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International Journal of Pharmaceutics 327 (2006) 58–64
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Enhancement of transdermal delivery of theophylline using microemulsion vehicle

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Received 9 April 2006; received in revised form 9 July 2006; accepted 17 July 2006
Available online 21 July 2006

Abstract

A microemulsion vehicle had been studied as a possible matrix for transdermal delivery of theophylline. The existence of microemulsion regions were investigated in pseudo-ternary phase diagrams, and various microemulsion formulations were prepared using oleic acid, Cremophor RH40/Labrasol (1:2) and water. The optimal formulation of the microemulsion was evaluated in vitro using Franz diffusion cells. The droplet size of microemulsion was characterized by photo correlation spectroscopy. Pharmacokinetic study in vivo was conducted using rabbits, and the results indicated that $AUC_{0\rightarrow\infty}$ of transdermal administration was 1.65-fold higher than that of oral solution administration. These studies showed that microemulsion system of theophylline might be promising vehicles for the transdermal delivery of theophylline.

Keywords: Microemulsion; Theophylline; Transdermal delivery; Enhancer; Bioavailability

1. Introduction

Theophylline (Fig. 1), a potent methylxanthine drug, which acted as a bronchiolar smooth muscle relaxant and a suppressor of non-bronchodilator response of airways, and whose bronchodilator mechanism was through the inhibition of phosphodiesterase by increasing cAMP, had been widely used for the treatment of asthma and chronic obstructive pulmonary disease by oral or intravenous route clinically (Choi et al., 1988). But theophylline had a low therapeutic index. In general, therapeutic serum theophylline concentrations ranged from 5 to 15 μg/ml. Small changes in plasma levels might result in therapeutic failure or adverse effects, such as nausea, vomiting, tachycardia, headache, seizure and agitation, therefore, the clinical use of theophylline was limited (Paloucek and Rodvold, 1988; Stavric, 1988; Minton and Henry, 1996).

Microemulsion system, composed of oil phase, aqueous phase, surfactant and co-surfactant at appropriate ratios, had a droplet size between 10 and 100 nm, possessed specific physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability (Gasco, 1997; Tenjarla, 1999; Lawrence and Rees, 2000).

Three main mechanisms had been proposed to explain the advantages of microemulsion for the transdermal delivery of drugs. First, the high solubility potential for both lipophilic and hydrophilic drugs of microemulsion systems might increase thermodynamic activity towards the skin. Second, ingredients of microemulsion, acting as permeation enhancers, might destroy the structure of stratum corneum and increase the flux of drug via skin. Third, the drug from microemulsion might be increased because the affinity of a drug to the internal phase could be modified easily (Delgado-Charro et al., 1997; Baroli et al., 2000; Kreilgaard, 2002). Recently many drugs such as ketoprofen, triptolide, apomorphine, lidocaine and estradiol using microemulsion for transdermal delivery had been reported (Kreilgaard et al., 2000; Peira et al., 2001; Rhee et al., 2001; Peltola et al., 2003; Chen et al., 2004; Sintov and Shapiro, 2004).

In transdermal delivery, choice of an appropriate vehicle for the transdermal delivery was an effective method to maximize the flux through the skin into systemic circulation (Berner and John, 1994; Hilton et al., 1994). The objective of this study was to find a stable microemulsion vehicle for theophylline transdermal delivery. A system consisting of oleic acid, Cremophor RH40, Labrasol and water was prepared, and its physicochem-

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doi:10.1016/j.ijpharm.2006.07.027

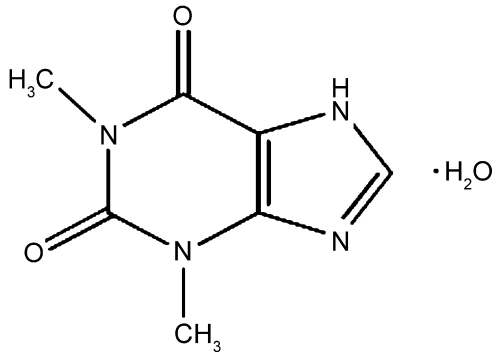

Fig. 1. The structure of theophylline.

ical properties and transdermal ability of theophylline in vitro and in vivo were evaluated.

2. Materials and methods

2.1. Materials

Theophylline was obtained from Shanghai Wandai Corporation (batch no. A031213, Shanghai, China). Oleic acid was purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). PEG-8 glycol caprylate (Labrasol) was kindly donated by Gattefossé, France. Polyoxyl 40 hydrogenated castor oil (Cremophor RH40) was donated by BASF, Germany. Water was deionized and filtered in our lab. All other chemical and solvent were analytical reagent grade.

2.2. Screening of oils and surfactants for microemulsion

In order to find out appropriate oils and surfactants that had good solubilizing capacity of theophylline and, thus, could be used as the oil phase and surfactants in microemulsion, the solubility of theophylline in various oils and surfactants were measured. Oils employed were sesame oil, olive oil, oleic acid, castor oil, salad oil and ethyllinoleate. Surfactants employed were lecithin, propanediol, span-80, tween-80, Labrasol, Cremophor EL35 and Cremophor RH40. An excess amount of theophylline was added to 10 ml of each oil or surfactants and turbine reciprocally at 60 °C, then balanced at 37 °C for 72 h and centrifuged, the theophylline concentration in the filtrate was determined using HPLC method after dilution with ethanol.

2.3. Construction of pseudo-ternary diagrams

Pseudo-ternary phase diagrams were constructed using H_2O titration method at 37 °C to obtain the components and their concentration ranges that could result in large existence area of microemulsion without the drug. Four phase diagrams were prepared with the 1:2, 1:1, 2:1and 4:1 weigh ratios of Cremophor RH40 to Labrasol, respectively. At each ratio of surfactant to co-surfactant (S/CoS), the ratio of oil to the mixture of surfactant and co-surfactant was varied as 0.3:9.7, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. Water was added drop by drop, under gentle magnetic stirring, to each oily mixture. After being equilibrated, the mixtures were assessed visually and determined as being microemulsion, crude emulsion or gel. Based on these diagrams, appropriate concentration of materials were selected and used in the preparation of microemulsion containing theophylline.

2.4. Preparation of microemulsion

For further studies, from the constructed pseudo-ternary phase diagrams, nine potential microemulsion vehicles were prepared at Km = 1:2 (Table 1). The theophylline microemulsion was prepared by adding theophylline in the oil phase and surfactant mixture, then water was added to them drop by drop under a 600 rpm magnetic stirring at 37 °C. Finally, permeation enhancer was added to the drug-load microemulsion formulation at the level of 3% (w/w).

2.5. Characterization and stability of microemulsion

The average droplet size and polydispersivity index of microemulsion were checked by photo correlation spectroscopy instrument (Marlven, UK) at 25 °C.

The morphology of theophylline microemulsion was also observed using transmission electron microscopy (TEM, H-7000, Japan). One drop of diluted samples was deposited on a film-coated 200-mesh copper specimen grid and allowed to stand for 10 min after which any excess fluid was removed with filter paper. The grid was later stained with one drop of 3% phosphotungstic acid (PTA) and allowed to dry for 5 min before examination under the electron microscope (Oyewumi and Mumper, 2003).

The viscosity of various microemulsion vehicles were measured at 25 °C, using a NDJ-8S digital viscometer (Shanghai Precision and Scientific Instrument, Shanghai, China) with a

Table 1
Compositions of the selected microemulsion formulations

Component	ME 1	ME 2	ME 3	ME 4	ME 5	ME 6	ME 7	ME 8	ME 9
Oleid acid (%)	5	5	5	5	12	10	5	5	5
Cremophor RH40 (%)	15	12	18	10	15	15	15	15	15
Labrasol (%)	30	24	36	20	30	30	30	30	30
Water (%)	49.8	58.8	40.8	64.8	42.8	44.8	49.6	49.4	49.2
Theophylline (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.6	0.8

no. 1 rotor set at 60 rpm. The centrifuge tests were carried out to assess the physical stability of microemulsion. Microemulsion vehicles were centrifuged for 30 min at 13,000 rpm and 4 h at 4000 rpm in the centrifuge test.

Microemulsion vehicles were stored at −4 °C, 60 °C and ambient temperature for 3 months. Then the clarity, phase separation and concentration of theophylline were investigated to judge the optimal storage temperature monthly.

2.6. In vitro permeation studies

Skins were obtained from male mice weighing 20 ± 2 g. After hair was removed carefully with a razor, a 2.5 cm × 2.5 cm patch of skin was excised from the abdomen region from each sacrificed mice and the subcutaneous fat and other extraneous tissues were trimmed. And then the skins were washed and examined for integrity. The skins were stored at 4 °C overnight and then used for experiments.

The extent and rate of skin permeation of theophylline from prepared microemulsion were determined using a diffusion cells (TP-4, Nanjing, China). The skins were clamped between the donor and the receptor chamber of vertical diffusion cells. The effective diffusion area was 1.54 cm^2. The receptor chamber was filled with 16 ml of physiological saline solution and its temperature was maintained at 37 ± 0.5 °C and stirred at 400 rpm throughout the experiment. After 1ml of the microemulsion was applied on the epidermal surface of the skin, 5 ml of the receptor medium was extracted for determination using HPLC with an injector at 0.5, 1, 2, 4, 6, 10, and 12 h after the application, and replaced immediately with an equal volume of physiological saline solution equilibrated at 37 ± 0.5 °C. Microemulsion without any theophylline was dealt with the same method as control simultaneity. The cumulative amount of theophylline permeated through excised mice skins was calculated by the equation:

$$Q_s = \frac{V}{S} \times C_n + \sum_{n-i}^{n-1} \frac{V_i}{S} C_i$$

where C_n was the drug concentration of the receiver solution at each sampling time, C_i the drug concentration of the sample, and V and V_i were the volumes of the receiver solution and the sample, respectively. S was the effective diffusion area ($S = 1.54\ cm^2$). The cumulative amount of theophylline permeated plotted as a function of time. The slope and intercept of the linear portion of the plot was derived by regression. The permeation rate of theophylline at steady state (J_S, μg/cm^2/h) through mouse skin was calculated from the slope of linear portion of the cumulative amount permeated through the mouse skins per unit area versus time plot.

In order to obtain the permeability coefficient K_p (cm/h), we used the equation:

$$K_p = \frac{J_S}{C_0}$$

where K_p was the permeability coefficient, J_S the flux calculated at steady state and C_0 represents the drug concentration which remains constant in the vehicle.

2.7. In vivo permeation studies

New Zealand male rabbits (2.0–2.2 kg) were obtained from the animal centre of China Pharmaceutical University and maintained at 25 °C for the study. The animals were housed in stainless steel metallic cages and provided with standard diet and water ad libitum. Necessary approvals were obtained for conducting this study.

Animals were fasted 24 h prior to the administration of drug formulations but had free access to water. One day prior to the experiment, hair on the backside area was clipped by a razor and washed with distilled water. Microemulsion containing theophylline (14 mg/kg) was applied on the skin surface (4.9 cm^2) in open containers glued to the skin by a silicon rubber. Theophylline solution was administered orally (14 mg/kg) with a soft plastic tube as control. Blood samples (1.0 ml) were collected at 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, 24.0, and 30.0 h after transdermal administration and 0, 0.2, 0.4, 0.6, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, and 24.0 h after oral administration through auricular vein into a heparinized glass tubes. The plasma was separated immediately and frozen at −20 °C until analysis.

Plasma levels of theophylline were estimated by HPLC. To 0.3 ml plasma, 0.1 ml (30.6 μg/ml) of vanillin was added and then extracted with 3.4 ml of ethyl acetate for 2 min. Organic layer (2 ml) was separated and evaporated to dryness at 60 °C under a stream of nitrogen. The residue was reconstituted with 0.5 ml of pure water and then theophylline was estimated using HPLC by ultraviolet detector at 272 nm. The lower detection limit by this method was 0.1 μg/ml. The R.S.D. for intra- and inter-day estimations were 2.43% and 1.93%, respectively, demonstrating good reproducibility.

PK parameters such as peak plasma concentration (c_{max}) and time of its occurrence (t_{max}) were read directly from the individual plasma concentration–time profiles. The other PK parameters, e.g. biological half-life ($t_{1/2}$), mean residence time (MRT) and area under the curve ($AUC_{0\to\infty}$), were calculated using a statistic soft which measured $t_{1/2}$ from the regression of the terminal phase of concentration time plot. MRT was calculated by dividing the $AUMC_{0\to\infty}$ by $AUC_{0\to\infty}$ and $AUC_{0\to\infty}$ was calculated by linear trapezoidal rule.

2.8. Statistical analysis

All skin permeation experiments in vitro were repeated three times and experiments in vivo were repeated using eight rabbits. Data were expressed as the mean value ± S.D. Statistical data were analyzed by one-way analysis of variance (ANOVA). A multiple comparison test was used to compare different formulations, and a P-value of 0.05 was considered to be significant.

3. Results and discussions

3.1. Screening of oils and surfactants for microemulsion

The solubility of theophylline was highest in oleic acid, followed by ethyllinoleate, olive oil, castor oil, salad oil and

sesame oil. Theophylline also had a higher solubility in Labrasol and Cremophor RH40 followed by Cremophor EL35, tween-80 and propanediol, but had a less solubility in lecithin and span-80. Kanikkannan (Kanikkannan et al., 2000) had reported that oleic acid could increase fluidity of lipid portion of the stratum corneum which resulted in a permeation enhancing effect, and Cremophor RH40 and Labrasol were good emulsifiers used in microemulsion. So oleic acid, Cremophor RH40 and Labrasol were subsequently used as the oil phase and surfactants for the formulations of microemulsion containing theophylline in this study.

3.2. Construction of pseudo-ternary diagrams

The construction of pseudo-ternary phase diagrams is used to determine the concentration range of components in the existence range of microemulsion. The pseudo-ternary phase diagrams with various weight ratios of Cremophor RH40 to Labrasol were described in Fig. 2. As shown in this figure, there were four areas in the diagram. With the proper ratio of oil phase and mixture surfactants, system changed from translucent water-in-oil (W/O) region to oil-in-water (O/W) region followed by the drop of water. The gel area which was transparent and high viscosity region also be seen in the diagram. The rest of the regions on the phase diagram represented the turbid and conventional emulsions based on visual observation. As S/CoS decreased, the area of existence in O/W microemulsion became enlarged, reaching a maximum at S/CoS of 1/2.

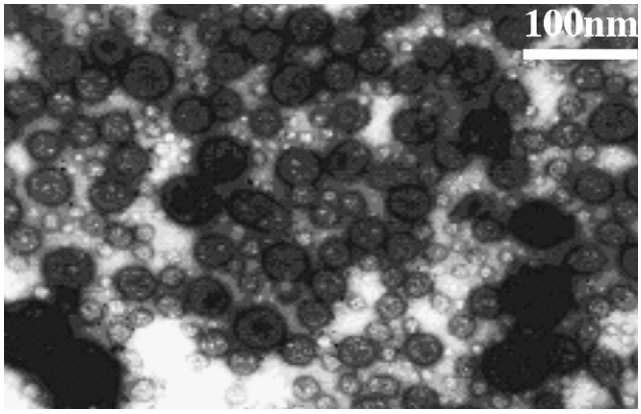

Fig. 3. Microphotograph of theophylline microemulsion by TEM (100,000×).

3.3. Characterization and stability of microemulsion

Morphology of theophylline microemulsion was characterized using TEM (Fig. 3). The average size of all microemulsion vehicles ranged from 22.3 to 78.5 nm. It was obvious that the average droplet size and viscosity of microemulsion with more oil increase significantly, while with more surfactant, no change

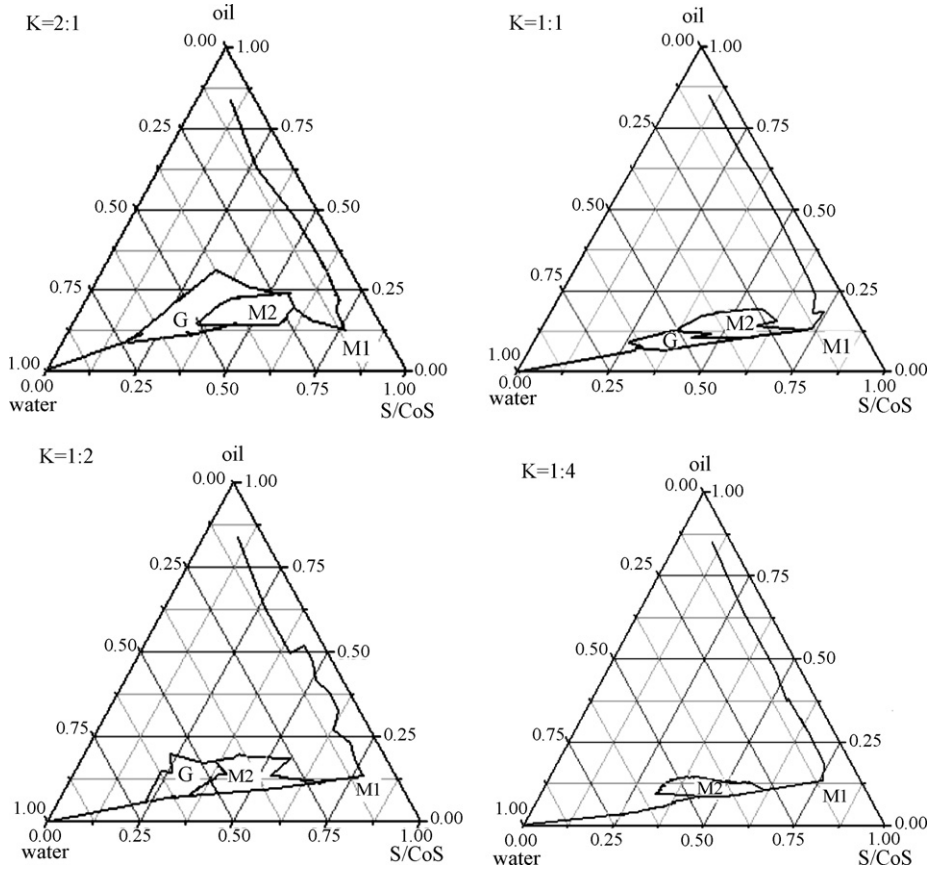

Fig. 2. Pseudo-ternary phase diagrams of microemulsion composed of oleic acid, surfactant (Cremophor RH40), co-surfactant (Labrasol) and water. K = S/CoS; M1, W/O region; M2, O/W region; G, gel region.

Table 2
Percutaneous permeation parameters of the tested vehicles (mean ± S.D.; $n = 3$)

Vehicles	J_S (μg/cm^2/h)	K_p (×10^3 cm/h)
ME 1	16.78 ± 0.75	9.87 ± 0.44
ME 2	15.60 ± 3.58	9.18 ± 2.11
ME 3	14.52 ± 1.05	8.54 ± 0.62
ME 4	18.70 ± 1.95[a]	11.00 ± 1.15[a]
ME 5	16.90 ± 2.38	9.94 ± 1.40
ME 6	15.36 ± 1.28	9.04 ± 0.75
ME 7	55.89 ± 9.65[b]	9.49 ± 1.64
ME 8	39.43 ± 7.98[b]	9.19 ± 1.86
ME 9	31.10 ± 4.91[b]	9.87 ± 1.56

[a] $P < 0.05$, compared with ME 1.
[b] $P < 0.01$, compared with ME 1.

in average droplet size and viscosity of microemulsion was observed. The ME 1 containing 5% oil, 15% Cremophor RH40 and 30% Labrasol without theophylline had the lowest average droplet size (23.6 nm) and viscosity (97.5 ± 0.3 mPa S). In addition, the average droplet size of microemulsion increased with the amount of theophylline. This is because theophylline might embed in the interfacial film (Sintov and Shapiro, 2004). Samples were diluted with distilled water before testing to avoid multiscattering phenomena. The droplet size of the diluted microemulsion was not significantly changed.

All microemulsion formulations were stable at ambient temperature in the presence or absence of theophylline. No changes of particle size, phase separation and degradation of theophylline were observed during 6 months. The centrifuge tests showed that all microemulsion had good physical stability. However, the phase separation and turbidity were observed when vehicles stored at −4 and 60 °C. The coagulation of the internal phase at low temperature and the cloud point of surfactant mixture at high temperature might lead to this instability. But these were easily recovered by storing in the ambient temperature for a while.

3.4. In vitro permeation studies

The effect of the content of oil and surfactant mixture on the skin permeation of theophylline was evaluated. The percutaneous permeation parameters of the tested microemulsion formulations were presented in Table 2. The skin permeation profiles were presented in Figs. 4 and 5.

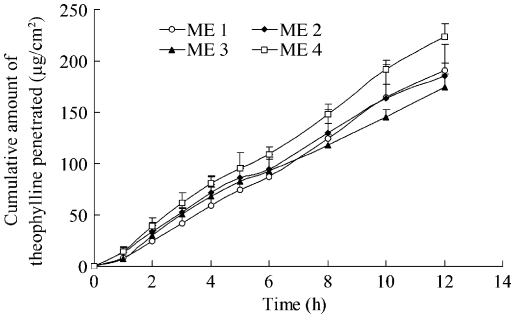

Fig. 4. The effect of the amount of mixture surfactants in microemulsion on the permeation rate of theophylline (mean value ± S.D.; $n = 3$).

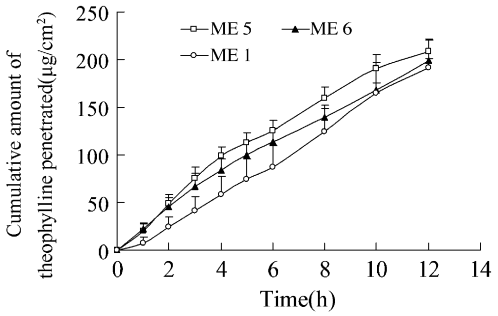

Fig. 5. The effect of the amount of oil phase in microemulsion on the permeation rate of theophylline (mean value ± S.D.; $n = 3$).

The content of surfactant mixture in microemulsion influenced the skin permeation rate of theophylline (Fig. 4.). As the content of surfactant mixture decreased from 54% to 30%, the skin permeation rate of theophylline increased 1.28 times. Theophylline was a poorly water-soluble drug, but soluble in the surfactant and co-surfactant. The thermodynamic activity of drug in microemulsion at the lower content of surfactant mixture was a significant driving force for the release and the penetration of the drug into skin (Walters et al., 1998; Rhee et al., 2001). So the increased concentration of surfactant in dispersed systems might decrease drug release and its permeation in the skin.

When the content of surfactant and co-surfactant was fixed to 45%, the skin permeation rate of the drug from the microemulsion containing different amount of oil (5, 10, and 12%) were tested. As shown in the Fig. 5, the skin permeation rate increased as the content of oil increased. Transdermal process was a passive diffusion process, which can be affected by transdermal permeation enhancer. Oleic acid was a powerful penetration enhancer, but it was encapsulated by surfactant mixture and hardly penetrated into stratum corneum. A high content of oleic acid could cause serious skin irritation (Boelsma et al., 1996). So 5% was chosen as the content of oil phase in this study.

Increasing the loading dose was an effective method to improve the skin permeation rate of various compounds. According to the Fick's first diffusion law, the permeation rate of drug was almost linearly improved as a function of loading dose of microemulsion. Fig. 6 showed the effect of the loading dose of theophylline in microemulsion on the permeation rate. The

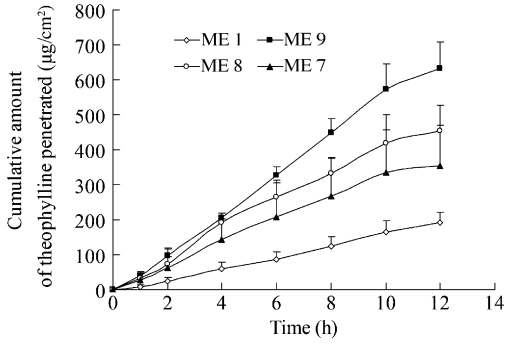

Fig. 6. The effect of different drug load of microemulsion on the permeation rate of theophylline (mean value ± S.D.; $n = 3$).

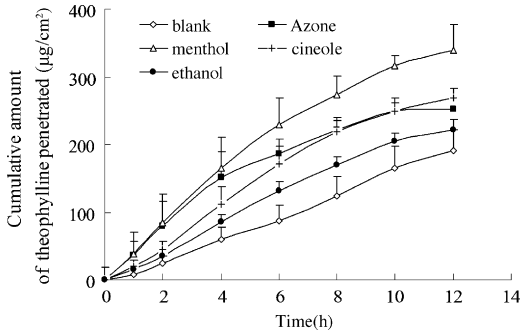

Fig. 7. The effect of different penetration enhancer on the permeation rate of theophylline (mean value ± S.D.; $n = 3$).

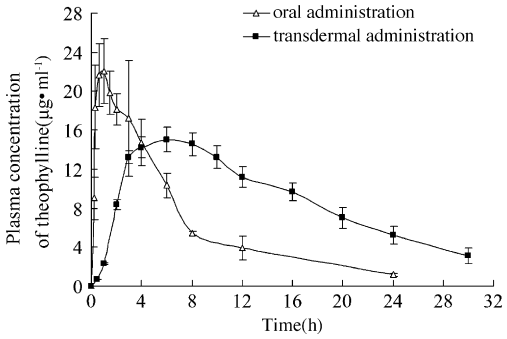

Fig. 8. Plasma concentration of theophylline following oral and transdermal administration to eight rabbits (mean value ± S.D.; $n = 8$).

permeation rate of theophylline increased from 16.78 ± 0.75 to 55.89 ± 9.65 μg/cm^2/h, as the loading dose of theophylline increased from 0.2% to 0.8%. The permeation rate of theophylline was almost linearly improved as a function of loading dose and the permeation of microemulsion accorded with the Fick's first diffusion law.

To improve the permeation rate of theophylline from microemulsion, various enhancers (menthol, eucalyptus oil, azone and ethanol) were added to the microemulsion. The obtained permeation profiles were shown in Fig. 7 and the permeation parameters calculated from the profiles were presented in Table 3. Menthol showed the most pronounced enhancing effect on the permeation rate of theophylline. When 3% menthol was incorporated into ME 1, the permeation rate of theophylline increased from 16.78 ± 0.75 to 29.15 ± 3.01, a 1.73 times increased in the permeation rate of theophylline was observed by compared to the control containing no enhancer, while other enhancer did not increase the skin permeation rate of the drug significantly. Menthol, a monocyclic terpene with pleasant odor, had been used as penetrate enhancer in many transdermal drug delivery system (Jain et al., 2002). The mechanism of enhancement might be attributed to the preferential hydrogen bonding of oxygen-containing monoterpene with ceramide head groups thereby breaking the lateral/transverse hydrogen bond network of lipid bilayer (Narishetty and Panchagnula, 2004).

3.5. In vivo permeation studies

The mean plasma concentration–time profiles of theophylline after oral and transdermal administration were shown in Fig. 8, respectively. The calculated PK parameters were given in Table 4. The results of the oral administration of theophylline solution indicated that theophylline could be rapidly absorbed from the rabbit gastrointestinal tract with a c_{max} of 22.0 ± 3.3 μg/ml at a t_{max} of 1.0 ± 0.1 h. Transdermal administration of theophylline microemulsion achieved steady state plasma concentration 14.6 ± 2.1 μg/ml after an initial lag time of approximately 8.8 h.

The transdermal administration microemulsion containing theophylline resulted in a bioavailability of 270.55 ± 31.8 μg/ml/h, whereas, the oral administration of theophylline solution resulted in an $AUC_{0\to\infty}$ of 164.0 ± 40.2 μg/ml/h. The $AUC_{0\to\infty}$ of transdermal administration was 1.65-fold higher than that of oral administration alone, and the statistic calculation provided a significant difference between both values ($P < 0.05$). Similarly, the MRT after transdermal administration was higher 12.75 ± 0.2 h compared to oral administration which was only 6.30 ± 0.2 h, which still provided a significant difference ($P < 0.05$).

Compared to oral administration, transdermal administration of theophylline microemulsion showed the low c_{max} and prolonged t_{max} in the plasma concentration–time curve, which was due to the barrier properties of the skin which lead to an accumulation of the drug in the skin. The summary of the AUC of theophylline after transdermal administration also increased compared with bioavailability following oral administration. It was due to avoidance of substantial amount of hepatic first pass metabolism associated with oral administration. The higher MRT values of transdermal delivery versus the oral route might be due to continuous replenishment of drug into the systemic circulation by constant and controlled delivery of drug from the theophylline microemulsion. Thus transdermal administration

Table 3
Percutaneous permeation parameters of the tested vehicles in the present of enhancer (mean ± S.D.; $n = 3$)

Vehicles	J_S (μg/cm^2/h)	K_p ($\times 10^3$ cm/h)
Control	16.78 ± 0.75	9.87 ± 0.44
Azone	19.58 ± 5.12	11.52 ± 3.01
Menthol	29.15 ± 3.01[a]	17.15 ± 1.77[a]
Cineole	24.40 ± 2.19[b]	14.35 ± 1.29[b]
Ethanol	19.85 ± 1.55	11.68 ± 0.91

[a] $P < 0.01$, compared with control.
[b] $P < 0.05$, compared with control.

Table 4
Pharmacokinetic parameters obtained after oral and transdermal administration of theophylline microemulsion in rabbits (mean ± S.D.; $n = 8$)

Parameter	Transdermal administration	Oral administration
c_{max} (μg/ml)	14.6 ± 2.1[a]	22.0 ± 3.3
t_{max} (h)	6.5 ± 1.6[b]	1.0 ± 0.1
$t_{1/2}$ (h)	8.8 ± 0.3	6.1 ± 0.2
AUC (μg/ml/h)	270.55 ± 31.8[a]	164.0 ± 40.2
MRT (h)	12.75 ± 0.2[a]	6.30 ± 0.2

[a] $P < 0.05$, compared with oral administration.
[b] $P < 0.01$, compared with oral administration.

of theophylline microemulsion had a sustained and enhanced absorption.

4. Conclusion

The theophylline microemulsion was formulated for transdermal application. The different microemulsion formulations were selected using the construction of pseudo-ternary phase diagram. Optimum formulation of the microemulsion was obtained by in vitro permeation studies. The addition of menthol to the microemulsion increased the permeation rate of theophylline via the skin. The permeation rate of theophylline from microemulsion accorded with the Fick's first law. The result of in vivo permeation experiments showed transdermal administration of theophylline microemulsion could provide sustained plasma concentration with minimal fluctuation.

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