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RESEARCH PAPER

Enhancement Effect of *p*-Menthane-3,8-diol on In Vitro Permeation of Antipyrine and Indomethacin Through Yucatan Micropig Skin

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ABSTRACT

The enhancing effect of *p*-Menthane-3,8-diol (MDO) on skin permeation of antipyrine (ANP) and indomethacin (IM) through Yucatan micropig skin in vitro was compared with l-menthol. *p*-Menthane-3,8-diol is a metabolite of l-menthol and has little odor. It is easy to combine the vehicle because of lower lipophilicity than l-menthol. All formulations contained 40% (v/v) ethanol. The permeation of ANP increased with MDO about three times that without enhancer by increasing ANP concentration in the skin. However, the MDO effect was about a quarter that of l-menthol. The permeation of IM with MDO was about 15 times that with no enhancer and it was almost the same as that with l-menthol. The lag time of permeation was not significantly changed by MDO, which was not so in the case of l-menthol. Skin concentration of IM increased about 11 times and six times with MDO and l-menthol, respectively. MDO and l-menthol partitioned to the skin relatively high concentrations, 5.9 and 2.5 mg/cm^3, respectively. The solubility of IM in the skin was improved by MDO, and consequently, the permeation of IM was enhanced.

Key Words: *p*-Menthane-3,8-diol; Skin permeation enhancer; Indomethacin; Antipyrine; Yucatan micropig skin; In vitro.

INTRODUCTION

Various methods of permeating sufficient amounts of drugs through skin have been studied. Coadministration of a chemical enhancer is a common method,[1] and many skin permeation enhancers, such as Azone,[2] oleic acid,[3] and nonionic surfactants[4] were investigated. Among them, terpens extracted from plants are

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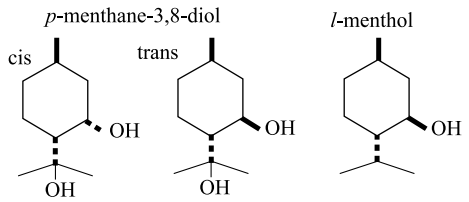

Figure 1. Structure of MDO and l-menthol.

good candidates for permeation enhancers because of their relatively low irritation. Many kinds of terpens, such as hydrocarbons (e.g., limonene), alcohols (e.g., l-menthol), or ketones (e.g., menthon), have been studied for their ability as enhancers.[5] *p*-Menthane-3,8-diol (MDO), mixture of cis:trans=65:35, originating in the leaf of *Eucalyptus citriodora*, is also a kind of alcohol-type terpen. Its chemical structure resembles l-menthol (Fig. 1), but is unique because it has two hydroxy groups. *p*-Menthane-3,8-diol was also known as one of the first main metabolites of l-menthol.[6] *p*-Menthane-3,8-diol is more hydrophilic [calculated logarithm of octanol/water partition coefficient (log P)=1.4] than other terpens reported as chemical enhancers (log P≅3), so the mixing with the vehicle might be easier. The allelopathic effect[7] and insect repellent effects[8] of MDO suggest its volatility; however, it is less than other terpens and has little odor. It experienced no change of formulation by evaporation of enhancer, and its reduced odor might be good for patients. Moreover, the two hydroxy groups are expected to induce high affinity to skin protein. *p*-Menthane-3,8-diol seems to be a candidate for a skin permeation enhancer; however, there are no reports about the effect of MDO on skin. Thus, we studied the enhancing effect of MDO on the skin permeation of drugs in vitro. The hydrophilic drug, antipyrine (ANP), and lipophilic drug, indomethacin (IM), were used as model drugs and their effects were compared with l-menthol.

MATERIALS AND METHODS

Materials

Antipyrine was obtained from Sigma (St. Louis, MO). Indomethacin (JP grade) was obtained from Nippon Bulk Yakuhin (Osaka, Japan). *p*-Menthane-3,8-diol and l-menthol were a gift from Takasago International (Tokyo, Japan). All other chemicals were reagent grade and were used without further purification.

Preparation of Samples for Skin Permeation Study

Drug and enhancer were dissolved in ethanol and added to water, and the mixture was kept at 37°C overnight, until immediately before the skin permeation study. The concentrations of ethanol and enhancer were fixed at 40% (v/v) and 3% (w/v), respectively. The drug concentration was fixed at 2% (w/v) for ANP and 0.5% (w/v) for IM. The formulation with 40% (v/v) ethanol and without enhancer was used as the control. The IM water suspension, which was prepared by suspending 0.5% (w/v) IM in water at 37°C overnight, was used for comparison with the control formulation.

Skin Permeation Study

Yucatan micropig skin (YMP skin set, Charles River Japan, Yokohama), after removal of the fat and subdermal tissue, was cut to an appropriate size and set on a modified Franz-type diffusion cell with an area of 1.1 cm^2. The thickness of the skin used was about 2 mm with a 20-μm stratum corneum and 20-μm viable epidermis. The receptor phase, which consisted of 16 mL of pH 7.1 isotonic phosphate buffer solution containing 0.01% (w/v) kanamycin, was kept at 37°C with stirring at 600 rpm. The solubilities of ANP and IM in the receptor solution were high, over 100 mg/mL and 1 mg/mL, respectively, so the sink conditions were maintained throughout the experimental periods. The sample (0.5 mL) was applied to the donor phase. A glass ball was used to occlude the upper portion of the donor phase. At predetermined intervals, 0.2 mL of the receptor phase was withdrawn and an equivalent volume of fresh solution was added. The drug concentrations in the receptor phase were determined by high-performance liquid chromatography (HPLC). After the permeation studies, skin samples were washed with purified water followed by 75% (v/v) methanol in water, and then homogenized (Hiscotron, Microtec Nichion, Chiba). Homogenates were centrifuged and the supernatants were filtered by membrane filter (Dismic-25, Advantec, Tokyo, Japan). The concentrations of drug and enhancer were determined using HPLC.

Determination of Drug and Enhancer by HPLC

The concentrations of drug and enhancer were determined using HPLC. The analytical system included

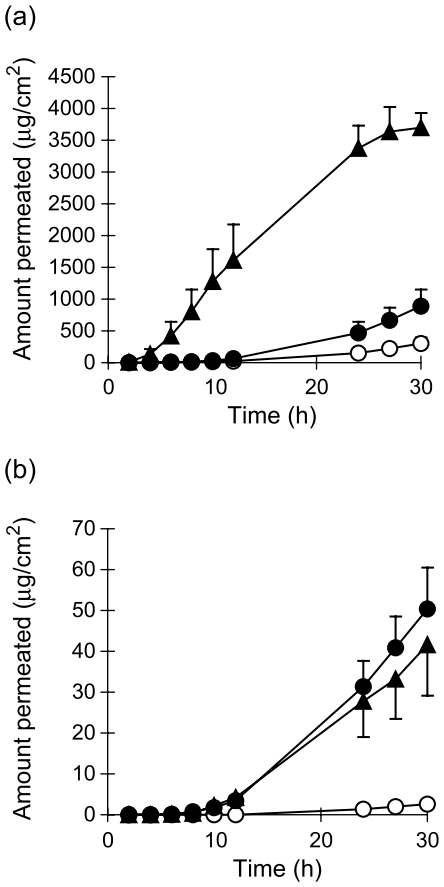

Figure 2. Permeation profiles of ANP (a) and IM (b) from various formulations. ○, control; ●, MDO; ▲, l-menthol. Each point represents the mean ± SD of at least three experiments.

a pump (LC-10A, Shimadzu, Kyoto, Japan), a UV detector (SPD-10AD) operated at 245 nm for ANP and at 264 nm for IM, and a refractive index detector (RID-10A) for MDO and l-menthol. Samples were injected using an autoinjector (SIL-10AD). The column (Wakosil-ll-5C18 HG, Wako Pure Chemical, Osaka, Japan) was eluted at ambient temperature with mobile phases of methanol: 0.1% (w/v) phosphoric acid (30:70) for ANP and (75:25) for IM, MDO, and l-menthol, at a flow rate of 1.0 mL/min.

Data Analysis

The cumulative amounts of drugs permeated through skin were plotted as a function of time. The permeation rate was calculated from the slope of the linear portion of the plots, and lag time was the x-intercept of the line. For statistical analysis, the data were subjected to analysis of variance (ANOVA) followed by Dunnet's test. The formulation with 40% (v/v) ethanol and without enhancer was used as the control. Differences of $p<0.05$ were considered significant.

RESULTS

Effect of Enhancers on the Permeation of ANP

Antipyrine has high solubility in both water and ethanol, and was in the solution in all formulations. *p*-Menthane-3,8-diol is slightly soluble in water; however, 40% (v/v) of ethanol was needed to obtain MDO 3% (w/v) solution at 25°C. On the other hand, l-menthol did not dissolve in 40% (v/v) ethanol, and divided into two phases, the l-menthol rich phase and aqueous phase.

Figure 2(a) shows the permeation profiles of ANP from the various formulations. The permeation rate from the formulation with l-menthol decreased after a 24-h application, because about half of the ANP applied penetrated the skin or receptor phase so that the ANP concentration in the donor phase decreased. Taking into account that it was a finite dose condition and that flux changes with time, it was calculated in the range of 6 and 24 h and afterwards it decrease. *p*-Menthane-3,8-diol enhanced the permeation of ANP

Table 1. Flux, lag time, and skin concentration of ANP.

Formulation	Flux (μg/cm²/h)	Lag time (h)	Skin concentration (mg/cm³)
Control	25.9 ± 11.4	17.5 ± 3.3	1.6 ± 0.5
MDO	69.2 ± 17.9	17.3 ± 1.4	2.6 ± 0.2[a]
l-menthol	211 ± 47[a]	4.3 ± 0.9[a]	6.8 ± 0.2[a]

Note: Data show the mean ± SD of at least three experiments.
[a]Significantly different from control.

Table 2. Flux, lag time, and skin concentration of IM.

Formulation	Flux ($\mu g/cm^2/h$)	Lag time (h)	Skin concentration ($\mu g/cm^3$)
Control	0.20±0.01	16.4±1.7	123±87
MDO	3.16±0.71[a]	13.9±1.3	1380±138[a]
l-menthol	2.59±0.50[a]	11.0±2.1[a]	684±63[a]
Water suspension	0.09±0.03	24.5±2.0[a]	71±2

Note: Data show the mean±SD of at least three experiments.
[a]Significantly different from control.

about three times more than the control did, although the effect was smaller than that of l-menthol. Table 1 shows the flux, lag time, and skin concentration of ANP. The flux with l-menthol was calculated in the range of 6 and 12 h. Lag time was not changed by MDO; in contrast, l-menthol shortened lag time by about one-fourth. On the other hand, the skin concentration of ANP increased 1.6 and 4 times that of the control with MDO and l-menthol, respectively. The flux was not significantly increased by MDO, thus, MDO has no apparent effect on the skin permeation of ANP, although it has some effect on increasing ANP skin concentration.

Effect of Enhancers on the Permeation of IM

The solubility of IM is low, about 10 μg/mL in water and 0.75 mg/mL in 40% (v/v) ethanol (control). When 3% (w/v) MDO was added to 40% (v/v) ethanol, solubility of IM was improved to 4.0 mg/mL. When 3% (w/v) l-menthol was added, IM concentration in the aqueous phase was 1.3 mg/mL, and the remaining IM was in the l-menthol rich phase.

Figure 2(b) shows the permeation profiles of IM. Indomethacin scarcely permeated in the control formulation. The permeation from the MDO-containing formulation was almost the same as that from the l-menthol-containing formulation, with the cumulative permeated amount after a 30-h application at about 50 $\mu g/cm^2$. Flux was improved about 15 times compared to that of the control. The lag time tended to be shortened, but not significantly. The skin concentration of IM with MDO was 11 times higher than that of the control, which was higher than that with l-menthol (Table 2).

DISCUSSION

We have reported the permeation of ANP and IM when the amount of application was set at 2 mL × 2 times, at initial and after a 12-h application, maintaining not only the concentration of drug but also those of ethanol and enhancer, to clarify the mechanism of enhancement.[9] In this study, the purpose was evaluation of the usefulness of MDO as a skin permeation enhancer, so the amount of application was fixed at 0.5 mL to be near the actual application amount.

The flux is expressed as follows:

$$J = DKCv/h$$

where D=the apparent diffusion coefficient in the skin, K=the partition coefficient of drug between vehicle and skin, Cv=the drug concentration in the vehicle, and h=the diffusion length. The main effects of the chemical enhancers reported were: 1) increasing of the diffusion coefficient by disruption of the stratum corneum, 2) increasing of the partition coefficient by the increasing activity of the drug in the vehicle, and 3) increasing the drug solubility in the skin.[10]

Concerning ANP, l-menthol decreased lag time and increased skin concentration, namely, it affected both the diffusion rate and the partition coefficient, enhancement factor [EF=(flux with enhancer)/(flux of control)] was 8.1. However, since MDO increased the skin concentration of ANP, it should affect only the partition coefficient. The EF of MDO was 2.7, but there was no significant difference compared to the control.

The skin concentration of IM after application of water suspension was low, 71 $\mu g/cm^3$; low solubility in the skin was considered one of the factors of low permeation.[11] All formulations were saturated with IM so that the skin concentrations determined represented the solubility of IM in the skin. Change in solubility of the skin should be the effect of the vehicle. When ethanol was added (control), the skin concentration was slightly high, but the effect of the ethanol was negligible. *p*-Menthane-3,8-diol and l-menthol were partitioned to the skin in relatively high concentrations, 5.9±1.3 and 2.5±1.5 mg/cm^3, respectively, because of their lipophilic character. The enhancers improved IM solubility in the skin, and consequently, the permeation of

IM was improved. The decrease in lag time with l-menthol suggested that l-menthol also affects diffusion in the skin. However, MDO had no effect on the diffusion rate in the skin and some effect in increasing the solubility of the skin.

p-Menthane-3,8-diol had little effect in enhancement of the skin permeation of the hydrophilic drug, ANP. Concerning IM, which is lipophilic and has low solubility in the skin, MDO showed an enhancement effect similar to that of l-menthol. The effect was not achieved by increasing the diffusion rate in the skin, but by increasing the skin solubility of IM. *p*-Menthane-3,8-diol was easy to mix with the vehicle and has little volatile character, which prevents changes in the formulation. Thus, MDO may be an effective enhancer for drugs that have skin permeation restricted by low solubility in the skin.

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