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A new enhancer-coenhancer system to increase skin permeation of morphine hydrochloride in vitro

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Summary

The effect of several chemical enhancers on the skin permeation of morphine hydrochloride (MPH) was investigated using the in vitro diffusion cell technique and excised hairless rat skin. Enhancers used were: laurocapram (Azone®), isopropyl myristate, Sefsol®-318, l-menthol and ethanol. l-Menthol emulsified in water (5%) showed a rapid and strong enhancement of penetration, as did Azone and Sefsol-318 aqueous emulsions, although not to be the same extent. The effects of ethanol and isopropylmyristate were moderate. In experiments, the use of a combination of l-menthol with ethanol showed greater penetration enhancement. The cumulative amount of MPH permeated through skin over 10 h with this combination system (2467 $\mu g/cm^2$) was far higher than the sum of those with 40% ethanol alone (73 $\mu g/cm^2$) and 5% l-menthol alone (533 $\mu g/cm^2$). The intact skin permeation rate of MPH when the combination system was used was much faster than that of stripped skin when it was not used. The enhancing effect of ethanol on stripped skin was greater than those of water or l-menthol emulsion. The experiment results on solvent (water and ethanol) and l-menthol permeation suggested that the high penetration enhancement was due to both the action of l-menthol against the stratum corneum barrier and the effect of ethanol against the viable skin layer beneath it. Ethanol and water as a second solvent may co-transport MPH across the skin when used with l-menthol. We therefore devised an enhancer-coenhancer system for this combination.

Introduction

Inert and non-irritating potential chemical enhancers are being sought for the development of transdermal therapeutic systems (TTS) containing any one of several potent drugs. Some of the kinds of enhancers tested are already in use in commercial TTSs. However, the effect of most of those evaluated was not always sufficient. Enhancer is known to act by several mechanisms (Barry, 1991) and we combined enhancers with different mechanisms or sites of action to obtain a synergistic effect in the hope that such an effect may provide a breakthrough in the research and development of newer TTSs.

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Sugibayashi et al. (1989) evaluated the impact of the simultaneous use of Azone (an oil-type enhancer) and *N*-methyl-2-pyrrolidone (a solvent-type enhancer) on in vitro and in vivo skin permeation and on the pharmacological effect of morphine hydrochloride. Addition of *N*-methyl-2-pyrrolidone shortened the period prior to onset of the penetration enhancing effect of Azone and increased skin permeation of the drug. The Azone-*N*-methyl-2-pyrrolidone system increased the blood concentration and pharmacological efficacy of morphine hydrochloride. This is an example in which appropriate selection of a combination of enhancers is a key factor in increasing skin permeation of a particular drug.

The present study evaluated the effects of single use of several potent chemical enhancers followed by the effect of several potent chemical enhancers used in combination (like Azone and *N*-methyl-2-pyrrolidone) on the skin permeation of morphine hydrochloride, the potent analgesic effect of which cannot be maintained by conventional dosage forms (Foley, 1985; Savarese et al., 1988). Experiments were performed using in vitro diffusion cell technique and excised hairless rat skin. The skin permeation of the solvents and the mechanism of the enhancing effect were of interest.

Experimental

Materials

Morphine hydrochloride (MPH) and naloxone hydrochloride were supplied by Takeda Pharmaceutical Co. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. Azone (Nelson-Sumisho Co., Tokyo), l-menthol (JP grade), isopropyl myristate (IPM, Wako Pure Chemical Industries, Osaka), Sefsol-318 (S-318, Nikko Chemicals, Tokyo), and ethanol (EtOH, Wako Pure Chemical Industries) were used as chemical enhancers. l-Carvone was purchased from Wako Pure Chemical Industries Ltd. Deuterium oxide (D_2O) and ethanol-d_6 (EtOD) were obtained from Merck Co. (Darmstadt, Germany) and Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), respectively. Other reagents were of reagent grade or HPLC grade products.

TABLE 1

Enhancer and its concentration (w / w)

Enhancer	Concentration (%)
l-Menthol	5
Azone®	3
Sefsol-318®	5
Ethanol	40
Isopropyl myristate	5

In vitro skin permeation experiments

Abdominal skin excised from hairless rat (WBN/ILA-Ht, male, average weight 150 g, Life Science Research Center, Josai University, Saitama Japan) was used in the permeation studies. Samples of whole skin or stripped skin (stratum corneum-stripped skin) were carefully mounted between half-cells of a two-chamber diffusion cell set (effective diffusion area: 0.95 cm^2) (Okumura et al., 1990). The stripped skin was prepared by the method of Washitake et al. (1973). One chamber facing the stratum corneum of the skin was filled with 1% MPH solution (with or without enhancer/coenhancer) and the other chamber facing the dermis was filled with distilled water; the volume of each was 2.5 ml. Concentrations of enhancer-coenhancer are listed in Table 1. Permeation experiments were carried out at 37°C with magnetic stirring. A constant volume of sample was withdrawn (0.1–2.0 ml each) from the dermal side solution at predetermined times to assay MPH, D_2O, EtOD or l-menthol. The same volume of distilled water was added to maintain a constant volume after sampling.

Determination of solubility of MPH and l-menthol

Excess MPH was added to the 5% l-menthol/aqueous ethanol of which concentration had been varied and equilibrated at 37°C for 24 h. Apparent solubility of MPH in the system was measured.

Excess l-menthol was added to the 40% ethanol solution and equilibrated at 37°C for 24 h. After standing for 24 h, the concentration of l-menthol in the lower layer was measured.

Assays

MPH was determined by an HPLC method. Each sample (100 μl) was added to the same volume of methanol containing naloxone hydrochloride as an internal standard. The HPLC system consisted of a pump system (LC-6A, Shimadzu Seisakusho, Kyoto, Japan), a UV detector (SPD-6A, Shimadzu Seisakusho), Chromatopac (CR-3A, Shimadzu Seisakusho) and a stainless-steel column (4.0 × 150 mm) packed with Nucleosil $5C_{18}$ (Macherey-Nargel, Germany). Conditions were: elution phase, 0.1% phosphoric acid/acetonitrile (65:35) containing 5 mM sodium dodecylsulfate; flow rate, 1.5 ml/min; room temperature; detection, UV 230 nm.

EtOD was measured by a gas-chromatographic (GC) assay. To 100 μl of sample, the same volume of distilled water containing *n*-propanol as an internal standard was added and 1 μl of the supernatant after centrifugation was injected in a GC system (GC-6A, Shimadzu Seisakusho). Conditions were: column, Gasukuropak 54; column, injection and detection temperature, 140, 180 and 180°C, respectively; carrier gas, N_2; flow rate, 50 ml/min.

l-Menthol was also measured by a GC assay. Sample containing l-menthol was added to the same volume of dioxane containing l-carvone as an internal standard. The supernatant (1 μl) after centrifugation was injected in a GC system (GC-14A, Shimadzu Seisakusho). Conditions were: column, OV-17 (GL Sciences); column, injection and detection temperature, 130, 160 and 160°C, respectively; carrier gas, N_2; flow rate, 50 ml/min.

D_2O was determined by IR spectrometry (IR-450, Shimadzu Seisakusho) with $CaCF_2$ cell (light distance, 0.025 mm; window, 30 mm diameter × 4 mm). Detection wave number was 2515 cm^{-1}. The amount of D_2O was calculated by subtracting the total (D_2O + EtOD) value from the EtOD value measured by GC (Sugibayashi et al., 1992).

Results and Discussion

The effect of several penetration enhancers on the skin permeation of MPH was first evaluated using excised hairless rat skin and two-chamber diffusion cells (Fig. 1). Fig. 1a shows the cumulative amount of MPH permeated through the skin from the aqueous solution with and without enhancer. Many reports have been published on Azone's effect (Stoughton, 1982; Stoughton and McClure, 1983; Sugibayashi et al., 1985). When used with aqueous base, a long lag time was generally observed before steady-state flux was achieved with respect to transdermal delivery (Shannon et al., 1985; Morimoto et al., 1986). This long lag time was also found in the present experiment, although Azone showed a strong enhancing effect on the skin permeation of MPH when added to a 1% drug aqueous solution (Fig. 1a). IPM, a potent penetration enhancer (Sato et al., 1988), had a similar lag time and less effect on the skin permeation of MPH, and S-318, a potent solubilizer and enhancer (Okumura et al., 1990), although more effective than IPM, was also less effective than Azone. Ethanol, which is used in Estraderm® and Duragesic® as a solubilizer and enhancer of estradiol (William et al., 1985) and fentanyl (Gale et al., 1986), respectively, had only 4.3-fold higher cumulative amount than in the control experiment (without enhancer, distilled water alone) with its 40% solution.

When l-menthol was added at a concentration of 5% in 1% MPH aqueous solution, a marked effect (about 30-times higher cumulative amount

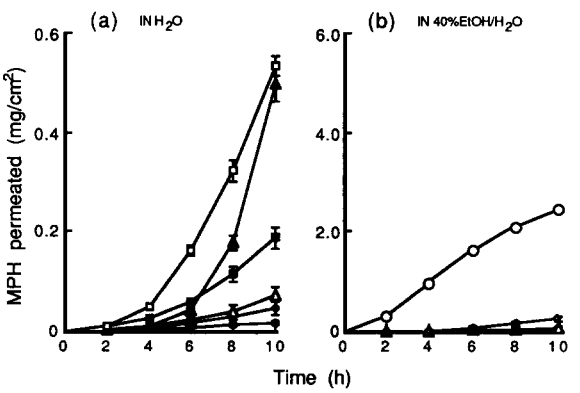

Fig. 1. Effect of several enhancers on the skin permeation of MPH. Each data point represents the mean and SE of three experiments. (a) In H_2O: (□) 5% l-menthol, (▲) 3% Azone, (■) 5% S-318, (△) 40% EtOH, (◆) 5% IPM, (●) control. (b) In 40% EtOH/H_2O: (○) 5% l-menthol, (◇) 5% IPM, (△) control (40% EtOH).

than control over 10 h) was found, similar to 3% Azone treatment. l-Menthol did have the advantage of a shorter lag time. It has been broadly used in Japan in many topical formulations containing nonsteroidal anti-inflammatory drugs to increase the pharmacological effects. The skin penetration enhancement by this substance has only recently been recognized, and reported together with that of other monoterpenes by Priborsky et al. (1991) and Williams and Barry (1991). Interestingly, MPH permeation was markedly increased by the synergism of 5% l-menthol and 40% ethanol (Fig. 1b), although slight synergism was found with the IPM-ethanol system. The cumulative amount of MPH permeated through skin per unit area over 10 h with the simultaneous use of l-menthol and ethanol (2468 $\mu g/cm^2$ per 10 h) was 145-times higher than control (water; 17 $\mu g/cm^2$ per 10 h). The enhancing ratio 145 was almost the same as the product of enhancing ratios, 4.3 and 31.2, which were obtained using 40% ethanol and 5% l-menthol alone, respectively. In addition to the permeation amount of MPH, the lag time was shortened to about 1 h by changing the vehicle from control vehicle (water) to the l-menthol-ethanol system.

Firstly, we investigated the relation between composition of l-menthol-ethanol system and the solubility of MPH in the system. The solubility was measured when the concentration of l-menthol had been held constant at 5% with the concentration of ethanol being made to vary (Table 2). Co-solvency was observed and MPH was most soluble in the system containing 40% ethanol. The solubility of l-menthol was about 4.0% in the 40% ethanol/water. Therefore, it was confirmed that l-menthol was saturated at 5% concentration in 40% ethanol/water. Consequently, the composition, 5% l-menthol/40% ethanol/water simultaneously achieved the highest solubility of MPH and the maximum activity of l-menthol. Detailed experiments on the effect of concentration of l-menthol and ethanol on the skin permeation of MPH are under way.

TABLE 2

Effect of EtOH concentration on the solubility of MPH in the system

EtOH concentration (%)	Solubility (mg/ml)
0	90.26
20	107.9
40	116.6
60	70.67
80	31.41
100	3.252

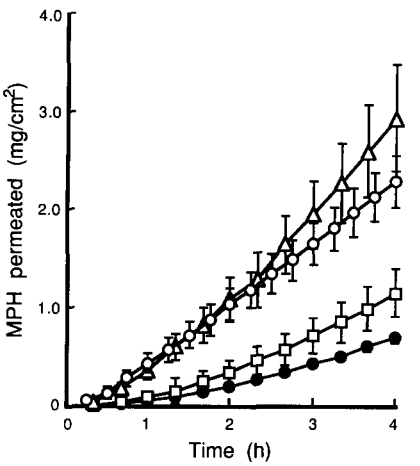

Fig. 2. Effect of several enhancing systems on the stripped skin permeation of MPH. Each data point represents the mean and SE of three experiments. (△) 40% EtOH/H_2O, (○) 5% l-menthol/40% EtOH/H_2O, (□) 5% l-menthol/H_2O, (●) H_2O.

Secondary, permeation of MPH through stripped skin was investigated (Fig. 2). Permeability through tripped skin is a good index by which to evaluate the effect of chemical enhancers on the skin main barrier, the stratum corneum (Morimoto et al., 1986). That is, the permeability was the same as that of control (without enhancer) when used with 5% l-menthol. In contrast, treatment with 40% ethanol or simultaneous use of 5% l-menthol and 40% ethanol showed about 3.5-times higher skin permeation than control. These results suggest that only ethanol can affect the stripped skin permeability of MPH. The fact that the intact skin permeability when 5% l-menthol and 40% ethanol were used suimultaneously was higher than that of stripped skin without the enhancer and coenhancer is of

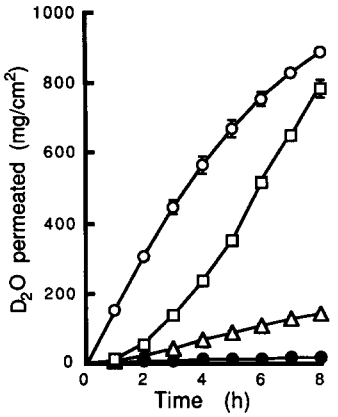

Fig. 3. Effect of several enhancing systems on the skin permeation of D_2O. Each data point represents the mean and SE of three experiments. (○) 5% l-menthol/40% EtOD/D_2O, (□) 5% l-menthol/D_2O, (△) 40% EtOD/D_2O, (●) D_2O.

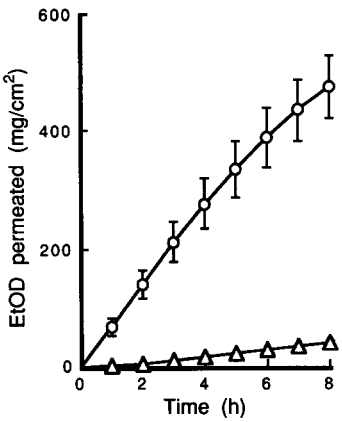

Fig. 4. Effect of several enhancing systems on the skin permeation of EtOD. Each data point represents the mean and SE of three experiments. (○) 5% l-menthol/40% EtOD/D_2O, (△) 40% EtOD/D_2O.

great interest. It was suggested that this simultaneous use could affect the barrier function of the stratum corneum much as the effect of ethanol on the viable epidermis and dermis came to be recognized.

Thirdly, the solvent flow was measured to evaluate the enhancing mechanism by the l-menthol-ethanol system. The cumulative amount of D_2O permeated through skin over 8 h was about 8.5-, 57- and 54-fold higher than that of control with the addition of 40% ethanol, 5% l-menthol, or their combination (Fig. 3). Taking into account the initial concentration of D_2O in donor, the permeability coefficient of D_2O for the case of 40% ethanol/water, 5% l-menthol/water of their combination was about 14-, 49- and 98-fold higher than that of control. The cumulative amount of EtOD permeated through skin over 8 h for this combined system was about 12-fold higher than that when 40% ethanol alone was used (Fig. 4). The rank orders of the solvent flow (Figs 3 and 4) were the same as for the skin permeation of MPH (Fig. 1). The increase in solvent flow partly contributed to the increase in permeability of MPH. The lag times of skin permeation of MPH and D_2O were 4 and 2 h, respectively (Figs 1 and 3) for 5% l-menthol aqueous emulsion, whereas they were 1 h and almost zero for the l-menthol-ethanol system. Since the penetration of ethanol into the skin for simultaneous use of l-menthol and ethanol was fast with no lag time, ethanol might facilitate the ready penetration of l-menthol into the skin.

Finally, permeation of l-menthol through intact skin was evaluated. The cumulative amount of l-menthol over 8 h from the simultaneous system was about 3-times higher than from 5% l-menthol alone. Thus, ethanol enhanced the permeation of l-menthol (Fig. 5). The action of ethanol of increasing l-menthol penetration and that of l-menthol of increasing ethanol penetration are believed to be interdependent. However,

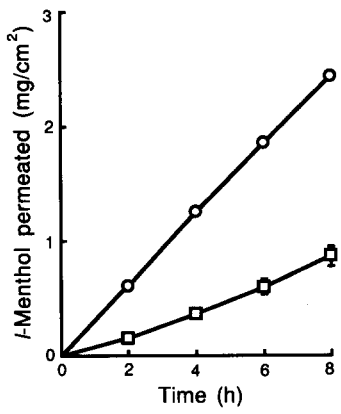

Fig. 5. Effect of EtOH on the skin permeation of l-menthol. Each data point represents the mean and SE of three experiments. (○) 5% l-menthol/40% EtOH/H_2O, (□) 5% l-menthol/H_2O.

it was suggested that the rapid penetration of ethanol might help to increase l-menthol penetration into the skin and that the resulting enhancement in l-menthol penetration might affect the permeation of MPH, although the enhancement effect of ethanol on MPH permeation was small. In this consideration, l-menthol and ethanol could be defined as an enhancer and a coenhancer, respectively.

These results suggest that the enhancement mechanism of the l-menthol-ethanol system is: (1) ethanol (coenhancer) facilitates the penetration of l-menthol (enhancer) into the stratum corneum; (2) l-menthol reduces the permeation resistance of all penetrants thorugh the stratum corneum; (3) permeation of the solvent is increased; (4) the permeation resistance of the epidermis beneath the stratum corneum is reduced; and (5) skin permeation of a drug (mainly a solvent-soluble drug) increases.

This enhancer-coenhancer system showed a marked effect on the skin permeation of MPH as we had been expecting, and the value of approx. 0.5 mg/cm^2 per h is the highest flux yet achieved as is known from the literature and our experimental results. The enhancer-coenhancer system therefore may be beneficial in increasing the skin permeation of many drugs. The enhancer-coenhancere system can be characterized either by parallel permeation of MPH with solvents or by high solubility of MPH in the system. Therefore, co-transport of solvents and MPH may be suggested. Drugs dissolved in an ethanol-water binary system would clearly be candidates. However, the mechanism of synergism requires further study.

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