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# Transdermal delivery of tolterodine by *O*-acylmenthol: *In vitro*/*in vivo* correlation

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ABSTRACT

The aim of the present investigation was to evaluate the percutaneous absorption of tolterodine (TOL) using *O*-acylmenthol derivatives as enhancers as well as to correlate their enhancing activity under *in vitro* and *in vivo* conditions. The *in vitro* permeation studies of TOL were conducted in isopropyl myristate (IPM) solution or from patches in side-by-side diffusion cells. TOL pharmacokinetic parameters were determined after intravenous administration and topical application of patches without enhancer or with *l*-menthol and (E)-2-isopropyl-5-methylcyclohexyl octadec-9-enoate (M-OA) as enhancers in rats. The *in vitro* permeation studies indicated that M-OA was the most promising enhancer for transdermal delivery, as 2-isopropyl-5-methylcyclohexyl 2-hydroxypanoate (M-LA) was merely effective in IPM solution. There was no significant difference between the control and *l*-menthol group in terms of the flux before patches were removed, while the skin reservoir effects of the enhancer-containing groups were significantly greater than that of the control group. The mean steady-state plasma concentrations after applying patches without enhancer or with *l*-menthol and M-OA as enhancers were 0.89, 0.84 and 1.47 μg/mL, respectively. The *in vivo* results observed from the three types of patches in rats were in good agreement with the plasma concentrations predicted from the *in vitro* data.



## 1. Introduction

Tolterodine (TOL) presented in Fig. 1 is a tertiary amine with anticholinergic and direct spasmolytic effects on the bladder smooth muscle. It is widely used in the treatment of urge urinary incontinence associated with detrusor instability (Abrams et al., 1998). However, oral administration of TOL is often limited on account of its dose-related adverse side effects, including dry mouth, tachycardia, dizziness, and gastrointestinal obstructive disorder (Wefer et al., 2001). TOL is extensively metabolized following oral administration, and its major metabolite acts in a similar fashion as the parent substance on receptors, which restricts its application in patients with liver cirrhosis. In addition, the oral route of drug delivery is not preferable for patients with severe nausea. Hence, the transdermal route could be an alternative route for these patients, because it bypasses first-pass metabolism (which is especially important in patients with liver disease), minimizes the gastrointestinal side effects, increases patient compliance, maintains a constant drug level in plasma and makes it possible to interrupt or terminate treatment when necessary (Kiptoo et al., 2006). However, there is no literature report on the skin permeation of TOL either *in vitro* or *in vivo*.

An essential prerequisite for the development of a transdermal drug delivery system (TDDS) is that the drug must be capable of passing through the skin at a sufficiently high rate to achieve therapeutic plasma concentrations. However, the outermost layer of skin, the stratum corneum (SC), forms a major barrier to most exogenous substances, including drugs (Hui et al., 2005). One popular approach to deliver an effective dose of drug through skin is to reversibly reduce the barrier function of the skin with the aid of penetration enhancers or accelerants (Hadgraft, 1999). More recently, new types of *O*-acylmenthol derivatives (Fig. 2) have been synthesized and their promoting activities have been evaluated using model drugs with a range of lipophilicity (Zhao et al., 2008a,b). According to the *in vitro* penetration results from IPM solution, 2-isopropyl-5-methylcyclohexyl heptanoate (M-HEP), 2-isopropyl-5-methylcyclohexyl tetradecanoate (M-TET), (E)-2-isopropyl-5-methylcyclohexyl octadec-9-enoate (M-OA) and 2-isopropyl-5-methylcyclohexyl 2-hydroxypanoate (M-LA) were selected to enhance the penetration of TOL in the present study. Moreover, as esterases are present in the human and animal epidermis (Montagna, 1955), the ester linkage of *O*-acylmenthol offers the possibility of degradation by skin esterases in the living epidermis which increased enhancer safety. The similar researches have been done by some other researchers (Vávrová et al., 2005; Hrabálek et al., 2006) who found the compound could be hydrolyzed into nontoxic metabolites *in vitro* using porcine esterase. A good *in vitro*/*in vivo* correlation is always the goal for developing a penetration enhancer, however, there have been no literature reports of the

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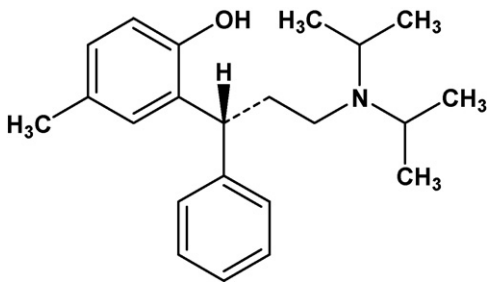


**Fig. 1.** The chemical structure of tolterodine.

enhancing activity of *O*-acylmenthol from patches *in vitro* or from animals *in vivo*. In order to further evaluate the effectiveness of the selected *O*-acylmenthol *in vitro* and *in vivo*, the parent compound *l*-menthol was also used as a reference enhancing promoter.

The aim of present investigation was subdivided into three objectives. Objective one was to compare the *in vitro* permeability of TOL from IPM solution and drug-in adhesive (DIA) patches across rat skin using *O*-acylmenthol as penetration enhancers, so that the most promising enhancer could be identified. In order to validate the *in vitro* investigation, the second objective was to conduct *in vivo* permeation studies in rats by evaluating the topical application of the patches, and it was important to establish a steady-state plasma concentration of the drug in the animals and to investigate the existence of a drug reservoir effect post-patch removal. Occasionally, there are discrepancies in enhancing activity between *in vitro* and *in vivo* results, and this is due to the potential interaction among permeants, enhancers and pressure-sensitive adhesives (PSAs) or difference between *in vitro* and *in vivo* conditions, especially cutaneous blood flow (Auclair et al., 1991). Therefore, the third objective was to correlate the enhancing activity of *O*-acylmenthol from IPM solution and patches, especially from patches *in vitro* and rats *in vivo* when the skin reservoir effects were taken into consideration.

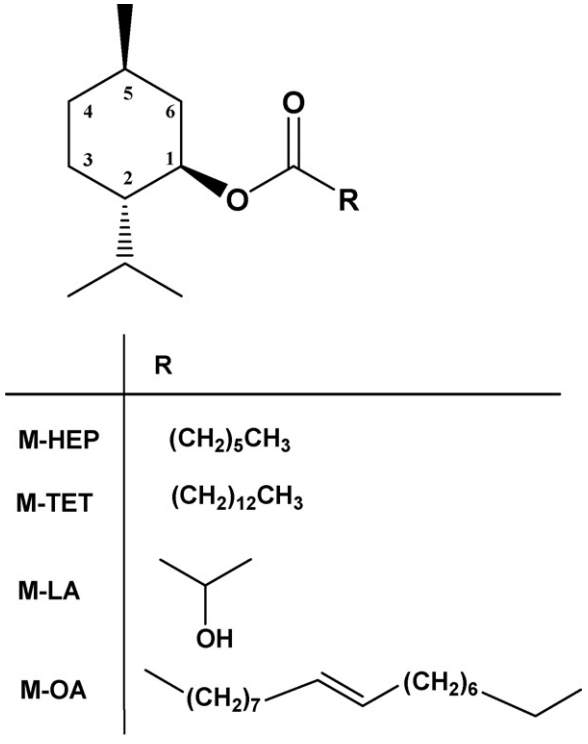


**Fig. 2.** The chemical structures of *O*-acylmenthol derivatives.

## 2. Materials and methods

### 2.1. Materials

Tolterodine tartrate was supplied by Jinghua Pharmaceutical Co., Ltd. (Beingjing, China); IPM, acetaniline, carbamazepine and *l*-menthol were purchased from China National Medicines Co., Ltd. (Shanghai, China); M-HEP, M-TET, M-OA, M-LA were synthesized in previous reports (Zhao et al., 2008a,b), and their structures were confirmed by NMR (ARX-300, Bruker, Switzerland) and HPLC-MS (ZQ-2000, Waters, USA). Duro-Tak® (DT) adhesives were obtained from National Starch and Chemical Co., USA. Methanol of HPLC grade was obtained from the Yuwang Pharmaceutical Co., Ltd. (Shandong, China). All other chemicals were of the highest reagent grade available.

### 2.2. Preparation of TOL from TOL tartrate

The TOL free base was prepared from TOL tartrate, which was available commercially. TOL tartrate suspension prepared in water was titrated with 0.1 M sodium hydroxide solution to pH 12, then the aqueous phase was extracted with ethyl acetate. The organic layer was separated and washed with water, followed by the addition of anhydrous sodium sulfate. The ethyl acetate was filtered and evaporated using a rotary evaporator (SB-2000, Eyela, Japan). The viscous colorless oil residue was transferred to an amber glass container and kept in a vacuum evaporator until the weight loss had ceased. The final product, TOL, was identified using NMR and HPLC.

### 2.3. Determining the partition coefficients of TOL and TOL tartrate

*n*-Octanol and water were mutually saturated for 24 h prior to the experiment. The TOL solution (200 μg/mL) was prepared using *n*-octanol saturated with water and the TOL tartrate solution (200 μg/mL) was prepared using water saturated with *n*-octanol. One milliliter of the solution was then transferred to a 10 mL centrifuge tube containing 1 mL of water saturated with *n*-octanol or 1 mL of *n*-octanol saturated with water. The tube was gently shaken for 48 h at 25 ± 0.5 °C and centrifuged at 16,000 rpm for 10 min. After centrifugation, the TOL or TOL tartrate concentrations in each phase were determined using a validated HPLC method.

### 2.4. Determination of TOL and TOL tartrate solubility in water and IPM

To determine the saturation solubility of TOL and TOL tartrate in water and IPM, 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 equilibrated at 25 ± 0.5 °C for more than 48 h. Finally, the suspensions were filtered through a 0.45 μm membrane filter and aliquots of the supernatant saturated solution were diluted and analyzed by HPLC. The experiments were performed in triplicate.

### 2.5. In vitro permeation studies across rat skin

#### 2.5.1. Preparation of donor solutions containing TOL tartrate or TOL

Donor solution of TOL tartrate was obtained by equilibration of 0.056% solute (w/v, the solubility of TOL tartrate calculated as TOL in IPM is 0.56 mg/mL) in IPM, donor solutions of TOL were prepared by equilibration of 0.056% and 10% solute (w/v) in IPM with 5% (w/w) *l*-menthol (or an equivalent molar amount

of *O*-acylmenthol with *l*-menthol) or without enhancer, then vortexed for 2 min followed by sonication for 10 min to dissolve the drug, both the TOL tartrate and TOL dissolved completely in the vehicle.

*2.5.2. Preparation of DIA patches containing TOL*

The DIA patches containing 10% TOL (w/w) were prepared with various PSA and enhancers, the amount of TOL was the same for all dosing formulations. A laboratory-coating unit (SLT200, Kaikai Co., Ltd., Shanghai, China) was used to prepare the DIA patches. An appropriate amount of TOL was dissolved in a suitable amount of ethyl acetate, which was later added to the PSA solution and mixed thoroughly with a mechanical stirrer. The resulting drug-PSA solution was coated onto a fluoropolymer-treated polyester release liner (ScotchPak® 1022, 3M, USA) at a thickness of 80 μm. After the solvent had been removed, it was laminated with a polyester backing film (ScotchPak® 9732, 3M).

*2.5.3. Skin preparation*

Male Wistar rats weighing 180–220 g (6–8 weeks old) were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All experiments were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and also in accordance with the guidelines for animal use published by the Life Science Research Center of Shenyang Pharmaceutical University. All efforts were made to minimize animal suffering and to limit the number of animals used. The rats were anesthetized with urethane (20%, w/v, i.p.) and the abdomen regions were carefully shaved with a razor after removal of hair by electric clippers (model 900, TGC, Japan). Full thickness skin (epidermis with SC and dermis) was excised from the shaved abdominal site. The integrity of the skin was carefully checked by microscopic observation, and any skin which was not uniform was rejected. The sub-dermal tissue was surgically removed and dermal-side was wiped with cotton swab dipped in isopropyl alcohol to remove adhering fat for 1 min. The skin with a thickness of approximately 400 μm was washed immediately with phosphate buffered saline, wrapped in aluminum foil and stored at −20 °C till further use (used within 1 week of preparation), this skin preparation procedure was in accordance with a previous report (Narishetty and Panchagnula, 2004). Before starting the experiments, the skin was allowed to reach room temperature for at least 10 h.

*2.5.4. Permeation experiments*

Skin permeation experiments of TOL in IPM solutions were performed according to the method of Fang et al. (2002). A diffusion cell consisting of two half-cells with a water jacket connected to a water bath at 32 °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 solutions and the receiver compartment with pH 7.4 phosphate buffer saline (PBS). In the patch application study, the dermis side of the skin was attached to one-half of the side-by-side diffusion cells. The TOL patch was applied to the SC side of the skin, and pH 7.4 PBS was used as the receptor medium. In both application studies, 2.0 mL of receiver solution was withdrawn at predetermined intervals for measurement of the permeated drug, and fresh pH 7.4 PBS was added to maintain a constant volume. The patches were removed from the skin at 24 h, and the whole volume of receiver phase was withdrawn, then the receiver compartment was washed three times and filled with fresh receiver medium. The receiver solution was sampled for evaluation of the reservoir capacity in the skin preparation until 48 h. The solution in the donor or receiver compartment was stirred with a star-head bar driven by a constant speed synchronous motor at 600 rpm.

*2.6. In vivo studies*

*2.6.1. Preparation of intravenous formulations*

TOL tartrate was prepared in a vehicle of sterile saline. A weighed amount of the drug was wetted with a drop of ethanol, and sterile saline was added to give a concentration of 2.5 mg/mL (calculated as TOL). Drug solutions were prepared immediately before each animal was dosed.

*2.6.2. In vivo studies in rats*

Male Wistar rats weighing 210 ± 10 g were used for these studies. Rats were anesthetized with urethane (1 g/kg, i.p.) and, following anesthesia, catheters were surgically implanted into the jugular vein for blood sampling. For i.v. bolus experiments, a dose of 12.5 mg/kg (2.5 mg/mL) TOL was infused over a period of 30 s. Blood samples of approximately 0.25 mL were drawn at 0.083, 0.167, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8 h following the intravenous doses. For topical delivery studies, patches without enhancer or with *l*-menthol and M-OA as enhancers (covering an area of 18 $cm^2$, equivalent to 80 mg TOL) were applied to the shaved abdomen of the rats. The blood samples were collected for 24 h while the patches remained on the animal, and for another 24 h after patches were removed. All animal studies were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were also approved by the Life Science Research Center of Shenyang Pharmaceutical University. The blood samples were immediately centrifuged at 5000 rpm for 5 min, and plasma was separated and stored at −70 °C until analysis by HPLC.

*2.7. Plasma sample extraction*

For this, 100 μL plasma and 50 μL carbamazepine water solution (equal to 0.05 μg of carbamazepine) were pipetted into a 10 mL centrifuge tube and vortex-mixed for 30 s. Then, 100 μL pH 11.2 PBS was added and vortex-mixed for another 30 s. The mixture was extracted with 4 mL diethyl ether/*n*-hexane (1:1, v/v) for 5 min using a vortex mixer. After centrifugation at 3000 rpm for 10 min, the supernatant was decanted into a clean test tube and evaporated under nitrogen at 37 °C. The residue was reconstituted with 100 μL mobile phase for *in vivo* analysis, vortexed, and sonicated for 3 min. A sample of the clear solution (20 μL) was injected into the HPLC system. The extraction efficiency was 95.81 ± 2.26% for TOL.

*2.8. Quantitative analysis*

The concentrations of TOL in the receptor medium or rat plasma were determined using a validated HPLC method. The HPLC system was equipped with an L-2420 variable-wavelength ultraviolet absorbance detector and an L-2130 pump (Hitachi High-Technologies Corporation, Tokyo, Japan). The reversed phase stainless-steel column (200 mm × 4.6 mm) was packed with Diamonsil C-18 (5 μm particle size; Dikma Technologies, Beijing, China). The mobile phase was a mixture of methanol and 20 mM ammonium formate buffer adjusted to pH 3.0 with methanoic acid (55:45) for permeation studies and (50:50) for pharmacokinetic studies. The wavelength was set at 283 nm for permeation studies and 272 nm for pharmacokinetic studies (an endogenous substance interfered determination of TOL at 283 nm). The internal standard for the permeation studies was acetanilide, while that for the pharmacokinetic studies was carbamazepine. The flow rate of the mobile phase was 1.0 mL/min and the injection volume was 20 μL. The sensitivity of the assay was 200 ng/mL for TOL.

### 2.9. Data treatment

#### 2.9.1. In vitro data analysis

The diffusion data were plotted as the cumulative amount of drug permeated per $cm^2$ as a function of time. The skin flux was determined by Fick's law of diffusion:

$$J_{ss} = \frac{dQ_r}{A\ dt} \quad (1)$$

where $J_{ss}$ is the steady-state flux, $Q$ is the accumulative amount of the drug, $A$ is the active diffusion area. The flux was calculated from the slope of the linear portion of the profiles and the lag time was determined by extrapolating the linear portion of the curve to the abscissa (Nash et al., 1992). Statistical analysis was carried out using analysis of variance (ANOVA). The level of significance was taken as $P < 0.05$.

#### 2.9.2. In vivo pharmacokinetic analysis

The pharmacokinetic analysis of the TOL plasma concentration–time profiles after intravenous bolus administration was carried out by fitting the data to a two-compartment model (DAS® 2.0, Boying Corporation, Beijing, China) with the following exponential expression:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (2)$$

where $C$ is the plasma concentration of drug, $A$ and $B$ are pre-exponential constants, $\alpha$ and $\beta$ are hybrid first-order rate constants for the distribution phase and elimination phase, respectively (Shargel and Yu, 1985), $t$ is time. The pharmacokinetic parameters: half-life for the elimination phase, $t_{1/2(\beta)}$, half-life for the distribution phase, $t_{1/2(\alpha)}$, steady-state volume of distribution, $V_{ss}$, area under the curve from o to infinity, $AUC_{0-\infty}$ were estimated using the software described above. The total body clearance (CL) was calculated by the following equation:

$$CL = \frac{dose}{AUE_{0-\infty}} \quad (3)$$

The $C_0$ (drug plasma concentration at zero time) after the i.v. bolus dose of TOL tartrate was used to calculate the volume of the central compartment ($V_d$) by Eq. (4):

$$V_d = \frac{dose}{C_0} \quad (4)$$

At zero time, $C_0$ could be calculated by Eq. (5):

$$C_0 = A + B \quad (5)$$

Following topical administration, data were analyzed by non-compartment analysis to determine the peak concentration ($C_{max}$), area under the curve from 0 to 48 h ($AUC_{0-48}$) and area under the curve from 0 to $\infty$ ($AUC_{0-\infty}$). The rate constants for the terminal phase after removal of patches ($\beta$) were calculated by a two-compartment model. The steady-state plasma concentration of TOL after the application of patches containing TOL was calculated by using the equation (Valiverti et al., 2005):

$$C_{ss} = \frac{AUC_{0-t}}{time} \quad (6)$$

The statistical significance of the *in vivo* data from intravenous administration or transdermal application of the patches was computed with a one-way ANOVA followed by Tukey's post hoc analysis using SPSS® program.

#### 2.9.3. Correlation analysis

The 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 and discussion

### 3.1. Preparation of TOL from TOL tartrate

The diffusion behavior of the drug molecules is one of the most important physicochemical properties in determining the feasibility of a transdermal delivery system for a drug. However, TOL tartrate, has a low lipophilicity represented as $\log K_{O/W}$ (−0.96), a high water-solubility (10.19 mg/mL) and melting point (decomposing at 205–210 °C), and is not easily permeable, mainly due to its low solubility in the lipophilic SC. It is generally accepted that the lipophilic free acid or free base of a drug has higher partition coefficient than the corresponding hydrophilic salt form. Therefore, the former may have higher skin permeability than the latter. In this regard, the free base form of TOL was prepared using an acid–base titration and extraction procedure because only the tartrate form of TOL is available commercially. The prepared product was identified as highly pure (>99.5%) by area normalization method of HPLC, and the NMR results were consistent with the previous publication (Kang et al., 2008). The efficiency of the conversion was 96%, and the $\log K_{O/W}$ of TOL was 2.05. The solubility of TOL and TOL tartrate in IPM were 637 and 0.56 mg/mL (calculated as TOL), respectively.

### 3.2. In vitro permeation of TOL tartrate and TOL from IPM solution

Table 1 shows the permeation parameters of TOL tartrate and TOL that permeated through the rat skin. Comparing the penetration results of TOL tartrate with TOL in IPM solution at the same concentration, TOL tartrate had higher permeability than TOL, which could be attributed that TOL tartrate had higher thermodynamic activity than TOL in IPM solution. Some researchers reported the experimentally determined permeability coefficients ($P$) generally decreased with increasing drug lipophilicity expressed as $\log K_{O/W}$ in the lipophilic vehicle IPM (Uchida et al., 1993; Fang et al., 2003). As $\log K_{O/W}$ increases, the drug becomes more like the vehicle (the drug should become more soluble in IPM) and the vehicle does not cause extensive partitioning of the drug out of the vehicle, the lower the tendency of the solute to leave the donor phase, the lower the flux. However, the flux is equal to the product of the $P$ value and the concentration of the drug in the donor side. Compared with saturated solution of TOL tartrate, a 41.1-fold increase in the flux was observed at concentration of 10% TOL (w/v) in IPM solution due to the low solubility of TOL tartrate, which confirmed the hypothesis that the lipophilic free base of TOL had better properties than the hydrophilic salt for skin permeation. Among the enhancer examined, M-LA had the highest enhancing

**Table 1**
Skin permeation rates of TOL tartrate or TOL through rat abdominal skin. The donor phases consisted of IPM; IPM:menthol (20:1) (w/w) and an equivalent molar amount of *O*-acylmenthol with *l*-menthol in IPM.

| Permeants | Enhancers | $J_{ss}$ (μg/cm²/h) | $Q_8$ (μg/cm²) | $T_{lag}$ (h) | ER[a] |
|---|---|---|---|---|---|
| TOL tartrate | Control[b] | 0.98 ± 0.21 | 6.87 ± 2.13 | 0.67 | – |
| TOL | Low control[c] | 0.21 ± 0.06 | 1.76 ± 0.28 | 2.13 | – |
| | High control[d] | 43.11 ± 8.79 | 277.10 ± 50.35 | 1.60 | 1.00 |
| | Menthol | 51.22 ± 3.58* | 371.92 ± 41.20* | 0.73 | 1.34 |
| | M-HEP | 42.48 ± 15.46 | 283.41 ± 84.66 | 0.77 | 1.02 |
| | M-TET | 31.50 ± 3.54 | 212.33 ± 29.82 | 1.29 | 0.77 |
| | M-LA | 115.47 ± 14.52* | 837.44 ± 127.21* | 0.66 | 3.02 |
| | M-OA | 64.36 ± 4.46* | 466.79 ± 51.36* | 0.68 | 1.68 |

Data are given as average ± S.D. ($n = 4$).

[a] ER is the enhancement ratio calculated as follows: ER = $Q$ (with enhancer)/$Q$ (without enhancer).

[b] TOL tartrate concentration in IPM is 0.56 mg/mL.

[c] TOL concentration in IPM is 0.56 mg/mL.

[d] TOL concentration in IPM is 100 mg/mL.

* Value is significantly different from TOL (100 mg/mL) in IPM ($P < 0.05$).

effect on the permeation of TOL through the skin, followed by M-OA and *l*-menthol ($P < 0.05$), while M-HEP and M-TET had no promoting effects ($P > 0.05$). The solubility parameters of *O*-acylmenthol derivatives were similar to that of IPM (Zhao et al., 2008b), which could insure that all the derivatives are fully compatible in IPM and, so, the mixture of derivatives and IPM could be delivered to the SC. When using IPM which is known to be a fluidizer of intercellular lipids (Lee et al., 2006; Leichtnam et al., 2006) as a vehicle, the enhancing activity of *O*-acylmenthol derivatives for TOL was relatively low (ER values <5), which could be attributed to the fact that IPM also had an enhancing effect. According to a previous report (Zhao et al., 2008b), M-TET, with a C14 alkyl chain had a significant effect on a drug with a high water-solubility, so in the case of TOL with a low water-solubility (1.16 mg/mL), no enhancing effect was observed. Moreover, the addition of M-OA had a greater enhancing effect on the skin permeation of TOL, and this result agreed well with our previous report which investigated the effects of M-OA on lipophilic and hydrophilic drugs (Zhao et al., 2008a). Indeed, experimental verification of postulation that distinct hydrophilic (polar) and lipophilic (nonpolar) pathways exist for hydrophilic and hydrophobic drugs has come from the work by Yamashita et al. (1994, 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 high hydrophobicity and viscosity which could contribute to their more pronounced effect on nonpolar pathways which were rich in the esterified fatty acids and ceramides within the SC that contribute to diffusional resistance (Golden et al., 1987). As far as M-LA was concerned, its effect is attributable to the α-hydroxy moiety which exerts a skin "hydration effect" and produces an increase in partition into the skin of highly water-soluble compounds as proposed by previous reports (Hood et al., 1999). However, Zhao et al. (2008a) found that M-LA could promote the permeation of isosorbide dinitrate (calculated water-solubility and $\log K_{O/W}$ were 0.52 mg/mL and 1.34) which had an even lower water-solubility than that of TOL. Accordingly, the "hydration effect" theory may also account for the enhancing activity of M-LA for TOL.

### 3.3. Effect of PSAs and O-acylmenthol on skin permeation of TOL

The advantage of fabricating the patches in the present study was to maintain intimate contact with the skin for at least 24 h, so that an *in vitro*/*in vivo* correlation could be obtained. The selection of an appropriate PSA is the most important factor in designing a TDDS (Tan and Pfister, 1999). For the formulation of a DIA patch containing TOL, the effect of different types of PSA on the skin permeation of TOL was evaluated using excised rat skin. The TOL concentration in the PSAs was fixed at 10% (w/w) and each DIA patch was prepared with a thickness of 80 μm. Table 2 shows the skin permeation rates of TOL from the DIA patches made from the different PSA. The skin permeation of TOL depends on the functional group of PSA employed. Whereas PSAs containing a carboxylic acid group had much lower skin permeation rates, PSAs containing no functional groups produced higher skin permeation rates of TOL. Other studies have also reported that different functional groups in acrylate PSAs could have different permeation rates (Guyot and Fawaz, 2000; Hai et al., 2008). The presence of carboxylic acid groups can increase the hydrophilicity of the matrix, which decreases the diffusivity of lipophilic TOL, and this phenomenon has been reported previously (Hai et al., 2008). In addition, the interaction between the amine moiety of TOL and the carboxylic acid of the PSA could also play an important role in the global reduction in penetration. Similarly, some researchers (Cho and Choi, 1998; Kim et al., 2000) also found that the interaction between a drug and PSA had significant effects on the diffusion of permeants from the patches.

Of the PSAs possessing no functional group, DT-4098 showed a higher skin permeation rate of TOL. Therefore, DT-4098 was selected as the PSA of choice for the DIA patch containing TOL. In order to develop a DIA transdermal delivery system for a drug, an appropriate enhancer is often needed to enhance the permeation rate and/or to increase the solubility of the drug in the patch. Since M-LA, M-OA and *l*-menthol produced high skin permeation rates of TOL in IPM solution, their enhancing effects on the skin permeation of TOL from DIA patches were investigated, and the results are shown in Table 3. The concentration of TOL was fixed at 10% (w/w) and the concentration of selected *O*-acylmenthol was the same molar concentration of *l*-menthol which was fixed at 5% (w/w). The skin permeation rate of TOL with the *l*-menthol group was almost the same as with the control. M-OA produced the highest increase in *Q* ($1046.44 \pm 88.23$ μg/cm$^2$) ($P < 0.05$). However, unlike solution formulations, the addition of M-LA failed to increase the *Q* value dramatically, but actually reduced the cumulative amount compared with the control.

This result suggests that the permeation rate of TOL depends not only on the promoting effect of enhancer, but also on the interactions among the drug, PSA and enhancer, especially the potential interaction between the –OH of M-LA and the –CO– (carbonyl) of DT-4098 which prevents M-LA releasing from the patch and interacting with the SC. The inter-molecular H-bonding between the hydroxyl group of M-LA and the amine of TOL may also play a negative part in the release and diffusion of the TOL from the patch as the lag time is longest for M-LA. A similar discrepancy in enhancing activity has also been observed by Cheong and Choi (2003) who

**Table 3**
Skin permeation rates of TOL through excised rat skin from the patches containing 10% TOL (w/w) and different enhancers in DT4098.

| Enhancers | $J_{ss}$ (μg/cm$^2$/h) | $Q_{24}$ (μg/cm$^2$) | $T_{lag}$ (h) | ER[a] |
|---|---|---|---|---|
| Control | 26.95 ± 2.95 | 694.80 ± 87.24 | 0.29 | 1.00 |
| Menthol | 28.81 ± 5.33 | 739.95 ± 121.68 | 0.43 | 1.07 |
| M-LA | 7.84 ± 1.24 | 141.93 ± 21.30 | 2.36 | 0.21 |
| M-OA | 40.72 ± 4.51* | 1046.44 ± 88.23* | 0.27 | 1.51 |

Data are given as average ± S.D. ($n = 4$).
[a] ER is the enhancement ratio calculated as follows: ER = *Q* (with enhancer)/*Q* (without enhancer).
* Value is significantly different from TOL in control ($P < 0.05$).

**Table 2**
Skin permeation rates of TOL from the patches made with different PSAs containing 10% (w/w) TOL through excised rat skin.

| PSAs | | | | | $J_{ss}$ (μg/cm$^2$/h) | $Q_{24}$ (μg/cm$^2$) |
|---|---|---|---|---|---|---|
| Trade name[a] | Chemical description | Function group | Theoretical Tg (°C) | Tack (oz/in.$^2$) | | |
| DT4098 | Acrylate–vinylacetate | None | −11 | 19 | 26.95 ± 2.95 | 694.80 ± 87.24 |
| DT2852 | Acrylate | –COOH | −26 | 40 | 2.54 ± 0.58 | 56.29 ± 17.29 |
| DT2677 | Acrylate | –COOH | −28 | 5 | 1.56 ± 0.28 | 42.04 ± 8.46 |
| DT9301 | Acrylate | None | −26 | 45 | 18.73 ± 1.48 | 379.05 ± 34.53 |

Data are given as average ± S.D. ($n = 4$).
[a] Duro-Tak series made by Nation Starch and Chemical Co., USA.

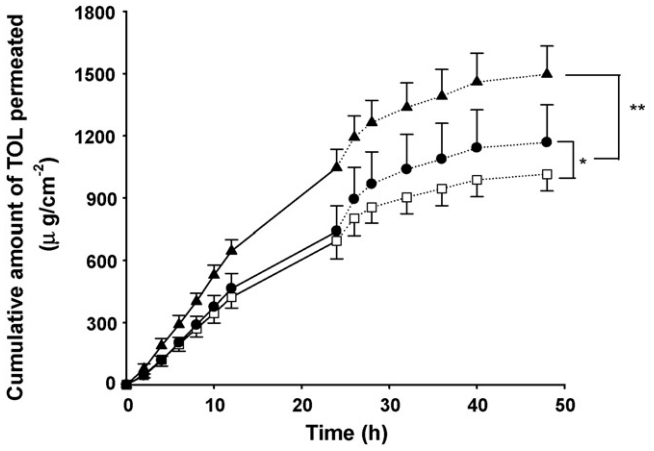


**Fig. 3.** Mean (±S.D.) cumulative amount of TOL permeated across rat skin ($n = 4$) after the application of transdermal patches containing M-OA, *l*-menthol as enhancers and without enhancer. The dotted line (…) indicates the cumulative amount after the removal of patches. Key: (□) control group; (●) containing *l*-menthol as enhancer; (▲) containing M-OA as enhancer. $^{*}P < 0.05$, $^{**}P < 0.01$.

investigated the effects of enhancers on the percutaneous absorption of piroxicam from PSA. However, more detailed investigation is required to further elucidate the mechanisms of interaction among the permeant, M-LA and DT-4098.

### 3.4. The effect of l-menthol and M-OA on the skin reservoir of TOL

Fig. 3 shows the cumulative amount of TOL that permeated through the rat skin from patches without enhancer or with *l*-menthol and M-OA as enhancers, before and after the removal of patches. Over the application wear time, it appears that the skin permeability was highest during the initial 12 h of TDDS application, then, the TOL content and concentration in the TDDS gradually decreased, resulting in a slow decline of TOL diffusion into the skin. It was indicated by Guy and Hadgraft (1992) that the surface concentration of permeant in contact with the skin was one of the most important factors affecting the drug penetration, which may account for the decline of permeation rate in the present work. In the case of the cumulative amount of TOL, there was no significant difference between the control group and the *l*-menthol group before patch was removed at 24 h, and to help investigate the existence of the skin reservoir effect, additional skin penetration studies were conducted until 48 h. There was a significant difference in TOL absorbed in the receptor fluid when data was compared at 48 h between the control and *l*-menthol group. The skin reservoir effects of enhancer-containing groups were significantly greater than that of the control group which could be attributed to the higher partition coefficient to the SC enhanced by *l*-menthol and M-OA. This is true since it was demonstrated that the reservoir effect was dominated by the lipophilicity of the chemical agent, the thickness of the skin layer and the partition coefficient in the skin layer (Kemppainen et al., 1991; Kubota and Maibach, 1994). It is interesting to note that the cumulative amount of TOL increased abruptly for a few hours just after removal of patches, which could be elucidated by the subsequent explanation: The driving force for permeation is the difference in concentration of TOL between skin and receiver compartment, so the maximum membrane concentration gradients (i.e., the driving force for diffusion) should be maintained. However, no sample was withdrawn during the time from 12 to 24 h which led to the driving force for permeation decreased. Therefore, the abruptly increasing in the cumulative amount of TOL could be observed just after the whole volume of receiver side was replaced with fresh medium due to the sharply increasing of concentration gradient between skin and receiver compartment. The lipophilic chemical, TOL was artificially retained in skin during the *in vitro* diffusion cell absorption studies due to the inability of TOL to freely partition from skin into the aqueous receptor fluid. Some researchers have recommended that the amount of permeant remaining in the skin at the end of a study should be included as part of the total dose absorbed unless the fate of the chemical in the skin is investigated and it is shown not to be available for systemic absorption (Bronaugh and Collier, 1991).

### 3.5. Intravenous administration of TOL

Pharmacokinetic parameters after intravenous administration of TOL in rats were required in order to predict the *in vivo* plasma levels of TOL in the rats from the *in vitro* skin permeability data. However, there are no pharmacokinetic data available for TOL in rats in the literature. Hence, pharmacokinetic parameters were calculated after intravenous administration of TOL tartrate (equal to 12.5 mg/kg of TOL) in rats. Fig. 4a shows the plasma profile of the observed concentrations after intravenous administration

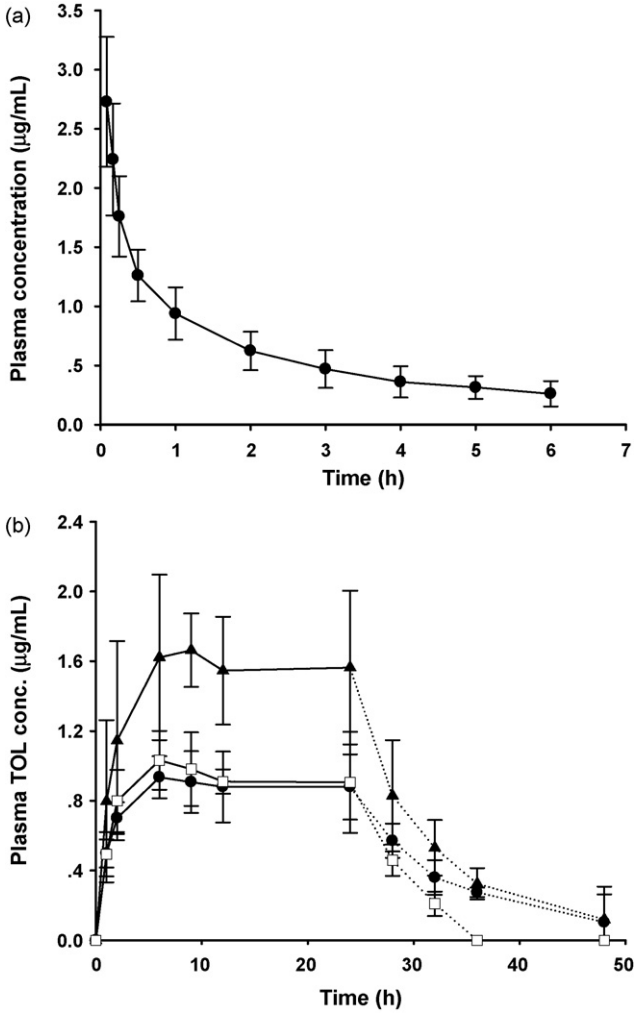


**Fig. 4.** Mean (±S.D.) plasma concentration-time profiles of TOL. Profile (a) is TOL plasma concentration after intravenous administration of TOL (12.5 mg/kg) in rats ($n = 7$); profile (b) is TOL plasma concentration after the application of transdermal patches containing M-OA, *l*-menthol as enhancers and no enhancer in rats ($n = 6$). The dotted line (…) indicates the plasma concentration after the removal of patches. Key: (□) control group; (●) containing *l*-menthol as enhancer; (▲) containing M-OA as enhancer.

**Table 4**
Pharmacokinetic parameters of TOL after intravenous administration (12.5 mg/kg) in rats.

| Parameter | Mean ± S.D. |
|---|---|
| $A$ | 2.51 ± 1.06 |
| $B$ | 0.95 ± 0.36 |
| $C_0$ (μg/mL) | 3.45 ± 1.04 |
| $AUC_{0-\infty}$ (μg h/mL) | 5.54 ± 1.28 |
| CL (L/h) | 0.51 ± 0.13 |
| $\alpha$ (1/h) | 4.29 ± 2.92 |
| $\beta$ (1/h) | 0.23 ± 0.10 |
| $t_{1/2(\alpha)}$ (h) | 0.22 ± 0.12 |
| $t_{1/2(\beta)}$ (h) | 3.46 ± 1.18 |
| $V_d$ (L/kg) | 3.57 ± 0.91 |
| $V_{ss}$ (L/kg) | 10.93 ± 3.08 |

Data are given as average ± S.D. ($n$ = 7).

of TOL. The observed data were in agreement ($r$ = 0.993) with a two-compartment open model. The pharmacokinetic parameters of TOL after intravenous administration are given in Table 4. The steady-state volume of distribution and clearance of TOL were 10.93 L/kg and 0.51 L/h, respectively. The concentration of TOL was too low to be detected in rat plasma up to 6 h of sampling.

### 3.6. Transdermal delivery of TOL from patches

The *in vivo* studies in rats were conducted in order to examine the ability of the TDDS of TOL to provide a steady-state plasma concentration of the drug as well to correlate the *in vitro* and *in vivo* permeability data. The plasma concentration profiles of TOL following the application of patches without enhancer or with *l*-menthol and M-OA as enhancers in rats are shown in Fig. 4b. The pharmacokinetic parameters, including $C_{max}$, $t_{max}$, $C_{ss}$, $AUC_{0-24}$, $AUC_{0-\infty}$ and MRT, are given in Table 5. The mean steady-state TOL plasma concentrations of 0.89, 0.84 and 1.47 μg/mL were maintained over 24 h for control, *l*-menthol and M-OA, respectively. M-OA provided the highest steady-state plasma concentration and the maximum plasma concentration of TOL (1.47 and 1.98 μg/mL). It can be seen from Fig. 4b that the plasma level of TOL declined after the removal of the patches, and it is noteworthy that the TOL of the control group was eliminated much faster than in the *l*-menthol group, although they had similar steady-state plasma concentrations and maximum plasma concentrations. Significant skin reservoir effects were observed, especially for the patches with *l*-menthol and M-OA as enhancers, as the rate constants for terminal phase presented in Table 5 after removal of the TDDS patches were much smaller than the rate constant for the elimination phase after i.v. bolus.

**Table 5**
Pharmacokinetic parameters of TOL after the application of patches without enhancer, with *l*-menthol and M-OA as enhancers in rats.

| Parameter | Control 18 $cm^2$ | Menthol 18 $cm^2$ | M-OA 18 $cm^2$ |
|---|---|---|---|
| $C_{max}$ (μg/mL) | 1.08 ± 0.13 | 0.98 ± 0.15 | 1.98 ± 0.23 |
| $AUC_{0-24}$ (μg h/mL) | 21.31 ± 2.62 | 20.11 ± 3.64 | 35.30 ± 1.83 |
| $AUC_{0-\infty}$ (μg h/mL) | 28.35 ± 4.16 | 32.36 ± 7.67 | 48.62 ± 3.99 |
| AUMC (μg $h^2$/mL) | 435.8 ± 62.08 | 768.3 ± 322.1 | 896.89 ± 176.97 |
| $C_{ss}$ (μg/mL) | 0.89 ± 0.11 | 0.84 ± 0.14 | 1.47 ± 0.08 |
| $t_{max}$ (h) | 13.5 ± 8.43 | 8.1 ± 2.45 | 11.1 ± 7.01 |
| MRT (h)[a] | 14.83 ± 1.18 | 23.13 ± 3.61 | 18.32 ± 2.62 |
| $\beta$ (1/h)[b] | 0.078 ± 0.046 | 0.049 ± 0.042 | 0.061 ± 0.054 |
| Enhancement facto $r$[c] | 1.00 | 1.14 | 1.71 |

Data are given as average ± S.D. ($n$ = 6).
[a] MRT, mean residence time.
[b] $\beta$, rate constant for the terminal phase after removal of patch.
[c] Enhancement factor calculated as follows: $AUC_{0-\infty}$ (with enhancer)/$AUC_{0-\infty}$ (without enhancer).

It should be noted that although the skin reservoir contributes to the total dose absorbed when the results are expressed as the area under the curve, it may not change the peak concentrations of TOL in blood because the absorption occurs as a continuous diffusion process over a long period of time. In conclusion, this study has shown that once the lipophilic chemical, TOL, has formed a reservoir which is significantly affected by the dosing enhancer in the skin after dermal application, it enters the body slowly over time, as demonstrated by the increase in the cumulative amount in the receptor solution and in the area under the curve after removal of patches.

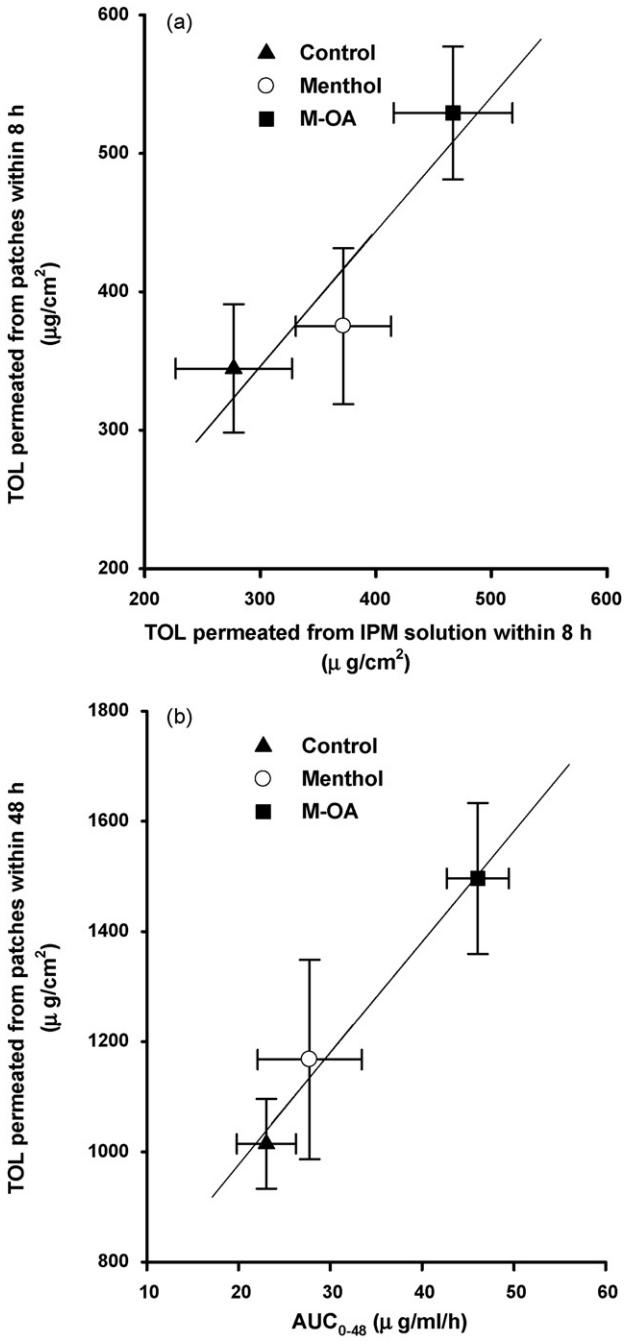


**Fig. 5.** Profile (a) is the relationship between the permeation amounts of TOL from patches and IPM solution, with or without enhancer, $r$ = 0.941; profile (b) is the relationship between the permeation amount of TOL from patches and the AUC after application of the patches, with or without enhancer, $r$ = 0.993. Skin permeation data (mean ± S.D., $n$ = 4); AUC data (mean ± S.D., $n$ = 6).

### 3.7. In vitro/in vivo correlation

To predict the steady-state plasma concentrations of TOL after the application of transdermal patches, with or without enhancers, from the *in vitro* permeation data, the following equation was used (Valiverti et al., 2005):

$$C_{ss}^{P} = \frac{J_{ss}A}{CL} \quad (7)$$

where "$C_{ss}^{P}$" is the predicted steady-state plasma concentration, "$J_{ss}$" is the steady-state flux across rat skin, "*A*" is area of the applied patch (18 $cm^2$) and "CL" is the total body clearance (510 mL/h). The mean steady-state fluxes of TOL following the application of patches were 26.95, 28.81 and 40.72 μg/$cm^2$/h for control, *l*-menthol and M-OA, respectively. The observed steady-state plasma concentrations of TOL after the application of patches without enhancer ($0.89 \pm 0.11$ μg/mL), with *l*-menthol ($0.84 \pm 0.14$ μg/mL) and M-OA ($1.47 \pm 0.08$ μg/mL) as enhancers in rats were comparable with the respective predicted steady-state plasma concentrations (0.95, 1.04 and 1.44 μg/mL) obtained over the period 0 and 24 h from the *in vitro* permeation data. This indicates that *in vitro* experiments with rat skin may be used for further transdermal drug delivery studies with TOL.

### 3.8. Correlation analysis of enhancing activity

The correlation analysis using the SPSS® program indicated that the penetration amount of the drug from IPM exhibited a relative good correlation with the cumulative amount of TOL from patches when TOL was in the control or using *l*-menthol and M-OA as enhancers, and the *r* is 0.941($P < 0.05$), as shown in Fig. 5a. As far as the *in vitro*/*in vivo* correlation was concerned, $AUC_{0-48}$ correlated well with $Q_{48}$ for the patches without enhancer or with *l*-menthol and M-OA as enhancers, the *r* being 0.993 ($P < 0.05$), as shown in Fig. 5b. The correlation analysis revealed that there was a good enhancing activity correlation between the $Q_8$ from IPM and the $Q_8$ from patches, especially between the $Q_{48}$ from patches *in vitro* and the $AUC_{0-48}$ *in vivo*.

The present study has indicated that more steady plasma levels of the TOL could be achieved via transdermal delivery, which would be an additional advantage in the treatment of urge urinary incontinence, and also, a good *in vitro*/*in vivo* correlation of transdermal TOL in rats using M-OA as an enhancer could be obtained, which would be meaningful for its future clinical application. As an ideal enhancer, M-OA should be effective, nonirritating, reversible and biodegradable, the experiments involving irritation tests and hydrolysis by esterase now in progress will be of importance in assessing enhancer suitability for clinical use.

## 4. Conclusions

The aim of the present study was to examine the transdermal delivery potential of TOL as well as to investigate the *in vivo* skin permeation enhancement effect of *O*-acylmenthol formulated in patches in rats. From the results of *in vitro* and *in vivo* studies, it is concluded that M-OA is the most promising enhancer among *O*-acylmenthol derivatives for transdermal delivery, although M-LA produced the highest enhancing activity in TOL in IPM solution. The observed 24-h mean steady-state concentrations for patches without enhancer, with *l*-menthol and M-OA as enhancers were in good agreement with the predicted plasma concentrations from the *in vitro* data. The correlation analysis revealed that there was a good enhancing activity correlation between the $Q_8$ from IPM and the $Q_8$ from patches, especially between the $Q_{48}$ from patches *in vitro* and the $AUC_{0-48}$ *in vivo* when using M-OA as a penetration enhancer.

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