

Influence of Composition of *l*-Menthol–Ethanol–Water Ternary Solvent System on the Transdermal Delivery of Morphine Hydrochloride

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The influence of concentration of each component in *l*-menthol–ethanol–water ternary solvent system (MEW system) on the skin permeation of morphine hydrochloride (MPH) was investigated in hairless rats. The cumulative amount of MPH permeated through the excised abdominal skin over 8 h (*Q*-8) was selected as an index of skin permeability. With changing MPH concentration over a wide range from 0.01 to 10% in a MEW system (5% *l*-menthol and 40% ethanol), the values of *Q*-8 were proportional to MPH concentration. The concentration was fixed at 1% for the following experiments. For the effect of the concentration of *l*-menthol at 40% ethanol, the maximum *Q*-8 was observed at 5% *l*-menthol, and no greater enhancement of *Q*-8 was obtained by increasing *l*-menthol concentration above that. In the ethanol effect at 5% *l*-menthol, the maximum *Q*-8 was observed at 45% ethanol. When 2-propanol and methanol, which are more lipophilic and hydrophilic than ethanol, respectively, were used instead of ethanol, the maximum values of *Q*-8 were observed at 40 and 55%. The maximum values for *Q*-8 were obtained in the vicinity of the solubility of *l*-menthol in the MEW system in all cases, suggesting that the skin permeation enhancing effect of the system is dependent on the thermodynamic activity of *l*-menthol.

Keywords morphine hydrochloride; skin permeation; penetration enhancer; *l*-menthol–ethanol–water ternary solvent system

Introduction

Morphine hydrochloride (MPH) has conventionally been used as an analgesic for the relief of acute surgical and cancer pain.¹⁾ The drug has been administered in the form of injections, oral dosage or suppositories. MPH administered by these forms is eliminated rapidly making frequent doses necessary,²⁾ and causing a great burden to the patient. Since these formulations bring about a rapid increase in blood concentration, there may be unfavorable side effects. An appropriate level of blood concentration must be maintained to suppress pain.

MPH administered by the transdermal therapeutic system (TTS) seems a very good way of overcoming these problems. The system has the additional advantages of possible administration to patients who cannot take medication orally and quick termination by simple removal. Since the skin permeability of water-soluble drugs like MPH is generally low,³⁾ an enhancement of the skin permeation rate is necessary to establish an effective blood concentration. There are several means of increasing skin permeability, one of which is the use of a chemical enhancer.

Various chemical enhancers have been reported: some act mainly on the nonpolar route of the stratum corneum, the uppermost skin layer, and others on the polar route.⁴⁾ A combination of enhancers was impressive to increase the penetration enhancing effect.⁵⁾ The effect is probably dependent on the composition of each ingredient.⁶⁾ On the other hand, excess enhancer may cause a side effect such as skin irritation.⁷⁾ It is therefore very important in the development of TTS to search for an optimum composition which is highly permeable through skin but has low potential for skin irritation.

The strong enhancement effect of a system containing 5% *l*-menthol and 40% ethanol on the permeation of MPH was described earlier.⁸⁾ Here, the influence of concentration of each element of the *l*-menthol–ethanol–water ternary

solvent system (MEW system) on MPH permeation was measured to learn the optimum composition.

Experimental

Materials MPH and naloxone hydrochloride (as internal standard) were purchased from Takeda Pharmaceutical Co. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. *l*-Menthol, methanol, ethanol and 2-propanol were purchased from Wako Pure Chemical Industries (Osaka) and used as received. All other reagents were of reagent grade and were used without further purification.

Animals WBN/ILA-Ht male hairless rats (150–180 g, Ishikawa Laboratory Animals, Saitama, Japan) were used in all animal experiments.

In Vitro Skin Permeation Experiments The abdominal skin was excised and immediately mounted in a 2-chamber diffusion cell.⁹⁾ Each half cell has a volume of 2.8 ml and an effective diffusional area of 0.95 cm². The stratum corneum of the skin was connected with the donor compartment, and the dermis with the receiver compartment. Each enhancer system containing MPH was kept overnight at 37°C and placed in the donor compartment, and distilled water in the receiver. In this study, all systems were one liquid- or two liquid-phase. This experiment was done at 37°C for 8 h. Unless otherwise noted, the concentration of MPH in the donor was 1%. A one ml sample was withdrawn from the receiver compartment every 2 h and used for analysis. The same volume of water was added to the receiver cell to keep the volume constant as the experiment continued.

Determination of Solubility The solubility of MPH in each enhancer system was determined by suspending its excess solid for at least 24 h at 37°C. After equilibrium, excess MPH was removed by a cellulose acetate-membrane filter (0.2 µm pore size, Toyo Advantec, Tokyo, Japan). The obtained filtrate was diluted with distilled water and assayed.

Analysis The concentration of MPH was assayed by a high performance liquid chromatograph (HPLC) system (pump, LC-9A; system controller, SCL-6B; auto injector, SIL-6B; UV detector, SPD-6A; Shimadzu Seisakusho, Kyoto, Japan). The column used was a LiChrospher 100RP-18(e) 5 µm (4.0 × 250 mm, Kanto Chemical Co., Tokyo). A mixture of 0.1% phosphoric acid–acetonitrile (65:35) containing 5 mM sodium dodecylsulfonate was used as a mobile phase, and the elution was at 1.2 ml/min and 40°C. Each sample (200 µl) was added to the same volume of methanol containing naloxone and stirred well. Then, the mixture was centrifuged at 10000 rpm for 10 min and a 20 µl aliquot of the supernatant was injected to HPLC. The peak was detected at UV 230 nm and the area was calculated by an integrator (C-R6A, Shimadzu Seisakusho).

Results and Discussion

We recently reported that the MEW system enhanced the permeation of MPH through hairless rat skin.⁸⁾ The effect of the concentration of each element in the system should be accurately estimated to achieve the desired results. Standard concentrations were set at 5 and 40% for *l*-menthol and ethanol, respectively. The cumulative amount of MPH permeated through skin over 8 h (*Q*-8) was selected as an index by which to evaluate skin permeability for the following reasons: The steady-state flux could not be clearly obtained, and the short lag-time of skin permeation and rapid onset of pharmacological effect were advantageous clinical characteristics of the analgesic. The effect of MPH concentration on the skin permeation was examined first (Fig. 1). The range of concentration of MPH in the system was from 0.01% to 10%, and the total amount of the system was adjusted with water. Compared with respective controls (water base), the MEW system containing 1% and 10% MPH showed a 170 and 350-fold increase, respectively. At 0.01% MPH, the skin permeation from the control vehicle was below the detectable level (<500 ng/ml). The relationship between *Q*-8 (*y*-axis) and the initial concentration of MPH (*x*-axis) of the vehicle is plotted in Fig. 2. The plot of $\log x$ vs. $\log y$ gave an almost straight line and the slope was 0.94, confirming that a constant permeability coefficient could be retained under this MPH concentration. The drug flux was almost proportional to the concentration of the drug in the vehicle; hence, it is possible to control plasma level by changing the drug concentration in the vehicle.

Investigation of the effect of *l*-menthol concentration followed. Compared with 40% ethanol solution without *l*-menthol, the enhancing effect was hardly visible in the vehicle (40% ethanol) containing 0.1% *l*-menthol, but those containing 1 and 2.5% *l*-menthol showed 26 and 100-fold increase in *Q*-8, respectively. The enhancing effect increased by *l*-menthol up to the concentration of 5%, but further increase (up to 10%) obtained no greater enhancement (Fig. 3). Vehicle as *l*-menthol-ethanol-water solution changed from a homogeneous (one-liquid phase) to a heterogeneous (two-liquid phase) system at a concentration between 2.5 and 5% *l*-menthol. The heterogeneous system was raised by the saturation of *l*-menthol, and under this condition brought about the maximum thermodynamic activity of *l*-menthol. The activity of *l*-menthol became constant at more than 5% *l*-menthol. This may be why the permeation of MPH from the 10% *l*-menthol was similar to that from 5% *l*-menthol.

Figure 4 shows the effect of ethanol concentration at a fixed concentration of 5% *l*-menthol on the skin permeation of MPH. As ethanol concentration increased up to 45%, the skin permeation of MPH also increased, but, in contrast, when ethanol concentration rose above 45%, the skin permeation decreased. Interestingly, an alteration in the phase condition (from one-liquid phase to two-liquid phase) of the vehicle occurred between 40 and 45% of ethanol. The thermodynamic activity of *l*-menthol of the vehicle is kept at its highest level up to 40% ethanol because of the saturation of *l*-menthol, but it decreases at an ethanol concentration above 45% because of the dissolution of *l*-menthol. This reduction in the activity of *l*-menthol may decrease the amount of MPH permeated through the skin.

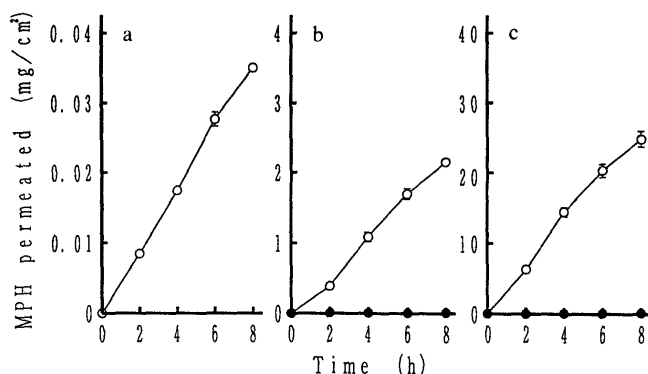


Fig. 1. Effect of MPH Concentration in Vehicle (a, 0.01%, b, 1%, c, 10%) on the Skin Permeation of MPH

○, MEW system (40% ethanol, 5% *l*-menthol); ●, control (without ethanol or *l*-menthol). Each value represents the mean \pm S.E. of at least 3 experiments.

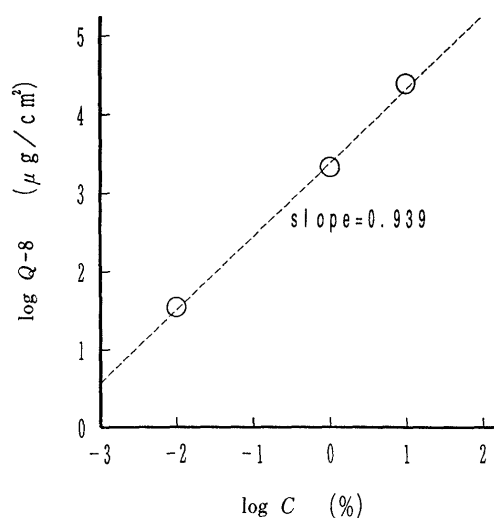


Fig. 2. Relationship between the Concentration of MPH in Vehicle and *Q*-8 of MPH Using the MEW System (40% Ethanol, 5% *l*-Menthol)

Each value represents the mean of at least 3 experiments.

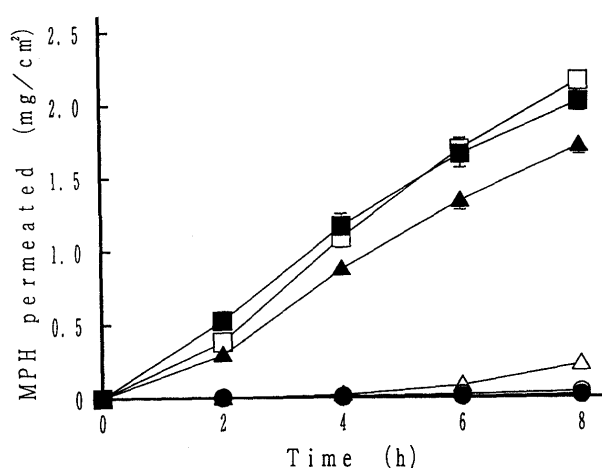


Fig. 3. Effect of Concentration of *l*-Menthol on the Skin Permeation of MPH Using 40% Ethanol

○, 0%; ●, 0.1%; △, 1%; ▲, 2.5%; □, 5%; ■, 10%. Each value represents the mean \pm S.E. of at least 3 experiments.

Based on these findings, it is assumed that the permeation of MPH is dependent both on the activity of *l*-menthol and on the concentration of ethanol in the system. Both substances have an individual enhancing effect (25.5-fold

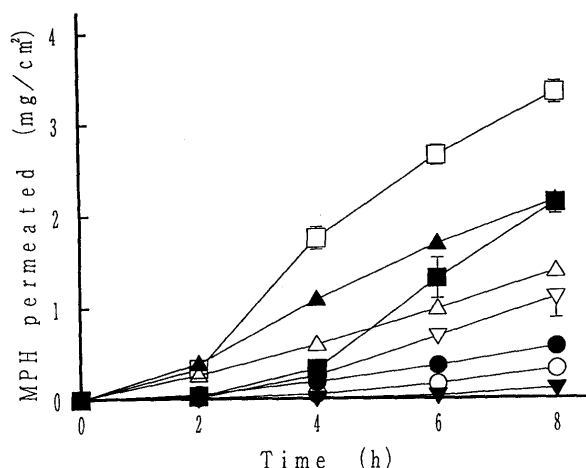


Fig. 4. Effect of Concentration of Ethanol on the Skin Permeation of MPH Using 5% *l*-Menthol

○, 0%; ●, 20%; △, 30%; ▲, 40%; □, 45%; ■, 50%; ▽, 60%; ▼, 80%. Each value represents the mean \pm S.E. of at least 3 experiments.

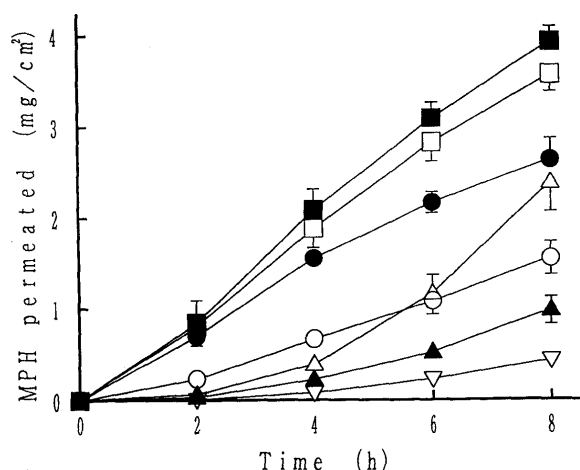


Fig. 5. Effect of Concentration of 2-Propanol on the Skin Permeation of MPH Using 5% *l*-Menthol

○, 20%; ●, 30%; □, 35%; ■, 40%; △, 45%; ▲, 50%; ▽, 60%. Each value represents the mean \pm S.E. of at least 3 experiments.

and 3.2-fold, respectively), and use of the two together brings about a multiple effect (171-fold). The maximum thermodynamic activity of *l*-menthol is apparently required for the greatest MPH skin permeation. Therefore, if ethanol as a coenhancer is replaced by another substance, the optimum concentration of coenhancer would be changed.

To confirm the above hypothesis, we used 2-propanol and methanol instead of ethanol; both showed different lipophilicity than ethanol and changed the solubility of *l*-menthol in the system. The results of these experiments are shown in Figs. 5 and 6. Each Q_8 was plotted against the initial concentration of the alcohol in the vehicle (Fig. 7). The maximum skin permeation was observed in the vicinity of the solubility of *l*-menthol in each system (methanol, 50–55%; 2-propanol, 35–40%). The results thus support the above hypothesis.

To compare the difference in the effect of alcohols on the skin permeation of MPH, Q_{\max} was applied using the following equation.^{5a)}

$$Q_{\max} = \frac{C_s}{C} \times Q_8$$

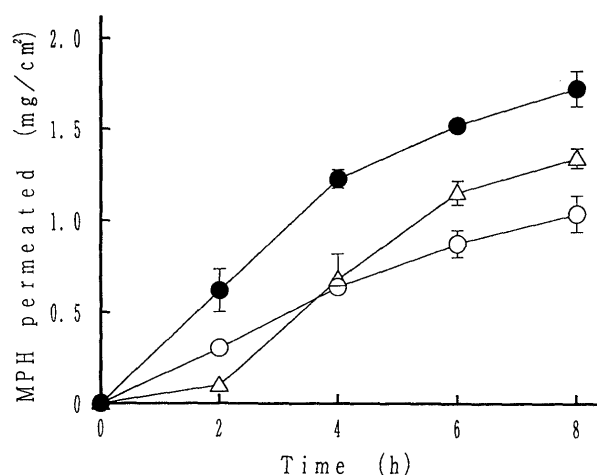


Fig. 6. Effect of Concentration of Methanol on the Skin Permeation of MPH Using 5% *l*-Menthol

○, 50%; ●, 55%; △, 60%. Each value represents the mean \pm S.E. of at least 3 experiments.

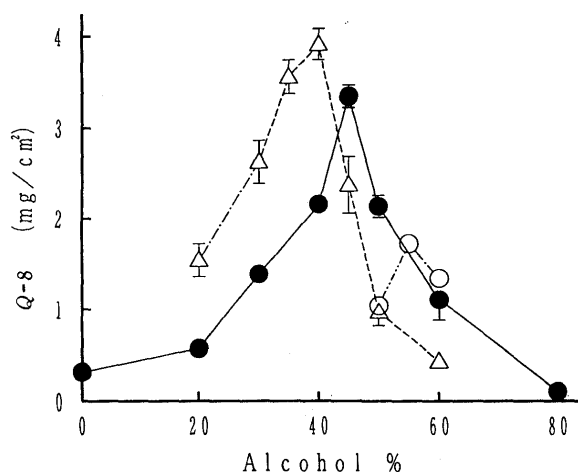


Fig. 7. Effect of Alcohols in the Enhancer System Containing 5% *l*-Menthol on the Skin Permeation of MPH

○, methanol; ●, ethanol; △, 2-propanol. Each value represents the mean \pm S.E. of at least 3 experiments.

TABLE I. Q_8 , Solubility and Q_{\max} at the Most Effective Concentration of Each Alcohol in an Enhancer System Containing 5% *l*-Menthol

	Q_8 ($\mu\text{g}/\text{cm}^2$)	Solubility of MPH (mg/ml)	Q_{\max} (mg/cm ²)
Methanol (55%)	1725 \pm 98	185.21 \pm 6.36	31.96 \pm 1.81
Ethanol (45%)	3346 \pm 124	106.03 \pm 3.04	35.48 \pm 1.32
2-Propanol (40%)	3917 \pm 170	75.24 \pm 2.22	29.41 \pm 1.27

Each value represents the mean \pm S.E. of 3 experiments.

where C_s and C are the solubility and concentration of MPH in each formulation, respectively. If the skin permeation of MPH can be explained by a solution-diffusion theory,¹⁰⁾ the effect of the alcohols can be compared by the Q_{\max} at the same thermodynamic activity of MPH. The Q_{\max} at the highest Q_8 for each alcohol is calculated as shown in Table I. These values were clearly independent of the kind of alcohol used.

The result suggests that the amount of solubilized *l*-menthol and its activity are the most important determinants for the MPH permeation, and that the

influence of alcohols is only related to the improvement of *l*-menthol solubility and is independent of the kind of alcohol. It is reported, however, that the main route for skin permeation of hydrophilic drugs such as MPH may be the pore pathway on the basis of the pore theory.¹¹⁾ Under the pore theory, the MPH permeation is not proportional to the activity, but to the concentration. Therefore, when the permeation was compared with the same concentration (1% MPH), its difference was dependent on the effect of the vehicle on skin and/or on the convective flow of solvents. Since the concentration and activity of *l*-menthol were almost equal among the vehicles in which the maximum of Q_8 was observed, the difference in the maximum of Q_8 should be caused by the difference in the effect of an alcohol. The similar Q_{\max} might be incidentally calculated. Since the mechanism of action by the MEW system on the skin barrier function and the skin permeation route of MPH are not yet clear, further detailed investigations are necessary to determine the roles of alcohol and *l*-menthol.

These results confirmed that the thermodynamic activity of *l*-menthol and the concentration of alcohol are related to the permeation of MPH through the skin. It was also confirmed that variation of the composition of the MEW system can make an enhancer system more effective, and it is suggested that the activity of *l*-menthol should be kept at the highest level to obtain maximal permeation of MPH

through skin.

We now intend to examine the relationship between the phase condition and the skin permeability of each element in the MEW system with the goal of designing the most effective permeation enhancing system for MPH.

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