24 ± 0.94	32197.43	178.14 ± 17.77*	0.34	5.53 ± 0.55*	10538.05	353.69 ± 53.36	0.53	33.56 ± 5.06*

Data are given as average ± S.E. ($n = 4$), MT is abbreviation of *l*-menthol.
* Value is significantly different from control group, $P < 0.05$.

for the enhancer-containing group divided by the same parameter for the control (no enhancer present). Controls were assigned a value of 1.00. All parameters were reported as the mean ± S.E. Statistical analysis was carried out using analysis of variance (ANOVA). The level of significance was taken as $P < 0.05$. A correlation analysis was performed with the help of the SPSS program, and correlation coefficients were examined for significance ($P < 0.05$) using Student's *t*-test.

3. Results

3.1. Percutaneous absorption of 5-FU in vitro

The cumulative and ER profiles of 5-FU are presented in Figs. 2 and 3a, respectively. The effects of *O*-acylmenthol on the percutaneous permeation parameters of 5-FU (solubility, flux, T_{lag} and P) through rat skin are shown in Table 3. Almost all the evaluated enhancers had no promoting effects on the percutaneous permeation of 5-FU ($P > 0.05$), except M-LA, compared with the control. It increased the 5-FU Q_8 value by 3.74-fold ($P < 0.05$), but the P value was decreased by 0.95-fold relative to the control.

3.2. Percutaneous absorption of ISDN in vitro

The effects of *O*-acylmenthol on the percutaneous permeation parameters of ISDN (solubility, flux, T_{lag} and P) through rat skin are presented in Table 3. Most of the evaluated enhancers had effects on the percutaneous permeation of ISDN ($P < 0.05$), except M-CA compared with the control. The highest increase in P was observed with M-OA (Fig. 3b), which increased the P by 1.64-fold followed by M-LA (1.46-fold) and M-SA (1.27-fold). M-OA provided the highest increase in Q_8 (1341.32 ± 148.81 μg/cm^2), followed by M-SA (1238.14 ± 84.68 μg/cm^2) and M-LA (953.70 ± 17.90 μg/cm^2), as shown in Fig. 2b. M-OA, M-SA and M-CA had a longer lag-time compared with the control.

3.3. Percutaneous absorption of LD in vitro

The cumulative and ER profiles of LD are presented in Figs. 2 and 3c , respectively. The effects of *O*-acylmenthol on the percutaneous permeation parameters of LD (solubility, flux, T_{lag} and P) through rat skin are presented in Table 4. The highest increase in P was observed with M-LA (3.20-fold), while the other three exhibited no obvious enhancement ($P > 0.05$). Similarly, M-LA also provided the highest increase in Q_8 (13023.18 ± 818.28 μg/cm^2), with an ER_{Q8} of 4.19-fold. All the enhancers had a shorter lag-time compared with the control.

3.4. Percutaneous absorption of KP in vitro

The effects of *O*-acylmenthol on the percutaneous permeation parameters of KP (solubility, flux, T_{lag} and P) through rat skin are shown in Table 3. M-SA increased the KP in P by 1.95-fold compared with the control (Fig. 3d), followed by M-OA

Table 4
Permeation parameters of LD and IM through rat abdominal skin. The receiver phase used an identical pH 7.4 PBS and donor phases consisted of IPM; equivalent molar *O*-acylmenthol derivatives with *l*-menthol in IPM (0.64:1) (mmol/g)

Enhancer	LD				IM			
	Solubiliy (mg/ml)	Flux (μg/cm^2/h)	T_{lag} (h)	P (cm/h) × 10^3	Solubiliy (μg/ml)	Flux (μg/cm^2/h)	T_{lag} (h)	P (cm/h) × 10^3
Control	171.88	502.21 ± 20.41	1.74	2.92 ± 0.12	1668.84	19.50 ± 2.37	0.83	11.68 ± 1.42
MT	264.43	648.46 ± 12.72*	0.82	2.45 ± 0.05	3486.37	31.63 ± 8.11*	0	9.07 ± 2.33
M-LA	170.60	1578.68 ± 79.66*	0	9.25 ± 0.47*	3779.31	1.86 ± 0.58	2.23	0.49 ± 0.15
M-CA	127.88	403.22 ± 46.34	1.00	3.15 ± 0.36	1642.50	16.21 ± 2.50	1.66	9.87 ± 1.52
M-SA	213.31	521.64 ± 49.24	1.52	2.45 ± 0.23	1547.69	20.47 ± 4.51	1.77	13.22 ± 2.91
M-OA	176.55	472.48 ± 30.23	0.51	2.68 ± 0.17	1216.65	23.62 ± 1.36*	1.79	19.42 ± 1.12*

Data are given as average ± S.E. ($n = 4$), MT is abbreviation of *l*-menthol.
* Value is significantly different from control group, $P < 0.05$.

(1.90-fold), while no significant difference was found between M-SA and M-OA ($P > 0.05$). The highest increase in the Q_8 was also provided by M-SA (3233.62.34 ± 100.35 μg/cm^2), followed by M-OA (2647.28 ± 370.11 μg/cm^2) as can be seen in Fig. 2d.

3.5. Percutaneous absorption of IM in vitro

The effects of *O*-acylmenthol on the percutaneous permeation parameters of IM (solubility, flux, T_{lag} and P) through rat skin are presented in Table 4. Only M-OA had effects on the percutaneous permeation of IM ($P < 0.05$), as show in Fig. 3e. The effects of M-SA and M-CA are not significant ($P > 0.05$) compared with the control, and the effect of M-LA is surprisingly lower than the control. The P of M-OA was increased by 1.70-fold; however, Q_8 provided by M-OA is not significant compared with the control ($P > 0.05$). Q_8 produced by all the *O*-acylmenthol is lower than that produced by *l*-menthol ($P < 0.05$), shown in Fig. 2e. All the *O*-acylmenthol enhancers had a longer lag-time compared with the control.

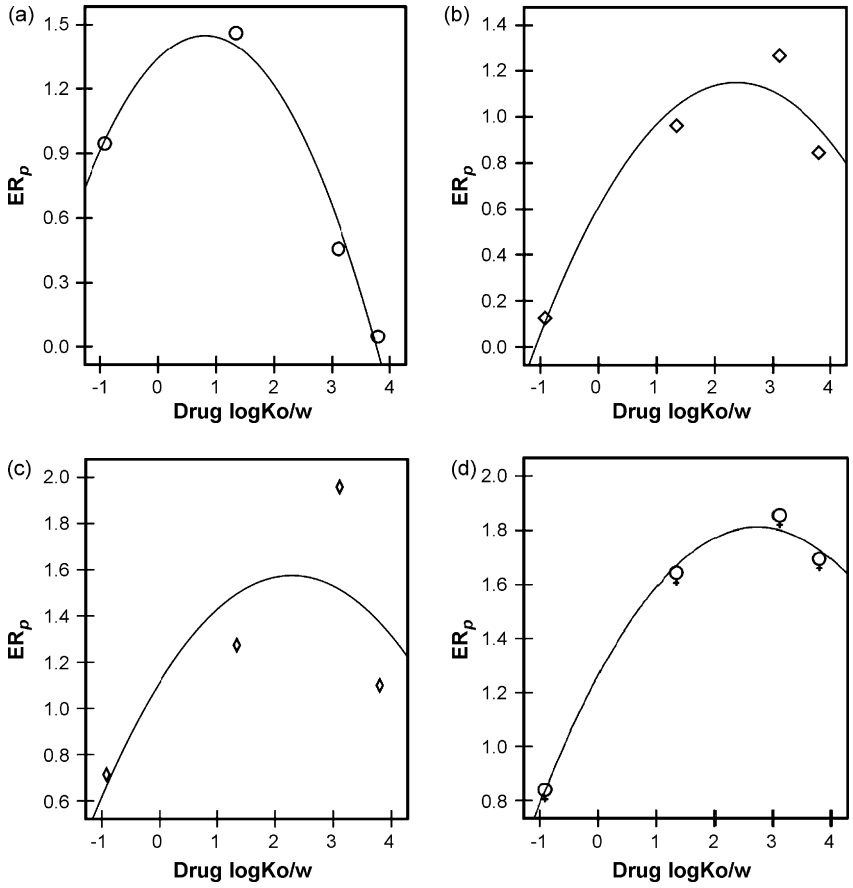

Fig. 4. The relationship between the log $K_{O/W}$ of model drugs and the ER$_P$ of the evaluated drugs (5-FU, ISDN, KP and IM), using M-LA, M-CA, M-SA and M-OA. Profile (a) is M-LA; profile (b) is M-CA; profile (c) is M-SA; profile (d) is M-OA.

3.6. Correlation of O-acylmenthol efficacy with log $K_{O/W}$ values of model drugs

In this study, the effects of *O*-acylmenthol enhancers on the percutaneous permeation of model drugs with different lipophilicity were investigated. Correlation analysis was performed between the lipophilicity of the model drugs and the ER_P. Fig. 4 presents the relationships between the lipophilicity of the four model drugs (5-FU, ISDN, KP, IM) and their ER_P with the *O*-acylmenthol enhancers. The relationship for these four enhancers was found to be parabolic {$r = 0.989$ ($P = 0.144$) for M-LA, $r = 0.965$ ($P = 0.216$) for M-CA, $r = 0.786$ ($P = 0.630$) for M-SA, and $r = 0.996$ ($P = 0.088$) for M-OA}.

4. Discussion

The vehicle, IPM, has been reported in the literature to be a penetration enhancer (Singh et al., 2005; Lee et al., 2006; Leichtnam et al., 2006), our previous study (Zhao et al., 2007) has also shown when using IPM as vehicle, the enhancing activity of *O*-acylmenthol derivatives for each of the model drugs was relatively low (ER values < 5), which was similar with the results in this study. This observation in penetration enhancement studies could be attributed to the fact that IPM also has an enhancing effect.

In our previous study (Zhao et al., 2007), the *O*-acylmenthol derivatives synthesized from *l*-menthol and fatty acids provided significant enhancement of the P and Q_8. In this study, some enhancing effects promoted by *O*-acylmenthol enhancers synthesized from *l*-menthol and excipient acids were also observed. M-LA, in particular, was the most effective enhancer in promoting the permeation of drugs having a high water-solubility (e.g. 5-FU and LD, Table 1). Since M-LA lacks the typical structure of a lipophilic chain and a polar group, the effect of M-LA may be different from some terpenes which attributed their activities to local disorder induced by its insertion within the SC intercellular lipid lamella (Vaddi et al., 2002; Kang et al., 2006). Probably the effect of M-LA is attributable to the α-hydroxy moiety which has hygroscopic properties (Kraeling and Bronaugh, 1997). It seems that M-LA exerts a skin hydration effect, which produces an increase in partition into the skin of highly water-soluble compounds (5-FU and LD) and in the permeation capacity through the skin. Nevertheless, for the most lipophilic compounds with low water-solubility (KP and IM), partition into the "hydrated" SC was made difficult—with a reduction in the permeation capacity through the skin (ER < 1). This effect seems to be confirmed by the increase in the lag-time value because of the inverse relationship between the diffusional parameter (D) and the lag-time ($t_L = L^2/6D$). The superior potency of M-LA as a hydrant possibly could be a result of increased retention within the SC reported by previous investigators (Copoví et al., 2006). In the present work, the reduction in SC corneocyte cohesion by the α-hydroxy moiety could also make a contribution to the global effect of M-LA which favors hydrophilic permeants as suggested by others (Kraeling and Bronaugh, 1999).

M-CA had no effect on any of the model drugs. It is possible that the two cyclic rings (cyclohexyl and phenyl group) providing steric hindrance allow for little intercalation with the tightly packed lipids of the SC and hence, no marked disruption of the lipids can take place. This result supports the observation of Hrabálek et al. (Hrabálek et al., 2005) who reported that the chain branching and cyclization had influence on the permeation-enhancing activity of esters of 6-aminohexanoic acid and concluded that a higher degree of branching, cyclization of the chain, and the presence of an aromatic ring resulted in a loss of the enhancing activity. It has also been reported that the promoting order of the cinnamene enhancer is cinnamic acid > cinnamic alcohol > cinnamaldehyde (Zhang et al., 2007), so the carboxyl group seems to be essential for enhancement of CA. However, the reason why M-CA had a surprisingly low effect on 5-FU is not understood.

M-SA only produced a significant increase for KP and ISDN. M-SA has a similar structure to M-CA, both of which have two cyclic rings (cyclohexyl and phenyl group), while M-SA contains a β-hydroxy group making it more polar compared than M-CA. This site may form hydrogen bonds with either acid carbonyl or hydroxyl group of the sphingosine molecule (which plays a major role in the skin lipid barrier function) (Narishetty and Panchagnula, 2004). It is postulated that hydrogen bonds between M-SA and the ceramide head group break the interlamellar hydrogen bonding networks between ceramides, then the distance between the two opposite lamellae increases and new pathways or channels are formed thus disrupting the SC barrier. This hypothesis agrees well with the observation by some other researchers (Jain et al., 2002; Zhang et al., 2007) who believed that the tight network could be destroyed by the enhancers possessing functional group which can donate or accept hydrogen bonding, so the mechanism of competitive hydrogen bond may exist. However, further investigation is required to prove this hypothesis.

The addition of M-OA had a greater enhancing effect on the transdermal permeation of drugs with a low water-solubility (ISDN, KP, IM) compared with drugs with a high water-solubility (5-FU and LD). This phenomenon agrees well with the report which investigated the effects of OA and its analogue esters on lipophilc and hydrophilic drugs (Song et al., 2001). Indeed, as suggested by the correlations between skin permeability and partition coefficients (Barry and Bennett, 1987), distinct hydrophilic (polar) and lipophilic (nonpolar) pathways exist for hydrophilic and hydrophobic drugs, respectively. Furthermore, experimental verification of this postulate has come from the work by Yamashita et al. (Yamashita et al., 1994; Yamashita et al., 1995) who analyzed the skin permeation of drugs based on a two-layer model with polar and nonpolar routes in the SC and found that the action of permeation enhancers can be discussed in terms of the drug diffusivity and partition coefficient in each domain. M-OA has a greater hydrophobicity and viscosity than OA and these factors could contribute to their more pronounced effect on nonpolar, esterfied fatty acid-rich pathways within the SC that contribute to diffusional resistance (Golden et al., 1987). Interestingly, a new hypothesis proposed recently (Heard et al., 2006) indicated there was close connection between the permeation rates of 1,8-cineole and mefenamic acid, so a "pull" effect whereby the permeation of the enhancer subsequently facili-

tates that of the solute, or more simply, where both species simultaneously permeate skin via a salvation or complexation interaction (Heard et al., 2003) may exist. Of course, this hypothesis whether exists in our study requires further investigation.

The development of biodegradable *O*-acylmenthol enhancers was undertaken to reduce their toxicity, and the most widely used biodegradable bond is the ester bond which has been reviewed by Vávrová et al. (Vávrová et al., 2005b) because esterases are known to exist in both human and animal skin (Montagna, 1955). It could be postulated that the *O*-acylmenthol esters exert their permeation enhancing effects on drug delivery, and then be degraded into *l*-menthol and pharmaceutical excipient acid which can also be viewed as another enhancer after they reach the metabolically active skin layers. To confirm the potential of *O*-acylmenthol could be enzymatically hydrolyzed in viable epidermis requires further experiments.

In this study, the penetration of LD was greatly enhanced by M-LA and similar results were obtained for another drug, 5-FU. 5-FU had an ER_{Q8} of 3.74 when coadministered with the enhancer M-LA as compared with the ER_{Q8} for LD (4.19) for the same enhancer. Although one is hydrophilic, with 5-FU having a $\log K_{O/W}$ of −0.95, the other is lipophilic having a $\log K_{O/W}$ of 2.56, both model drugs have a high water-solubility greater than 6 mg/ml. The other three model drugs in this study had a water-solubility of between 0.07and 0.52 mg/ml (Table 1). So, it could be postulated that the chief transdermal route of LD is the same as 5-FU by a polar pathway rather than the nonpolar pathway which facilitates the penetration of lipophilic drugs.

It is noteworthy that the lipophilicity of the *O*-acylmenthol had a significant influence on the enhancement ratios of the evaluated model drugs at $\log K_{O/W}$ values 2.89–9.22, notwithstanding lipophilicity is not the only determinant factor. Pathways of diffusion through the rate-limiting barrier of the SC and the enhancing effect of different *O*-acylmenthol enhancers are important in explaining this phenomenon. Polar and nonpolar substances diffuse through the SC by different molecular mechanisms (Blank et al., 1967). The nonpolar (lipophilic) pathway involves penetration of the drug through the continuous intercellular lipid phase of the SC. The polar pathway refers to the alternative passage of a drug through the hydrophilic cellular proteins and intercellular lipids of the SC. The degree of hydrophilicity or lipophilicity of the drug being delivered transdermally determines how it will partition into and through the SC barrier. It is postulated in this work that M-LA mainly influences the polar pathway while M-CA could not facilitate either the polar or nonpolar pathway; M-SA and M-OA enhance the permeation of lipophilic drugs (KP and IM) across the skin mainly by affecting the nonpolar pathway. Of course, to confirm this hypothesis requires further investigation. As well as the enhancement effects, skin irritation should be evaluated for the development of safer and more effective promoting agents. Experiments involving daily primary irritation indices for erythema and edema induced by 24-h application of enhancer (100%) are under investigation now, and will be published at a later date.

5. Conclusions

From the results of this investigation, it is concluded that some newly designed percutaneous absorption enhancers synthesized from *l*-menthol and pharmaceutical excipient acids have the potential to enhance the penetration of drugs of different lipophilicity. The lipophilicity of *O*-acylmenthol enhancers had a significant effect on the percutaneous permeation of the model drugs, notwithstanding lipophilicity is not the only determinant factor. M-LA, in particular, was the most effective enhancer in promoting the permeation of drugs having a high water-solubility (e.g. 5-FU and LD) while M-CA was ineffective for all the model drugs. M-SA and M-OA generally had a greater effect on drugs which have a low water-solubility (ISDN, KP, IM) than those with a high water-solubility. Furthermore, model drug lipophilicity had a significant impact on the derivative enhancer promoting activity, and a parabolic relationship within four drugs was found between the ER_P and $\log K_{O/W}$ of the model drugs tested, except LD.

Acknowledgements

The authors wish to thank Professor Yasunori Morimoto, Faculty of Pharmaceutical Sciences, Josai University, Japan, for providing the 2-chamber diffusion cells. The authors are grateful to Professor Maosheng Cheng, Faculty of Pharmaceutical Chemistry, Shenyang Pharmaceutical University, China, for providing the MARVIN software to calculate the parameters of *O*-acylmenthol derivatives.

References

Alvalez, C., Torrado, J.J., Cadorhigna, R., 1999. Stereoselective drug release from ketoprofen and ricobendazole matrix tablets. Chirality 11, 611–615.

Barry, B.W., Bennett, S.L., 1987. Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin. J. Pharm. Pharmacol. 39, 535–546.

Blank, I.H., Scheuplein, R.J., MacFarlane, D.J., 1967. Mechanism of percutaneous absorption. 3. The effect of temperature on the transport of non-electrolytes across the skin. Invest. Dermatol. 49, 582–589.

Brodin, A., Nyqvist-mayer, A., Wadsten, T., Forslund, B., Broberg, F., 1984. Phase diagram and aqueous solubility of lidocaine-prilocaine binary system. J. Pharm. Sci. 73, 481–484.

Bronaugh, R.L., Stewar, R.F., Wester, R.C., Bucks, D., Mabach, H.I., Anderson, J., 1985. Comparison of percutaneous absorption of fragrances by humans and monkeys. Food. Chem. Toxicol. 23, 111–114.

Copoví, A., Díez-Sales, O., Herráez-Domínguez, J.V., Herráez-Domínguez, M., 2006. Enhancing effect of alpha-hydroxyacids on “in vitro” permeation across the human skin of compounds with different lipophilicity. Int. J. Pharm. 314, 31–36.

Cornwell, P.A., Barry, B.W., 1993. The routes of penetration of ions and 5-fluorouracil across human skin and the mechanisms of action of terpene skin penetration enhancers. Int. J. Pharm. 94, 189–194.

Deppeler, H.P., 1981. Hydrochlorothiazide. In: Florey, K. (Ed.), Analytical profiles of drug substances, vol. 10. Academic press, New York, pp. 443–469.

Fang, L., Kobayashi, Y., Numajiri, S., Kobayashi, D., Sugibayashi, K., Morimoto, Y., 2002. The enhancing effect of a triethanolamine -ethanol-isopropyl myristate mixed system on the skin permeation of acidic drugs. Biol. Pharm. Bull. 25, 1339–1344.

Francoeur, M.L., Golden, G.M., Potts, R.O., 1990. Oleic acid: its effects on stratum corneum in relation to transdermal drug delivery. Pharm. Res. 7, 621–627.

Gao, S., Singh, J., 1998. In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J. Control. Release 51, 193–199.

Golden, G.M., Mckie, J.E., Potts, R.O., 1987. Role of stratum corneum lipid fluidity in transdermal drug flux. J. Pharm. Sci. 76, 25–28.

Hancock, B.C., York, P., Rowe, R.C., 1997. The use of solubility parameters in pharmaceutical solid dosage form design. Int. J. Pharm. 148, 1–21.

Heard, C.M., Gallagher, S.J., Harwood, J.L., Maguire, P.B., 2003. The in vitro delivery of NSAIDs across skin was in proportion to the delivery of essential fatty acids in the vehicle—evidence that solutes permeate skin associated with their salvation cages? Int. J. Pharm. 261, 165–169.

Heard, C.M., Kung, D., Thomas, C.P., 2006. Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect. Int. J. Pharm. 321, 167–170.

HO, H., Chen, L., Lin, H., Sheu, M., 1998. Penetration enhancement by menthol combined with a solubilization effect in a mixed solvent system. J. Control. Release 51, 301–311.

Hrabálek, A., Doležal, P., Vávrová, K., Zbytovská, J., Holas, T., Klimentová, J., Novotný, J., 2006. Synthesis and enhancing effect of transkarbam 12 on the transdermal delivery of theophylline, clotrimazole, flobufen, and griseofulvin. Pharm. Res. 23, 912–919.

Hrabálek, A., Vávrová, K., Doležal, P., Macháček, M., 2005. Esters of 6-aminohexanoic acid as skin permeation enhancers: the effect of branching in the alkanol moiety. J. Pharm. Sci. 94, 1494–1499.

Jain, A.K., Thomas, N.S., Panchagnula, R., 2002. Transdermal drug delivery of imipramine hydrochloride I. Effect of terpenes. J. Control. Release 79, 93–101.

Kang, L., Ho, P.C., Chan, S.Y., 2006. Interactions between a skin penetration enhancer and the main compoents of human stratum corneum lipids. Isothermal titration calorimetry study. J. Therm. Anal. Calorimetry 83, 27–30.

Kirschbaum, J., 1980. High-pressure liquid chromatography of triamcinolone acetonide: effect of different octadecylsilane columns on mobility. J. Pharm. Sci. 69, 481–482.

Kobayashi, D., Matsuzawa, T., Sugibayashi, K., 1993. Feasibility of use of several cardiovascular agents in transdermal therapeutic systems with l-menthol-ethanol system on hairless rat and human skin. Biol. Pharm. Bull. 16, 254–258.

Kraeling, M.E.K., Bronaugh, R.L., 1997. In vitro percutaneous absorption of alpha hydroxyl acids in human skin. J. Soc. Cosmet. Chem. 48, 187–197.

Kraeling, M.E.K., Bronaugh, R.L., 1999. Percutaneous absorption of alpha-hydroxy acids in human skin. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption. Marcel Dekker, New York, pp. 713–731.

Krevelen, V., Krevelen, D.W., 1990. Properties of Polymers. Elsevier Science Publishing Co., Amsterdam.

Lee, P.J., Ahmad, N., Langer, R., Mitragotri, S., Shastri, V.P., 2006. Evaluation of chemical enhancers in the transdermal delivery of lidocaine. Int. J. Pharm. 308, 33–39.

Leichtnam, M.L., Rolland, H., Wüthrich, P., Guy, R.H., 2006. Identification of penetration enhancers for testosterone transdermal delivery from spray formulations. J. Control. Release 113, 57–62.

Li, G.F., Quan, Y.S., Fumio, K.Y., 2002. Influence of different layers of skin on the percutaneous absorption of drugs with different lipophilicity. Chin. Pharm. J. 37, 833–836.

Loden, M., 2000. Keratolytics. In: Gabard, B., Elsner, P., Surber, C., Treffel, P. (Eds.), Dermatopharmacology of Topical Preparations. Springer, Berlin, pp. 225–280.

Moghimi, H.R., Williams, A.C., Barry, B.W., 1996. A lamella matrix model for stratum corneum intercellular lipids. II. Effect of geometry of the stratum corneum on permeation of model drugs 5-fluorouracil and oestradiol. Int. J. Pharm. 131, 117–129.

Montagna, W., 1955. Histology and cytochemistry of human skin IX. The distribution of non-specific esterases. J. Biophys. Biochem. Cyol. 1, 13–16.

Morimoto, Y., 1992. Prediction of skin permeability of drugs: comparison of human and hairless rat skin. J. Pharm. Pharmacol. 44, 634–639.

Morimoto, Y., Wada, Y., Seki, T., 2002. In vitro permeation of morphine hydrochloride during the finite application of penetration-enhancing system containing water, ethanol and *l*-menthol. Biol. Pharm. Bull. 25, 134–136.

Nakamura, Y., Takayama, K., Higashiyama, K., Suzuki, T., Nagai, T., 1996. Promoting effect of *O*-ethylmenthol on the percutaneous absorption of ketoprofen. Int. J. Pharm. 145, 29–36.

Nanayakkara, G.R., Barlett, A., Forbes, B., Marriott, C., Whitfield, P.J., Brown, M.B., 2005. The effect of unsaturated fatty acids in benzyl alcohol on the percutaneous permeation of three model penetrants. Int. J. Pharm. 301, 129–139.

Narishetty, S.T., Panchagnula, R., 2004. Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J. Control. Release 95, 367–379.

Negishi, J., Takayama, K., Higashlyama, K., Chida, Y., Isowa, K., Nagai, T., 1995. Promoting effect of *O*-alkylmenthol and *O*-acylmenthol derivatives on the percutaneous absorption of ketoprofen in rats. STP Pharm. Sci. 5, 156–161.

Niazy, E.M., 1996. Differences in penetration-enhancing effect of Azone through excised rabbit, rat, hairless mouse, guinea pig and human skins. Int. J. Pharm. 130, 225–230.

Obata, Y., Li, C.J., Fujikawa, M., Takayama, K., Sato, H., Higashiyama, K., Nagai, T., 2001. Evaluation and structure-activity relationship of synthesized cyclohexanol derivatives on percutaneous absorption of ketoprofen using artificial neural network. Int. J. Pharm. 212, 223–231.

Pilgram, G.S., Vanpelt, A.M., Spies, F., Bouwstra, J.A., Koerten, H.K., 1998. Cryo-electron diffraction as a tool to study local variations in the lipid organization of human stratum corneum. J. Mocrosc. 189, 71–78.

Scheuplein, R.J., 1978. Site variations in diffusion and permeability. In: Jarret, A. (Ed.), The Physiology and Pathophysiology of Skin. Academic Press, New York, pp. 1693–1730.

Sebastiani, P., Nicoli, S., Santi, P., 2005. Effect of latic acid and iontophoresis on drug permeation across rabbit ear skin. Int. J. Pharm. 292, 119–126.

Singh, B.N., Singh, R.B., Singh, J., 2005. Effects of ionization and penetration enhancers on the transdermal delivery of 5-fluorouracil through excised human stratum corneum. Int. J. Pharm. 298, 98–107.

Song, J.F., Lau-cam, C.A., Kim, K.H., 2001. Monohydroxylation and esterification as determinants of the effects of *cis*- and *trans*-9-octadecenoic acids on the permeation of hydrocortisone and 5-fluororacil across hairless mouse skin in vitro. Int. J. Pharm. 212, 153–160.

Strichartz, G.R., 1990. Fundamental properties of local anesthetic II Measured octanol:buffer partition coefficients and pka values of clinical used drugs. Anesh. Analg. 71, 58–70.

Takanashi, Y., Higashiyama, K., Komiya, H., Takayama, K., Nagai, T., 1999. Thiomenthol derivatives as novel percutaneous absorption enhancers. Drug. Devel. Ind. Pharm. 25, 89–94.

Thomas, S., Narishetty, K., Panchagnula, R., 2004. Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. J. Control. Release 95, 367–379.

Vaddi, H.K., Ho, P.C., Chan, S.Y., 2002. Terpenes in propylene glycol as skin-penetration enhancers: permeation and partition of haloperidol, Fourier transform infrared spectroscopy, and differential scanning calorimetry. J. Pharm. Sci. 91, 1639–1651.

Vávrová, K., Hrabálek, A., Doležal, P., Holas, T., Klimentová, J., 2005a. Biodegradable derivatives of tranexamic acid as transdermal permeation enhancers. J. Control. Release 104, 41–49.

Vávrová, K., Hrabálek, A., Zbytovská, J., 2005b. Amphiphilic transdermal permeation enhancers: structure-activity relationships. Curr. Med. Chem. 12, 2273–2291.

Williams, A.C., Barry, B.W., 2004. Penetration enhancers. Adv. Drug. Dev. Rev. 56, 603–618.

Yamashita, F., Bando, M., Koyama, Y., Kitagawa, S.M., Takakura, Y., Hashida, M., 1994. In vitro and in vivo analysis of skin penetration enhancement based on a two-layer diffusion model with polar and nonpolar routes in the stratum corneum. Pharm. Res. 11, 185–191.

Yamashita, F., Koyama, Y., Kitano, M., Takakura, Y., Hashida, M., 1995. Analysis of in vivo skin penetration enhancement by oleic acid based on a two-layer diffusion model with polar and nonpolar routes in the stratum corneum. Int. J. Pharm. 117, 173–179.

Zhang, C., Yang, Z., Luo, J., Zhu, Q., Zhao, H., 2007. Effect of cinnamene enhancers on transdermal delivery of ligustrazine hydrochloride. Eur. J. Pharm. Biopharm. 67, 413–419.

Zhao, L., Fang, L., Xu, Y., He, Z., 2007. Transdermal delivery of penetrants with differing lipophilicities using *O*-acylmenthol derivatives as penetration enhancers. Eur. J. Pharm. Biopharm., doi:10.1016/j.ejpb.2007.10.015.

Zheng, J., Xu, R., Tang, T., 2007. Targeting distribution of 5-FU magetic nanoparticles in nude mice bearing hepatoma. Acta. Med. Univ. Sci. Technol. Huazhong 36, 202–205.

Zhu, W.F., Zhang, Y.H., Liu, H.N., Xu, L.Y., Yang, W.L., Xie, P., 2003. Studies on in vitro transdermal absorption of cinnamic acid. Chin. Tradit. Patent Med. 25, 443–445.