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Note

Comparison of skin permeation enhancement by 3-*l*-menthoxypropane-1,2-diol and *l*-menthol: the permeation of indomethacin and antipyrine through Yucatan micropig skin and changes in infrared spectra and X-ray diffraction patterns of stratum corneum

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Abstract

3-*l*-Menthoxypropane-1,2-diol (MPD) is a derivative of *l*-menthol, which has an enhancement effect on drug permeation through skin. In this study, the effect of MPD on drug permeation through skin was compared with that of *l*-menthol. MPD or *l*-menthol at final concentrations of 3% in 40% ethanol was added to the drugs indomethacin or antipyrine and each mix then applied to Yucatan micropig skin in vitro. Drug concentrations in the skin were higher in the presence of either MPD or *l*-menthol, however, only *l*-menthol shortened the lag time of permeation. MPD enhanced the skin permeation of the drugs only by increasing the skin concentration of the drugs. In contrast, *l*-menthol enhanced the skin permeation of the drugs by increasing both the skin concentration and the diffusion rate in skin. The infrared (IR) spectra and X-ray diffraction patterns of stratum corneum after treatment with MPD did not differ from those of intact stratum corneum. A change in the IR spectra of stratum corneum after treatment with *l*-menthol was observed at the CH band, and the peaks representative of the lipid structure in the X-ray diffraction patterns decreased in intensity. These results suggest that *l*-menthol, but not MPD, disrupts the intercellular lipid structure of stratum corneum. Thus, MPD is expected to be a moderate skin permeation enhancer.

Keywords: 3-*l*-Menthoxypropane-1,2-diol (cooling agent 10); Skin permeation; Enhancer; *l*-Menthol; Indomethacin; Antipyrine; Yucatan micropig skin

Transdermal delivery systems are an attractive option for both topical and systemic therapeutics. However, the barrier function of the skin in protecting the body from physical and chemical attack makes delivering the required drug dose through the skin and to the target organ difficult. Thus, many studies have attempted to improve the permeation of drugs through the skin. The use of chemical enhancers is one of the most popular methods for improvement of transdermal absorption. Terpenes are contained in essential oils, insoluble in water, volatile and have flavor. Many kinds of terpenes, such as limonene,

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cineole, menthol, and carvon have been investigated as chemical enhancers, and it has become clear that they enhance drug permeation through the skin with relatively low irritation (Barry and Williams, 1993).

l-Menthoxypropane-1,2-diol (MPD), also known as cooling agent 10, is a derivative of *l*-menthol (Eccles, 1994). As the name suggests, it has a cooling effect but has no volatile or odor characteristics, and thus, is widely used in cosmetics, toothpaste and chewing gum. MPD is produced by combining glycerine with *l*-menthol by ether conjugation, resulting in MPD being more hydrophilic than *l*-menthol, with a lower calculated logarithm of octanol/water partition coefficient ($\log P$) of 2.5, compared to 3.2 for *l*-menthol. We previously investigated MPD as a skin permeation enhancer and found that MPD enhanced the skin permeation of indomethacin (IM) through hairless rat skin, although this effect was 2/3 as potent as that of *l*-menthol. However, MPD was very effective when added to both hydrophilic and lipophilic vehicles, which was not the case with *l*-menthol (Fujii et al., 1996). Thus, MPD became a new candidate molecule for skin permeation enhancement.

In the present study, we compared the effect of MPD on the skin with that of *l*-menthol in vitro. Yucatan micropig (YMP) skin was used as model skin due to its similarities with human skin (Kurihara-Bergstrom et al., 1986, Fujii et al., 1997). First, the permeation IM through full-thickness, epidermal membrane and stripped skin was investigated. IM is a lipophilic drug, with $\log P = 2.9$ and with extremely low solubility in water. In addition, we investigated the permeation of antipyrine (ANP), which is a hydrophilic drug, with $\log P = 0.3$ and high solubility in water, in spite of the lack of an ionic form at neutral pH. Also, the permeation of ethanol, which is a small hydrophilic molecule ($\log P = -0.2$) used as solvent, was determined.

The drug (IM 0.5% or ANP 2%) and enhancer (MPD 3% or *l*-menthol 3%) were dissolved in ethanol and added to purified water (final concentration of ethanol: 40%), and then the formulations were maintained at 37 °C overnight, until immediately before the permeation studies. Formulations containing drug and 40% ethanol without enhancer were used as controls. IM was a suspension and ANP was a solution when applied to the skin.

YMP skin sets frozen at −80 °C were purchased from Charles River Japan, Inc. (Kanagawa, Japan). A piece of skin was thawed at room temperature for approximately 30 min, followed by removal of the adhering fat layer using scissors and a grater (full-thickness skin). Stripped skin was prepared by removing stratum corneum with adhesive tape. Epidermal membrane and stratum corneum were obtained by the method reported by Kligman and Christophers (1963).

Skin permeation was measured in a modified Franz-type diffusion cell apparatus (effective area: 1.1 cm^2; receptor: 16 ml isotonic phosphate buffered solution (pH 7.1) maintained at 37 °C mixed with a star-head magnet at 600 rpm). The piece of skin was mounted on the cell directly except epidermal membrane. A membrane filter (0.8 μm, cellulose acetate, Advantec Toyo, Tokyo, Japan) was used to reinforce the epidermal membrane. A 2.0 ml aliquot of test formulation was poured into the donor phase. At predetermined times, 200 μl aliquots were withdrawn from the receptor compartment. The same volume of fresh solution was added to the receptor compartment after withdrawal to maintain constant volume. The donor phase was changed to a fresh test formulation 12 h after the start of the experiment. After the skin permeation studies, concentrations of drug and enhancer in the skin were determined. The concentration of drugs and enhancers were measured by HPLC (Shimadzu, Kyoto, Japan). Ethanol was determined using a GC system equipped with a FID detector (Shimadzu GC-14B).

The fluxes and lag times were calculated from the slope and intercept of the linear parts of the permeation profiles. Skin concentrations were calculated using an epidermal thickness of 40 μm and a dermal thickness of 2 mm, which were the averages of the values measured by microscope from skin cross sections. Statistical analysis was performed using one-way analysis of variance, and then Dunnet's test was performed using the no enhancer formulation as the control. Statistical significance was established at the $P < 0.05$ level.

The permeation profiles of IM in various formulations through various type of skin are shown in Fig. 1. The permeation of IM in the control formulation through full-thickness skin was low, at only 14 μg/cm^2 after 30 h. When MPD was added, permeation increased about three-fold, while *l*-menthol markedly enhanced the permeation of IM, to about 10 times that of the control. Permeation through epidermal

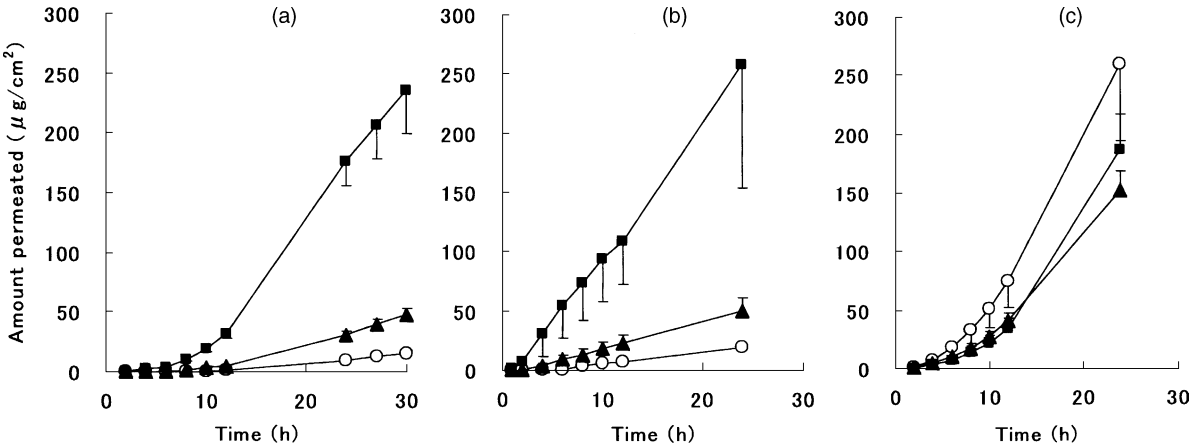

Fig. 1. Permeation profiles of IM through Yucatan micropig full-thickness skin (a), epidermal membrane (b), and stripped skin (c) in various formulations containing 40% ethanol: (○) control; (▲) with 3% MPD; (■) with 3% *l*-menthol. Data represent the mean ± S.D. of at least three experiments.

membrane was similar to that through full-thickness skin, except in lag time. Permeation through stripped skin is fast, regardless of the formulation.

Table 1 shows the permeation parameters. The fluxes through full-thickness skin of MPD- or *l*-menthol-containing formulations were significantly higher than that of the control. The lag time (index of diffusion coefficient) was shorter with *l*-menthol. Skin concentration was increased with both enhancers. The flux through epidermal membrane did not differ from that through full-thickness skin if the same formulation was applied. Lag times through epidermal membrane were shorter by about 8 h than those through full-thickness skin. The thickness of epidermal membrane was about 40 μm (20 μm of stratum corneum and 20 μm of viable epidermis), while that of full-thickness skin was about 2 mm, and thus, the diffusion coefficient in stripped skin was about 100 times greater than that of epidermal membrane. A comparison of lag times through epidermal membrane revealed that the lag time after application of *l*-menthol-containing formulations was significantly shorter than that of control. Skin concentrations of IM in epidermal membrane were higher than those in the cases of full-thickness skin, and were increased by both enhancers. In the case of stripped skin, neither of the enhancers had an effect on flux, lag time or skin concentration.

Table 1
Permeation parameters of IM and skin concentration of enhancer in various formulations through various types of skin

Skin type	Formulation	Flux (μg/cm²/h)	Lag time (h)	Skin concentration (mg/cm³)	Skin concentration of enhancer (mg/cm³)
Full-thickness	Control	0.85 ± 0.19	12.9 ± 0.4	1.2 ± 0.3	
	MPD	2.69 ± 0.56*	12.4 ± 1.8	2.5 ± 0.3*	15.3 ± 2.9
	l-Menthol	10.82 ± 0.84*	9.0 ± 0.4*	2.4 ± 0.5*	14.2 ± 6.7
Epidermal membrane	Control	0.98 ± 0.05	4.8 ± 0.6	6.4 ± 2.9	
	MPD	2.26 ± 0.25	3.7 ± 0.9	18.2 ± 3.1*	309.0 ± 186
	l-Menthol	11.24 ± 4.20*	1.6 ± 0.6*	22.4 ± 3.4*	315.0 ± 47
Stripped skin	Control	14.60 ± 3.42	6.4 ± 0.4	4.7 ± 1.1	
	MPD	8.66 ± 0.98*	6.7 ± 0.2	4.3 ± 1.2	21.0 ± 2.1
	l-Menthol	11.43 ± 1.67	7.9 ± 0.3*	4.1 ± 0.3	10.6 ± 1.6

* Statistically significant difference in MPD and *l*-menthol vs. control ($P < 0.05$).

Table 2
Permeation parameters of ANP in various formulations through full-thickness and stripped skin

Skin type	Formulation	Flux (μg/cm^2/h)	Lag time (h)	Skin concentration (mg/cm^3)
Full-thickness	Control	39 ± 8	7.2 ± 1.3	2.3 ± 0.3
	MPD	135 ± 27*	8.3 ± 0.6	5.0 ± 1.7*
	l-Menthol	448 ± 50*	6.3 ± 0.5*	6.8 ± 0.3*
Stripped skin	Control	322 ± 33	2.6 ± 0.4	10.1 ± 1.1
	MPD	331 ± 1	2.4 ± 0.2	9.3 ± 0.2
	l-Menthol	433 ± 15*	1.9 ± 0.1*	10.0 ± 0.7

* Statistically significant difference in MPD and *l*-menthol vs. control ($P < 0.05$).

The skin concentrations of the enhancers are shown in Table 1. Both enhancers penetrated in relatively high concentration to all types of skin. The skin concentrations of MPD and *l*-menthol were similar in both full-thickness skin and epidermal membrane; however, the MPD concentration in the stripped skin was higher than that of *l*-menthol.

Table 2 shows the permeation parameters of ANP through full-thickness skin and stripped skin. MPD enhanced the permeation of ANP to three times that of the control, while *l*-menthol enhanced permeation by about 11 times. MPD only increased the skin concentration of ANP, whereas *l*-menthol increased the skin concentration and shortened the lag time through full-thickness skin. Permeation through stripped skin did not differ among formulations. Fluxes through stripped skin were almost the same as those through full-thickness skin with *l*-menthol-containing formulations.

Fig. 2 shows the permeation of ethanol. Permeation through stripped skin was independent of formulation. The fluxes of ethanol from control through full-thickness skin were 1.3 ± 0.3 mg/cm^2/h, which agrees with the results of human skin permeation tests (Scheuplein and Blank, 1973; Berner et al., 1989; Magunusson et al., 1997). The fluxes increased to 2.1±0.4 and 6.4±1.4 mg/cm^2/h from MPD-containing and *l*-menthol-containing formulation, respectively.

In the case of stripped skin, MPD and *l*-menthol exist in the skin in relatively high concentration; however, the permeation rate and skin concentration of IM, ANP and ethanol did not differ among the formulations. These findings suggest that both MPD and *l*-menthol have no enhancing effect on viable

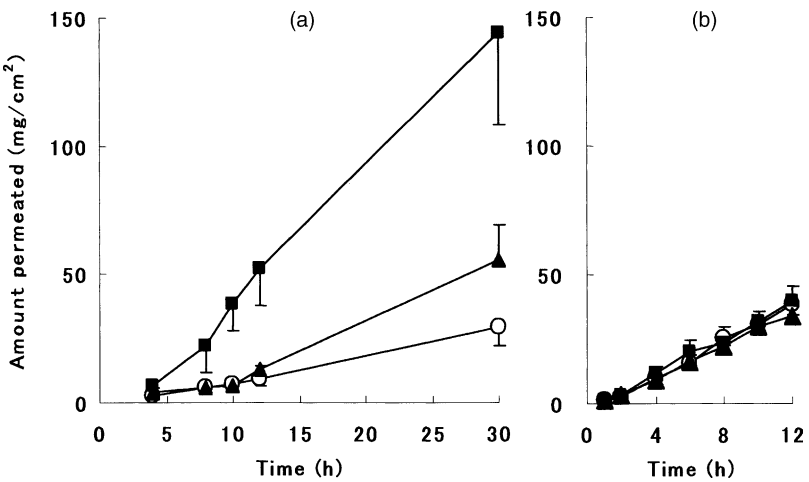

Fig. 2. Permeation profiles of ethanol through Yucatan micropig full-thickness skin (a), and stripped skin (b) in control formulation (○), MPD-containing formulation (▲), and *l*-menthol-containing formulation (■). Data represent the mean ± S.D. of at least three experiments.

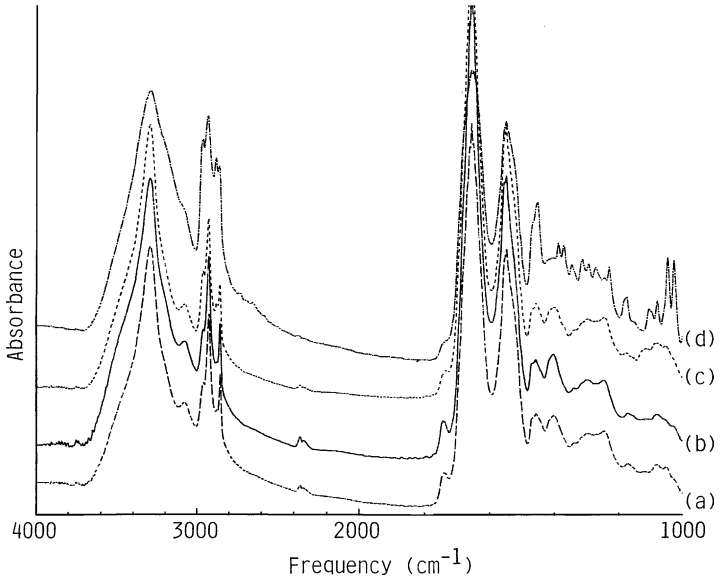

Fig. 3. FT-IR spectra of intact stratum corneum (a) and stratum corneum after treatment with control formulation (b), MPD-containing formulation (c) and *l*-menthol-containing formulation (d).

epidermis and dermis. The enhancers affected on epidermis including stratum corneum.

The effects of MPD and *l*-menthol on stratum corneum were studied by FT-IR spectra and X-ray diffraction patterns. The concentrations of enhancers were fixed at 3%, because the skin concentrations of enhancers were almost the same. Dried stratum corneum was used because defining the water content of stratum corneum is difficult and Bouwstra et al. (1991) reported that water does not affect the lipid structure.

Fig. 3 shows the IR spectra of stratum corneum. A stratum corneum sheet approximately 1 cm × 1 cm was placed into the FT-IR equipment (FT-IR-8000, JASCO, Tokyo, Japan) and absorbance measured from 4600 to 400 cm^{-1}. The sheet was then cut into four pieces and each soaked in a different test formulation for 24 h. They were dried over night and the IR spectrum measured again. Treatment with control or MPD-containing formulations produced no differences from intact skin. However, treatment with *l*-menthol-containing formulations resulted in differences at 1000, 1450 and 2900 cm^{-1}. The peak at 1000 cm^{-1} is a *l*-menthol-originating peak. The peaks originating from the lipids of the stratum corneum at 2919 cm^{-1} (νCH_2 antisymmetric) and 2851 cm^{-1} (νCH_2 symmetric) shifted 2–3 cm^{-1} higher, and new peaks appeared at 2955 (νCH_3 antisymmetric), 2872 (νCH_3 symmetric) and 1451 cm^{-1} (δCH_3 antisymmetric). However, *l*-menthol crystal shows peaks at almost the same wave number as that of CH_3, and thus, these peaks cannot be readily explained. After removal of *l*-menthol by soaking the sheet in 40% ethanol for about 24 h, IR spectrum was not different from that of intact skin.

X-ray diffraction patterns are shown in Fig. 4. Stratum corneum sheets, washed in acetone–ether mixture at 5 °C for 5 s, were soaked in test solution for 3 h and dried for 24 h. The sheets were then cut into pieces 1 cm × 0.3 cm and piled (ca. 200 pieces) into the sample folder. An X-ray diffractometer (Ru-300, Rigaku, Tokyo, Japan) was used with Cu Kα, scanning from $2\theta = 0.01$ to 4°. The spaces were calculated using Bragg equation. Intact skin showed a peak at 12 nm, which may represent double lipid bilayers. Peaks were also observed at 6, 4, and 3 nm, which are the second, third and forth peak, respectively, from the peak at 12 nm. After treatment with control formulation, no obvious change from intact skin was observed. After treatment with MPD-containing formulations, peaks were observed at the same 2θ, but seemed a little smaller than that in the case of intact skin. The peaks

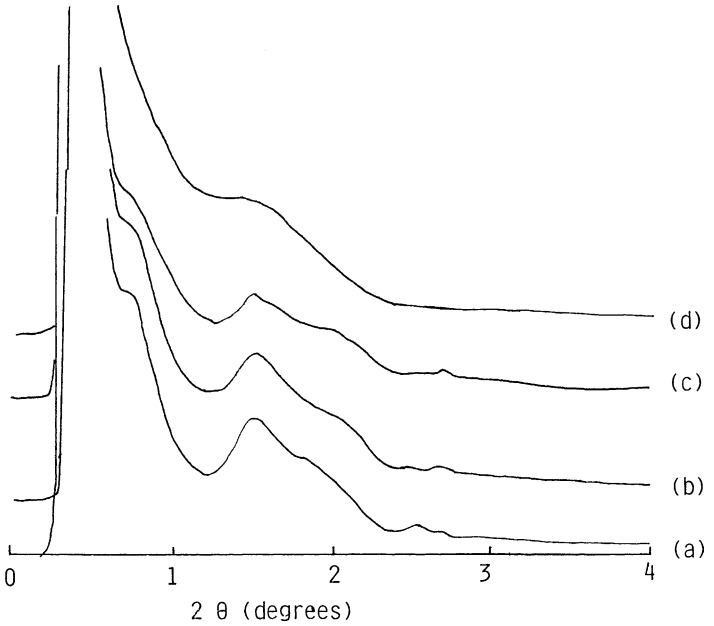

Fig. 4. X-ray diffraction patterns of intact stratum corneum (a) and stratum corneum after treatment with control formulation (b), MPD-containing formulation (c) and *l*-menthol-containing formulation (d).

at 12, 4 and 3 nm disappeared upon treatment with *l*-menthol-containing formulations. After soaking this stratum corneum with 40% ethanol, the peaks observed were the same as those resulting from treatment with control formulation, as with the IR spectrum.

The samples treated with control and MPD-containing formulation showed no obvious changes with intact skin, except slightly decreasing of intensity of the peaks in the X-ray diffraction patterns after treatment of MPD-containing formulation. Ghanem et al. (1987) examined the lipid extraction effect of ethanol, and found that extraction become obvious at ethanol concentrations of 50% or higher. In the present study, the ethanol concentration was 40%, and thus, the extraction effect was not marked. MPD partitioned relatively high concentration in stratum corneum; however, it did not disrupt lipid structure. After treatment with *l*-menthol-containing formulation, the fluidization of lipid was suggested (Suhonen et al., 1999). The X-ray diffraction peaks disappeared and re-appeared after washing with 40% ethanol (data not shown), thus, the effect of *l*-menthol is not extraction of lipid, but disruption of the lipid structure, and thus, may be reversible.

l-Menthol significantly increased (by five times) the permeation of ethanol. Also, the permeations of both IM and ANP were enhanced about 10 times those of control. Lag times were shorter and the skin concentrations of IM and ANP were two times those of controls. Thus, *l*-menthol increased the flux of drugs not only by improving the partition of drugs but also by increasing diffusion coefficient, as reported by Kobayashi et al. (1994), Kunta et al. (1997) and Gao and Singh (1998). The lipophilisity of IM and ANP are different, but enhancing effect seemed to be the same. The fluxes of IM, ANP and ethanol through full-thickness skin were as the same as those through stripped skin, *l*-menthol changes the permeability of stratum corneum high as that of viable skin. One of the reasons of these phenomena is disruption of lipid structure in stratum corneum, but it is not clear if this is the only reason for enhancement or not.

In contrast, MPD slightly enhanced (1.6 times) the permeation of ethanol. Also, the permeations of both IM and ANP were enhanced about three times those of control. Lag times were not changed and the skin concentrations of IM and ANP were two times those of controls. MPD partitioned in skin increased the

partition of drugs especially in epidermis, and consequently the permeations of drugs were improved. MPD did not change intracellular lipid structure, and thus, can be used as a moderate skin permeation enhancer.

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