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ORIGINAL ARTICLE

The effect of component of microemulsion for transdermal delivery of nicardipine hydrochloride

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Abstract

Purpose: Nicardipine hydrochloride has been used widely for the treatment of angina pectoris and hypertension. Because of its extensive first pass metabolism after oral administration, the transdermal administration of nicardipine microemulsions was developed in this study. *Methods*: Microemulsions consisted of isopropyl myristate (IPM), surfactant mixture of Tween 80/Span 80 and/or Tween 80/Span 20, co-surfactant (ethanol) and aqueous phase. Pseudo-ternary phase diagrams were constructed using water titration method. The effect of component of microemulsion on the percutaneous absorption of drug was evaluated by in vitro permeation study. *Results*: The area of microemulsion isotropic region in the presence of ethanol was comparably larger in the absence of ethanol. The mean droplet size of nicardipine microemulsions ranged from 70 to 123 nm. With addition of ethanol, the droplet size became smaller. The permeation rate and extent of nicardipine microemulsion transport across rat skin was affected by the components of microemulsion. Nicardipine microemulsion had higher flux at surfactant mixture with lower hyrophile-lipophile balance (HLB) value and Tween content. *Conclusions*: The microemulsion consisted of 52% IPM, 35% surfactant mixture and 13% water had higher permeation rate through rat skin above 122.53±1.87 μg/cm2/h and was expected to develop a transdermal delivery system.

Key words: *Nicardipine hydrochloride; Microemulsion; In-vitro study; Transdermal delivery; Flux*

Introduction

Nicardipine hydrochloride, a dihydropyridine calcium channel antagonist, is used in the treatment of angina pectoris and hypertension[1,2]. The oral bioavailability ranges from 20 to 33%, because of extensive hepatic first-pass metabolism. The elimination half-life is also very short at about 1 hour, and the drug has to be given frequently (30 mg three times daily), which often results in significant fluctuation in the plasma concentration[3–5]. Therefore, the development of a nicardipine transdermal dosage form would be beneficial for an effective and safe therapy of hypertension.

In the development of a transdermal dosage form, the most difficult aspect is to overcome the barrier of stratum corneum against foreign substances. It is well known that the permeation rate of drugs through the stratum corneum can be increased with appropriate vehicles and transdermal permeation enhancers, because of their ability to increase the solubility of drug and/or enhancers in pharmaceutical formulations and to change the structure of lipophilic and/or keratinized domains in stratum corneum[6,7]. Nowadays, numerous studies have reported that microemulsions consisting of an oily phase, aqueous phase, and surfactant have several advantages such as ease of manufacturing, thermodynamic stability, enhanced drug solubilization, and increase of the drug permeation rate[6,8–12] and they have been used as drug carriers for transdermal delivery such as triptolide, apomorphine, estradiol, hesperitin, and 8-methoxsalen[8,13–16]. However, the emulsifying agent (surfactant) is the most important component of the emulsion in terms of achieving stability and other characteristics such as size, viscosity, and permeability capacity. Therefore, the effect

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of component of surfactant in microemulsion on the characteristics and transdermal ability was evaluated in this study. The microemulsion systems were made up of isopropyl myristate (IPM) as oil phase, a mixture of polyoxyethylene sorbitan (Tween 80) and sorbitan surfactant (Span 80 or Span 20) as surfactant, and ethanol as cosurfactant and an aqueous solution. The in vitro permeation study was used to evaluate the effect of component of microemulsion on the rate and extent of nicardipine transport across rat skin.

Materials and methods

Materials

The following reagents were used: nicardipine hydrochloride, sorbitan monolaurate (Span 20, hydrophile-lipophile balance (HLB) 8.6), and sorbitan monooleate (Span 80, HLB 4.3) (Tokyo Chemical Industry, Japan); *p*-phenylphenol (Sigma-Aldrich, USA); polyoxyethylene sorbitan monooleate (Tween 80, HLB 15.0) (Showa, Japan); IPM and nimodipine (Merck Chemicals, USA). All other chemicals and solvents were of analytical reagent grade.

Construction of phase diagrams and nicardipine microemulsion preparation

Pseudo-ternary phase diagrams were constructed using the water titration method at ambient temperature to ascertain the concentration range of components for the existing range of microemulsions. Combinations of Tween and Span at 2/3, 3/2, and 4/1 weight ratios were prepared and used as mixed surfactant. Ethanol at a level of 40% aqueous phase was used as cosurfactant. The microemulsions with and without cosurfactant were prepared. The mixtures of oil (IPM) and mixed surfactant at certain weight ratios (1/9, 2/8, and 8/2) of 1 g were diluted with aqueous phase with or without cosurfactant drop wise, under moderate agitation for 1 minute. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions or crude emulsions.

Preparation of microemulsions

After the microemulsion regions in the phase diagrams were identified, the microemulsion formulations were selected at different component ratios as described in Table 1. The surfactant mixture of Tween 80/Span 80 or Tween 80/Span 20 and IPM was mixed well. Then water with or without cosurfactant (ethanol) was added to the oily phase drop by drop. The water-in-oil microemulsions were obtained using a vortex shaken at room temperature. Nicardipine at different levels was dissolved in the final microemulsion formulations.

Determination of droplet size of microemulsions

The average particle sizes of nicardipine microemulsions were determined by photocorrelation spectroscopy by laser light scattering (Zetasizer 3000HSA, Malvern, UK) using a helium–neon laser with a λ of 633 nm. Samples were loaded into 1 cm^2 cylindrical cuvettes and placed in a thermostated scattering chamber. Light scattering was monitored at a fixed angle of 90° and fixed temperature of 25°C.

Solubility of drug

An excess of nicardipine was placed in contact with 2 mL of water, 40% ethanol, or IPM in sealed glass tubes. The tubes were shaken continually at room temperature for 24 hours. The saturated solution was

Table 1. Composition, particle size, and permeation parameter of nicardipine microemulsions.

Surfactant	Ethanol	HLB	Drug (%)	Flux (μg/cm^2/h)	Q_{24h} (μg/cm^2)	Particle size (nm)
Control			1	5.68 ± 1.33	109.3 ± 27.2	—
T80/S80 (4/1)	+	4.50	2	87.31 ± 15.80	1643.1 ± 576.6	109.9 ± 3.5
T80/S20 (3/2)	−	4.35	2	267.81 ± 27.19	5471.0 ± 663.2	95.9 ± 6.8
T80/S20 (2/1)	−	4.50	2	166.29 ± 74.55	3697.6 ± 852.0	89.1 ± 8.3
T80/S20 (3/2)	+	4.35	2	147.83 ± 74.28	3083.7 ± 967.1	70.2 ± 6.3
T80/S20 (2/1)	+	4.50	2	125.26 ± 20.24	2672.7 ± 146.2	76.2 ± 1.7
T80/S80 (4/1)	+	4.50	3	116.10 ± 38.09	2146.4 ± 800.9	122.4 ± 22.7
T80/S20 (3/2)	−	4.35	3	—	—	—
T80/S20 (2/1)	−	4.50	3	—	—	—
T80/S20 (3/2)	+	4.35	3	154.25 ± 57.29	3460.1 ± 698.4	77.5 ± 5.5
T80/S20 (2/1)	+	4.50	3	157.30 ± 31.88	3116.0 ± 890.5	81.3 ± 8.3

Control contains 60% IPM and 40% surfactant mixture of Tween 80/Span 20 (suspension type). Microemulsion consists of 52% IPM, 35% surfactant mixture of Tween 80/Span 80 or Tween 80/Span 20 at different ratios, and 13% aqueous solution with or with ethanol as cosurfactant. HLB of surfactant mixture = ((volume percent Tween in formulation × HLB) + (volume percent Span in formulation × HLB))/100.

centrifuged and the supernatant was filtered through a 0.45 μm membrane. The concentration of drug in the saturated solution was determined by HPLC[17] after appropriate dilution with the selected solvents.

In vitro permeation study

The extent and rate of skin permeation of nicardipine from microemulsions were determined using a modified Franz diffusion cell fitted with excised rat skin. The skin was mounted on the receptor compartment with the stratum corneum side facing upward into the donor compartment and the dermal side facing downward into the receptor compartment. The donor cell was filled with 1 mL of nicardipine microemulsion and occluded by paraffin. The receptor compartment was filled with 20 mL of buffer (pH 7.4) containing 40% ethanol and its temperature was maintained at 37 ± 0.5°C by thermostatic water pump during the experiment. The effective diffusion area was 3.46 cm^2. Approximately 0.5 mL of the receptor medium was withdrawn at predetermined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the permeation data. Each data point represents the average of three determinations. The sample withdrawn from the receptor compartment was then analyzed by HPLC. Briefly, a HPLC equipped with a Waters LC Module 1 pump (Waters, Milford, MA, USA), a Hitachi model U-2001 detector (Hitachi, Tokyo, Japan), and Merck Lichrocart® 100RP-18 column (Merck, Germany) (150 × 4 mm I.D., particle size 5 μm) was performed. *p*-Phenylphenol was prepared as the internal standard. The mobile phase was a mixture of McIlvaine buffer (pH 5.0) and methanol in the ratio of 75:25 at the flow rate of 1 mL/min. The UV detection was at 240 nm. A calibration plot in the range 0.5–20 μg/mL was constructed. The coefficients of variation and relative error value were less than 4.98% and 2.19% for intra-day assay, and 3.02% and 3.23% for inter-day assay. The limit of detection was 0.1 μg/mL.

Results and discussion

Phase studies

Microemulsions consisted of IPM, surfactant mixture of Tween 80/Span 20 or Tween 80/Span 80, and aqueous phase in the presence and in the absence of cosurfactant (ethanol). For demarcation of the concentration range of components for the existence range of microemulsions, phase diagrams were constructed. The pseudo-ternary phase diagrams with various weight ratios of Tween 80/Span 20 or Tween 80/Span 80 are presented in Figure 1. The translucent microemulsion region is presented in phase diagrams. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. From Figure 1, it can be seen that the areas of microemulsions isotropic region produced by different ratios (2/3, 3/2, and 4/1) of Tween 80/Span 20 or Tween 80/Span 80 of surfactant mixture were different. The area formed by Tween 80/Span 20 of surfactant mixture was larger than that of Tween 80/Span 80 of surfactant mixture, even at similar HLB values, showing that the formation of microemulsions was affected by the surfactant mixture. The result is consistent with an earlier study, which reported that the formation of nanoemulsion was affected by the type, HLB value, and amount of surfactant mixture at specific oil phase[18].

In addition, the area of microemulsion isotropic region in the presence of cosurfactant (ethanol) was comparably larger than in the absence of cosurfactant. This is consistent with previous studies[19,20], which reported that cosurfactant acting predominantly in the aqueous phase is generally more effective at producing microemulsions over a wide range of compositions than those mainly acting in the interfacial film region. The phenomenon may be due to the fact that the cosurfactant could decrease the hydrophilicity of the polar solvent; hence the water-in-oil microemulsions were capable of solubilizing high water content[18,20].

Droplet size of microemulsion

The droplet sizes of tested microemulsion formulations are reported in Table 1. The droplet size of microemulsion vesicles was small with all the formulations having a mean vesicle size between 70 and 123 nm. It was found that the droplet size increased with increase of drug loading concentration. With the addition of cosurfactant, the droplet sizes became smaller. The phenomenon might be due to the fact that the cosurfactant increased the interfacial fluidity and flexibility of surfactant film by penetrating into the surfactant film, and then formed finer droplets[21,22].

Drug solubility

The solubility of nicardipine in water, IPM, and 40% ethanol was 4.47 ± 0.17, 0.14 ± 0.02, and 66.83 ± 196 mg/mL, respectively. Most microemulsions (Table 1) containing 2% or 3% nicardipine were clear and transparent solutions; no precipitate was observed, indicating that microemulsion can increase the solubility of the drug[14,15,23] and most nicardipine dissolved in the inner phase. Further, microemulsion with cosurfactant could dissolve more amount of the drug.

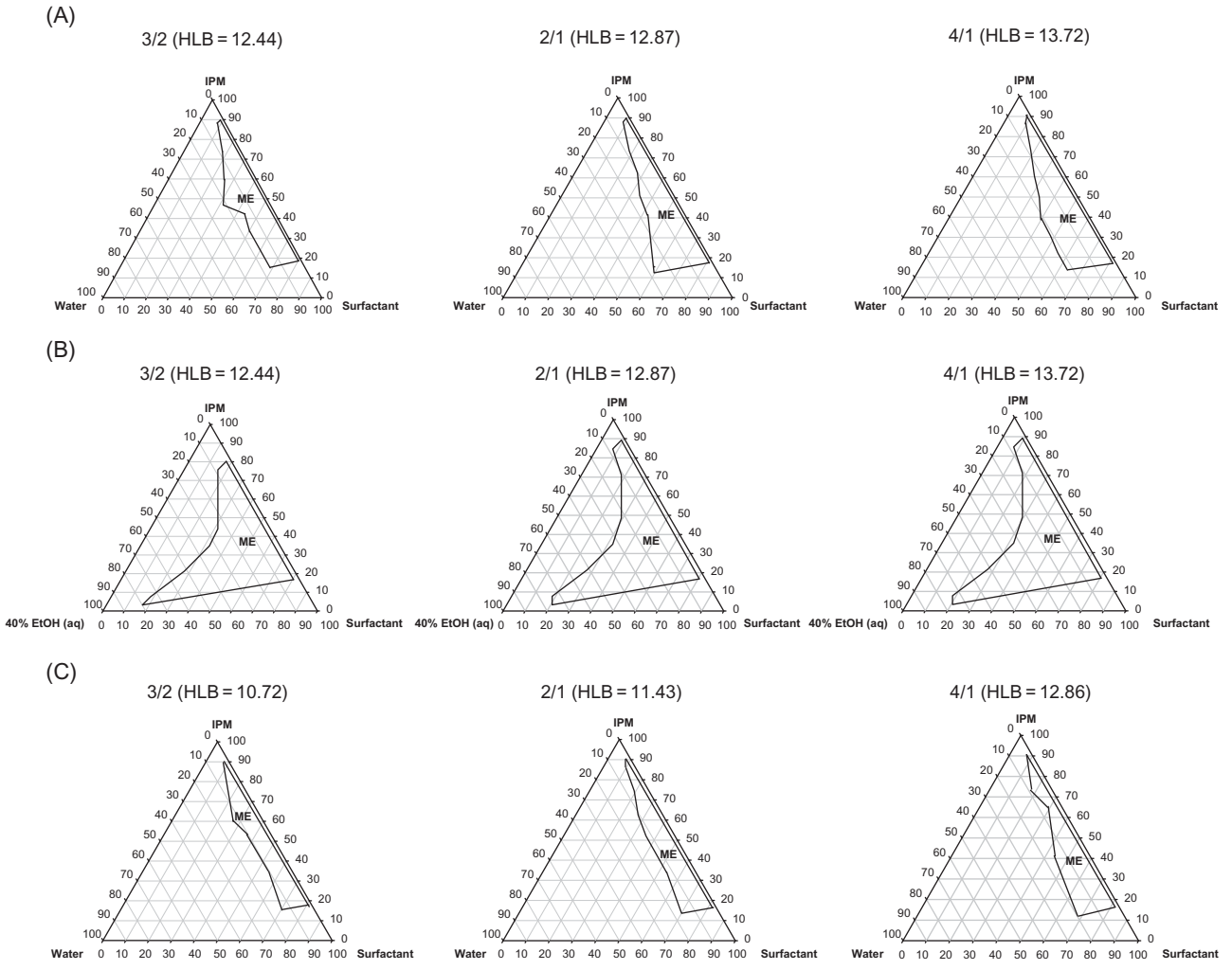

Figure 1. Pseudo-ternary phase diagrams of microemulsion composed of IPM, surfactant mixture, and aqueous phase: (A) surfactant mixture of Tween 80/Span 20 at ratio of 3/2, 2/1, and 4/1; (B) surfactant mixture of Tween 80/Span 20 at ratio of 3/2, 2/1, and 4/1 with cosurfactant; (C) surfactant mixture of Tween 80/Span 80 at ratio of 3/2, 2/1, and 4/1.

In vitro permeation study

The permeation profiles of nicardipine microemulsions through rat skin are shown in Figure 2. The permeation parameters are listed in Table 1. The permeation rate and 24-hour cumulative amount (Q_{24h}) of nicardipine microemulsions transport across rat skin ranged from 87 to 268 μg/cm^2/h and from 2146 to 5471 μg/cm^2, respectively, showing that the drug permeation characteristics through rat skin were significantly affected by the composition of microemulsion formulation. Moreover, the enhancement ratios of permeation rate of microemulsions were 15.4- to 47.2-fold higher than the control group (1% nicardipine dispersed in oil phase containing surfactant indicated microemulsion could increase drug permeation rate)[8,14–16]. When comparing the effect of composition of microemulsions on nicardipine permeation (Table 1 and Figure 2), it was found that the permeation rate and extent increased with the increase in drug content. This is due to the concentration gradients toward the skin. With the addition of ethanol as cosurfactant, it can be seen that the permeation rates and extent in microemulsions with ethanol were lower than those in microemulsions without ethanol. The partition coefficient of drug in IPM/water and IPM/40% ethanol were 12.26 and 0.33, respectively, indicating that a larger amount of drug dissolved in the inner phase in water-in-oil microemulsion with ethanol, thus resulting in the decrease of drug permeation. The other possibility was the increase of thermodynamic activity of the drug in microemulsion with cosurfactant which led to the reduction of the permeation rate[18,24].

Furthermore, the permeation rate and extent of microemulsion with lower HLB value were significantly

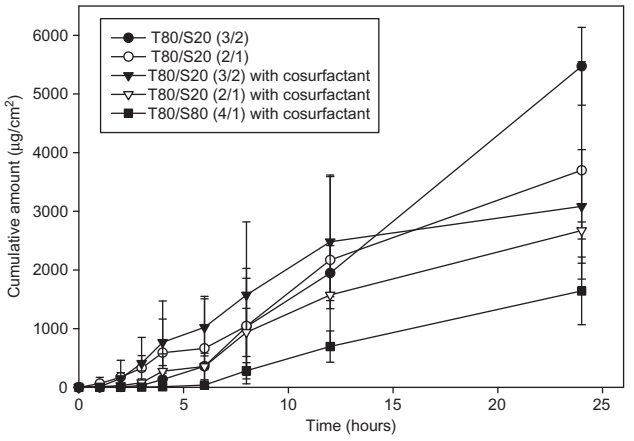

Figure 2. In vitro permeation–time profile of 2% nicardipine microemulsions through rat skin ($n = 3$).

higher than that of microemulsion with higher HLB value. This is consistent with a previous study reporting that the rate and extent of hydrophilic compound transport across hairy mouse skin were highly dependent on the HLB of the surfactant mixture in the nano-emulsion[18]. In addition, the microemulsions prepared with 52% surfactant mixture of Tween 80/Span 20 (2/1) showed higher permeation rate than those prepared with 52% surfactant mixture of Tween 80/Span 80 (4/1). This result might be due to the sorbitan surfactants being more hydrophobic than polysorbate surfactants, and may be expected to have a greater effect on the permeability of the stratum corneum[25].

In addition, Krishnaiah et al.[26] reported that a nicardipine total irritation score (TTS) patch containing 5% menthol had a permeation rate of 122.53 ± 1.87 μg/cm^2/h through rat skin in in vitro skin permeability studies. And the nicardipine TTS patch could provide a steady-state concentration of 21.39 ± 1.15 ng/mL for 26 hours at an administration area of 25 cm^2 in in vivo (health human volunteers) evaluation, for which plasma concentration lies in the steady-state Cmax values of 13.4 to 58.4 ng/mL after oral administration of Cardene SR (nicardipine hydrochloride) of 30 to 60 mg every 12 hours[27]. In this study, most nicardipine microemulsion formulations with higher permeation rate were above 122.53 ± 1.87 μg/cm^2/h through rat skin, demonstrating that the tested microemulsions were good carriers for nicardipine, and the nicardipine microemulsion might have the possibility of developing a transdermal delivery system.

Conclusion

The rate and extent of nicardipine transport across rat skin were found to be dependent on the type surfactant, HLB value, and amount of the surfactant mixture and cosurfactant in the microemulsion. The microemulsion consisting of 52% IPM, 35% surfactant mixture of Tween and Span, and 13% water had higher permeation rate through rat skin and was expected to develop a transdermal delivery system.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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