Effect of *l*-Menthol on the Permeation of Indomethacin, Mannitol and Cortisone through Excised Hairless Mouse Skin

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The effect of *l*-menthol on the skin permeability of mannitol, cortisone or indomethacin was examined by an *in vitro* penetration technique with hairless mouse skin. The donor solution was prepared with phosphate buffered saline, ethanol : buffered saline (20 : 80, v/v) or ethanol : buffered saline (20 : 80, v/v) containing 1% (w/v) *l*-menthol. Although ethanol showed little enhancing effect, *l*-menthol in an aqueous ethanol vehicle at pH 7.4 increased the permeability coefficients of mannitol and indomethacin by about 100 times that of the control (an aqueous vehicle) and increased that of cortisone by about 10 times. *l*-Menthol, however, scarcely enhanced the penetration of indomethacin at pH 3.0, the majority of the species being in unionized form. These results suggested that the menthol–ethanol–aqueous system enhanced skin permeability through a direct effect on the polar and/or lipid pathways, while the thermodynamic activity of the penetrant molecule in the delivery vehicle might also influence the effectiveness of the penetration enhancer.

Keywords indomethacin; cortisone; mannitol; hairless mouse; *l*-menthol; penetration enhancer; skin permeability

Introduction

The systemic transdermal delivery of drugs has become of great interest in recent years. However, many drugs may be prevented from penetrating the skin because of the low permeability of the stratum corneum. The improvement of permeability by using penetration enhancers, therefore, may be desirable. Recently, cyclic monoterpenes have been shown to be effective penetration enhancers for the percutaneous absorption of drugs.[1-6] Hydrocarbon terpenes are effective for both lipophilic and hydrophilic compounds. Oxygen-containing terpenes are also effective for hydrophilic compounds, whereas for lipophilic compounds they are less effective. Whether or not these discrepancies can be attributed to different enhancing mechanisms among terpenes remains unclear. The activities of penetration enhancers may also depend on the choice of donor vehicles.

In the present study, we investigated the effects of *l*-menthol on the permeability of model penetrants having different polarities. By using an aqueous ethanol as a donor vehicle, the penetrant–vehicle relationship was also examined.

Experimental

Materials D-[1-^{3}H]Mannitol (specific activity 274 MBq mg^{-1}) was purchased from NEN Research Products (Boston, U.S.A.) and indomethacin was obtained from Sigma Chemical Co. (St. Louis, U.S.A.). *l*-Menthol and cortisone were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were of reagent grade and used without further purification.

Skin Permeation Study The hairless mouse (7 to 11 weeks old) was sacrificed by dislocating the spinal cord. Excised abdominal skin with adhering fat removed was mounted in two 30 °C water-jacketed diffusion cells (the surface area = 0.864 cm^2). Both cells, to ensure well hydration, were filled with 2.5 ml of isotonic phosphate buffered saline (pH 7.4) and were magnetically stirred at about 500 rpm before the permeability experiments. Fourteen hours later, the mediums were carefully sucked off and the test solutions were introduced to both the cells. The donor solution containing indomethacin (0.1%), mannitol (0.05%) or cortisone (0.015%) was prepared with isotonic phosphate buffered saline with a pH of 7.4 (vehicle I) and phosphate buffered saline : ethanol (80 : 20, v/v) (vehicle II). The phosphate buffered saline : ethanol (80 : 20, v/v) containing *l*-menthol (1%) was applied as an emulsion to maintain constant thermodynamic activity throughout the experiments (vehicle III). For indomethacin, phosphate buffered solutions at pH 3.0 and 5.0 were also applied. The acceptor solution consisted of isotonic phosphate buffered saline (pH 7.4). The temperature of both the donor and acceptor solutions was maintained at 30 °C throughout the experiments. At appropriate intervals for 10 h, an aliquot (0.5 ml) of the acceptor medium was drawn and this volume was replaced with fresh medium.

Analytical Method Indomethacin and cortisone were determined by a Shimadzu model LC-6A HPLC system equipped with an SPD-6A spectrophotometric detector (Shimadzu, Kyoto, Japan). The column (15 cm × 4.0 mm i.d.) was packed with LiChrosorb RP-18 (5 μm particle size; Merck, Darmastadt, West Germany). The mobile phase consisted of acetonitrile and 0.05% phosphoric acid (45 : 55 v/v for indomethacin and 35 : 65 v/v for cortisone). The flow rates for indomethacin and cortisone were 1.5 and 0.5 ml/min, respectively. Tolbutamide was used as the internal standard for indomethacin and peak area ratios were calculated on the wavelength at 230 nm. For cortisone, dexamethasone was used as the internal standard and the wavelength for detection was set to 245 nm. For mannitol, 4 ml of scintillation cocktail (Atomlight, NEN Research Products) was added to the acceptor solution (0.2 ml), and the radio-labeled drug was determined by liquid scintillation counting (Beckman LS 3801 scintillation counter).

Estimation of Permeability Coefficient According to Fick's law of diffusion through the skin with two boundary conditions (*i.e.*, a constant drug concentration in the donor compartment and the acceptor phase as a perfect sink), $\bar{q}$ (the Laplace transform of the amount of penetrant per unit area which penetrates through the skin) is expressed as follows,

$$\bar{q}=\frac{K_p(6LT)^{1/2}C_0}{s^{3/2}\sinh(6LTs)^{1/2}} \quad (1)$$

where K_p and LT are the permeability coefficient and lag time, respectively. C_0 is a constant concentration in the donor phase and s is the Laplace operator. The individual penetration profile was adapted to Eq. 1 using MULTI (FILT)[7] and the penetration parameters (K_p and LT) were obtained.

Results and Discussion

The penetration profiles of mannitol, cortisone and indomethacin from the donor vehicles at pH 7.4 to the acceptor solutions through the hairless mouse skin are shown in Figs. 1, 2 and 3, respectively. Although ethanol showed little enhancing effect, the co-application of *l*-menthol and ethanol in an aqueous vehicle significantly enhanced the penetration of cortisone and indomethacin as well as mannitol.

The estimated penetration parameters are summarized in Table I. *l*-Menthol in an aqueous ethanol vehicle at pH 7.4

(vehicle III) increased the permeability coefficients of mannitol and indomethacin by about 100 times that of the control (vehicle I) and by about 10 times for cortisone. On the other hand, the time lag values with *l*-menthol treatment varied over a wide range. From these values, however, it would be difficult to predict the enhancing mechanisms, because they could be affected not only by changes in the diffusivities of penetrants but also by the time-delayed enhancing effect. Thus, we focused the discussion on the skin penetration at a steady state.

l-Menthol had been effective in the penetration of a water-soluble material from *N*-methylpyrrolidone as the donor vehicle but not to a lipophilic material.[4] Similar results had been shown with other oxygen-containing terpenes.[2,6] Our studies with an aqueous ethanol vehicle partially support this view; *l*-menthol more effectively promoted the penetration of a hydrophilic penetrant, mannitol, than a relatively non-hydrophilic penetrant, cortisone (log octanol/water partition coefficient; $\log P = 1.1$). However, no simple explanation for the selectivity of the enhancing effect has been offered. This study also demonstrated that *l*-menthol enhanced the penetration of indomethacin from the donor vehicle at pH 7.4 or 5.0. However, *l*-menthol scarcely enhanced the penetration of indomethacin at pH 3, the majority of indomethacin existing in unionized form, as shown in Table I.

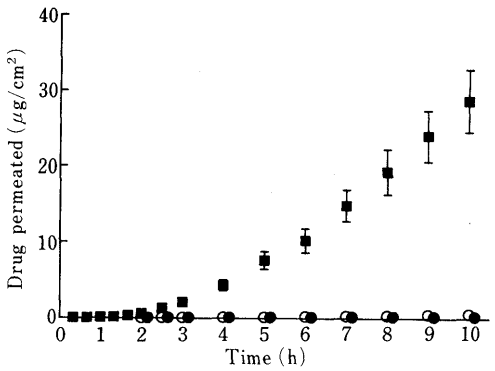

Fig. 1. Effect of *l*-Menthol on the Transport of D-Mannitol through the Skin of Hairless Mice

●, vehicle I; ○, vehicle II; ■, vehicle III. Each point represents the mean ± S.E.M. The number of each experiment was shown in Table I.

Ethanol and water are completely intersoluble and give a single homogeneous solution. However, the addition of *l*-menthol to the vehicle forms a two-phase ethanol–water–menthol system, *i.e.*, an ethanol-rich phase and a water-rich phase. Penetrants are distributed into the two phases with various ratios, dependent on their physico-chemical properties. Almost 100% of mannitol existed in the water-rich phase, while the content of cortisone in the phase is only about 51%. The partition of weak electrolytes also varies with pH values. Indomethacin was preferentially distributed in the water-rich phase at pH 7.4 (98% in the water-rich phase), whereas in the ethanol-rich phase it was distributed at pH 5 and 3 (13.1 and 6.8% in the water-rich phase, respectively).

It has been pointed out that a drug diffuses in the skin through particular penetration pathways (*i.e.*, the polar and lipid pathways).[8-10] Some information on the particular pathway(s) accelerated by certain enhancer(s) has been also presented.[11-13] In this study of a multi-phase system, the species in each phase penetrate into the skin at different

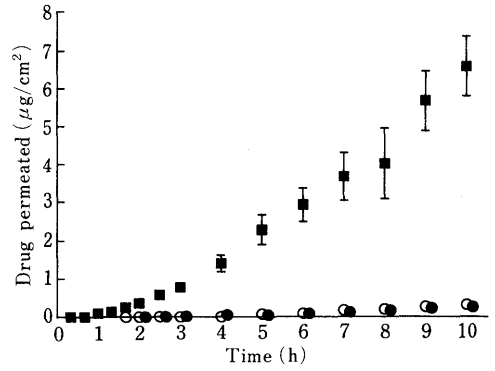

Fig. 2. Effect of *l*-Menthol on the Transport of Cortisone through the Skin of Hairless Mice

●, vehicle I; ○, vehicle II; ■, vehicle III. Each point represents the mean ± S.E.M. The number of each experiment was shown in Table I.

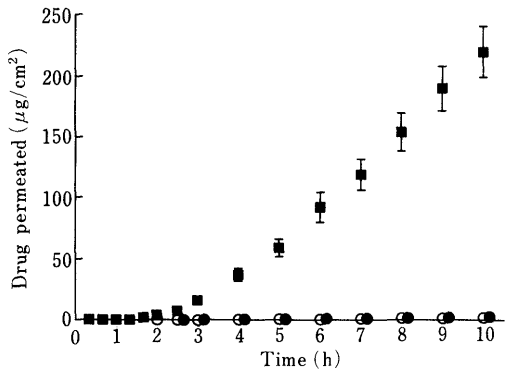

Fig. 3. Effect of *l*-Menthol on the Transport of Indomethacin at pH 7.4 through the Skin of Hairless Mice

●, vehicle I; ○, vehicle II; ■, vehicle III. Each point represents the mean ± S.E.M. The number of each experiment was shown in Table I.

TABLE I. Permeability and Lag Time of Mannitol, Cortisone and Indomethacin through Hairless Mouse Skin[a]

	Permeability coefficient (cm/h) × 10^5			Lag time (h)		
	Vehicle I	Vehicle II	Vehicle III	Vehicle I	Vehicle II	Vehicle III
Mannitol (pH 7.4)	7.8 ± 1.1 (8)	15.7 ± 1.8 (5)	998 ± 141 (8)	2.17 ± 0.28 (8)	2.41 ± 0.24 (5)	4.38 ± 0.23 (8)
Cortisone (pH 7.4)	51.1 ± 10.8 (7)	58.2 ± 19.3 (3)	576 ± 81 (3)	6.39 ± 1.47 (7)	6.40 ± 2.30 (3)	2.76 ± 0.20 (3)
Indomethacin (pH 7.4)	45.2 ± 6.1 (3)	25.7 ± 2.1 (4)	3690 ± 423 (7)	4.79 ± 0.89 (3)	6.54 ± 0.86 (4)	3.95 ± 0.35 (7)
Indomethacin (pH 5.0)	52.3 ± 9.7 (3)[b]	126 ± 44 (3)[b]	3650 ± 387 (3)	2.40 ± 1.03 (3)	3.85 ± 0.72 (3)	0.47 ± 0.24 (3)
Indomethacin (pH 3.0)	32.5 ± 3.9 (3)[b]	46.9 ± 15.9 (3)[b]	59 ± 15 (3)	2.85 ± 0.50 (3)	2.81 ± 0.36 (3)	1.08 ± 0.37 (3)

a) Values represent the means ± S.E.M. with the number of experiments given in parentheses. *b*) Indomethacin was applied as a suspension in the donor vehicle. These values might be underestimated because of using the value of 0.1% as C_o.

rates and the composition of the delivery vehicle may also influence the release of penetrants. However, the penetration of a hydrophilic material, mannitol, must pass preferentially through the polar pathway regardless of the presence of *l*-menthol. Thus, the permeability of any penetrant, including hydrophobic materials, through the polar pathway from the water-rich phase may be predicted by the permeability of mannitol. The penetrant concentration in the water-rich phase (C_w) was calculated from two volume fraction of the phase (*i.e.*, 98.8%) and the content in the phase described above. Therefore, when the steady state flux ($K_p \times C_0$) was divided by C_w, the new effective permeability coefficient (K_p') could be obtained. The K_p' values for mannitol and cortisone with *l*-menthol treatment were 9.98×10^{-3} and 11.4×10^{-3} (cm/h), respectively. Thus, these close K_p' values suggested that the penetration of cortisone with *l*-menthol treatment could also be accounted for by the enhancement through the polar pathway from the water-rich phase, though its penetration without *l*-menthol might be primarily through the lipid pathways. Furthermore, cortisone in the ethanol-rich phase appeared to contribute very little to the overall penetration. It must be due to the low thermodynamic activity of the penetrant, *i.e.*, the low escaping tendency of cortisone from the ethanol-rich phase to the skin. The penetration of hydrophobic materials with an aqueous vehicle is greater than with an aqueous ethanol vehicle, which has been well documented.[14)]

The vehicle effect on the penetration of indomethacin at pH 3.0 might be more remarkable due to the high lipophilicity of the unionized form ($\log P = 4.1$) and the enhancing effect of *l*-menthol was not apparently obtained. Similar observations were reported by Okabe *et al.*,[1)] who found that *l*-menthol had little effect on the percutaneous absorption of indomethacin when applied with gel ointments as donor vehicles. However, the K_p' for indomethacin at pH 3.0, 5.0 and 7.4 in the presence of *l*-menthol were 86.3, 278 and 37.7 ($\times 10^{-3}$ cm/h), respectively. All these values were much larger than those of mannitol and cortisone, which indicated that the enhancing effect of *l*-menthol on the penetration of indomethacin could not be explained only in terms of the acceleration of the polar pathway, as described above. The activation of the lipid pathway by *l*-menthol must also contribute to the skin penetration of indomethacin. However, it can be seen that the K_p' values of indomethacin are not correlated to the fractions of unionized forms. As possible explanations, the discrepancy might come from the pH dependence of the accelerating effects of *l*-menthol. Variations in pH values between the donor vehicle and hairless mouse skin may also be involved in these circumstances. Further studies on pH dependence of the enhancing effect should be done. This is now under investigation.

From our results, it is concluded that the enhancing effect of *l*-menthol is primarily through the polar pathway. It may be a potential enhancer which also affects the lipid pathway, but the delivery vehicle influences the effectiveness of the penetration enhancer and the enhancing effect can occasionally be masked by the vehicle effect.

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