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The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems

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Abstract

The percutaneous permeation of hydrocortisone (HC) was investigated in hairless mouse skin after application of an alcoholic hydrogel using a diffusion cell technique. The formulations contained one of 12 terpenes, the selection of which was based on an increase in their lipophilicity (log P 1.06 − 5.36). Flux, cumulative receptor concentrations, skin content, and lag time of HC were measured over 24 h and compared with control gels (containing no terpene). Furthermore, HC skin content and the solubility of HC in the alcoholic hydrogel solvent mixture in the presence of terpene were determined, and correlated to the enhancing activity of terpenes. The in vitro permeation experiments with hairless mouse skin revealed that the terpene enhancers varied in their ability to enhance the flux of HC. Nerolidol which possessed the highest lipophilicity ($\log P = 5.36 \pm 0.38$) provided the greatest enhancement for HC flux (35.3-fold over control). Fenchone ($\log P = 2.13 \pm 0.30$) exhibited the lowest enhancement of HC flux (10.1-fold over control). In addition, a linear relationship was established between the log P of terpenes and the cumulative amount of HC in the receptor after 24 h (Q_{24}). Nerolidol, provided the highest Q_{24} (1733 ± 93 μg/cm^2), whereas verbenone produced the lowest Q_{24} (653 ± 105 μg/cm^2). Thymol provided the lowest HC skin content (1151 ± 293 μg/g), while cineole produced the highest HC skin content (18999 ± 5666 μg/g). No correlation was established between the log P of enhancers and HC skin content. A correlation however, existed between the log P of terpenes and the lag time. As log P increased, a linear decrease in lag time was observed. Cymene yielded the shortest HC lag time, while fenchone produced the longest lag time. Also, the increase in the log P of terpenes resulted in a proportional increase in HC solubility in the formulation solvent mixture.

Keywords: Hydrocortisone; Terpenes; Hydroxypropylmethyl cellulose; Log P; Hydrocortisone skin content; Flux; Lag time

1. Introduction

The transdermal route offers several advantages over other routes for the delivery of drugs with systemic activity. For example, it provides a con-

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tinuous mode of administration at rates approaching zero-order similar to that provided by an i.v. infusion. Moreover, the delivery is non-invasive, and no hospitalization is required. Additionally, the transdermal route as compared with the oral route reduces drug degradation at the site of administration due to its lower metabolic activity (Roy, 1999). Once the drug is absorbed, the hepatic circulation is bypassed, thus avoiding another major site of potential drug metabolism (Barry, 1983; Wester and Maibach, 1992).

Despite the important advantages of transdermal drug delivery, this route of administration presents unique challenges. The greatest obstacle is the stratum corneum (SC), the uppermost layer of the skin, that provides the rate limiting step for drug transport. It is composed of dead, flattened cells, filled with keratin, in the form of a regular array of protein rich cells embedded in an intercellular multilamellar lipid domain running parallel to the skin (Elias, 1996). Several experiments showed that lipoidal domains, the integral components of the transport barrier, must be breached if the drug is to be delivered at an appropriate rate (Mao-Qiang et al., 1993; Gao and Singh, 1998). Several enhancement techniques have been developed to overcome the impervious nature of the SC through physical approaches (iontophoresis Valpato et al., 1998, electroporation Wang et al., 1998, phonophoresis Asano et al., 1997, heat enhancement Kuratomi and Miyauchi, 1988 or the application of supersaturated drug systems Davis and Hadgraft, 1991), and biochemical means (prodrugs Bando et al., 1997, liposomal vesicles Cevc, 1996). However, the most widely implemented technique is the use of chemical enhancers. The aim of these agents is to allow drug permeation through the skin at the appropriate rate and for a suitable time. In this way, many compounds were developed and later examined for their percutaneous enhancing activity, such as Azone, its analogues, pyrrolidones, polyunsaturated fatty acids, alkanols, polymeric enhancers, non-ionic surfactants, and terpenes (Iwasa et al., 1991; Kim et al., 1992; Cornwell and Barry, 1994; Michniak et al., 1994; Yamane et al., 1995; Akimoto et al., 1997; Tanojo et al., 1997; Sato et al., 1998). However, the skin as a primary barrier, has a special role: protecting the living body from cutaneous exposure to toxic chemicals; hence, the safety of permeation enhancers is of a prime consideration. Consequently, the search continues for an ideal enhancer that is pharmacologically inactive, non-irritant, non-damaging for the skin, potent, and cosmetically acceptable (Pfister et al., 1990). Terpenes, naturally occurring volatile oils, appear to be promising candidates for clinically acceptable enhancers (Williams and Barry, 1991). They were reported to have good toxicological profiles, high percutaneous enhancement abilities, and low cutaneous irritancy at low concentrations (1–5%), (Opdyke, 1979; Okabe et al., 1990; Obata et al., 1991). Moreover, a variety of terpenes have been shown to increase the percutaneous absorption of both hydrophilic and lipophilic drugs (Cornwell and Barry, 1991a; Moghimi et al., 1996, 1997; Gao and Singh, 1998). Differential scanning calorimetry and X-ray diffraction studies suggest that terpenes increase the drug percutaneous permeation mainly by disrupting the intercellular packing of the SC lipids (Williams and Barry, 1991; Cornwell and Barry, 1994; Cornwell, 1993). Furthermore, this interaction with the SC lipids has been reported to be reversible and produce lower skin irritation if compared with Azone® (Okabe et al., 1990). Williams and Barry (1991) studied the reversibility of the action of terpenes on the skin. They reported that 5-fluorouracil flux in terpene-treated skins decreased with time after washout of the terpenes from the skin surface.

In this study, ethanol and hydroxypropylmethyl cellulose (HPMC, a gelling agent) were incorporated into gel formulations. Ethanol, one of the most widely used vehicles has been found to affect terpene enhancing activity. Morimoto et al. (1993) examined the effect of ethanol and L-menthol on the skin permeation of morphine hydrochloride. This combination provided higher skin permeation for morphine hydrochloride compared with either alone. HPMC belongs to a family of inert hydrophobic non-ionic polymers that is widely used in oral and topical pharmaceutical formulations and is available in several grades, which vary in viscosity, molecular weight, and extent of substitution. In topical formulations, HPMC poly-

mers are used as emulsifiers, suspending agents, and stabilizing agents for topical gels and ointments (Wu et al., 1998). As protective colloids, they can prevent droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments. They were reported to be non-irritant and non-toxic (Wade and Weller, 1995). The purpose of this study was to evaluate the enhancing effect of 12 naturally occurring terpenes on the in vitro percutaneous absorption of hydrocortisone (HC) from HPMC gels containing ethanol, distilled water, and glycerol. An attempt was made to correlate the log P of the studied terpenes with the resultant percutaneous parameters of HC. In addition, the effect of terpenes on the content of HC into the skin and the solubility of HC in the alcoholic hydrogel solvent mixture were measured and related to the enhancing capacity of terpenes.

2. Materials and methods

2.1. Chemicals

Terpinen-4-ol, verbenone, fenchone, carvone, menthone, α-terpineol, cineole, geraniol, thymol, cymene, D-limonene, and nerolidol were all obtained from Aldrich Chemical (Milwaukee, WI). Hydrocortisone was purchased from Sigma Chemical (St Louis, MO). Acetonitrile, methanol, and water used were of HPLC grade and were supplied by EM Science (New Briggs, NJ). Ethyl alcohol USP was purchased from Aaper Alcohol and Chemical (Shelbyville, KY). HPMC and glycerol were obtained from Spectrum Chemical Manufacturing (Gardena, CA). Female hairless mice strain SKH1 (hr/hr), 6–8 weeks old were obtained from Charles River Laboratory, (Wilmington, MA).

2.2. Preparation of gel

The procedure for the preparation of HPMC gels was followed as described by Giannakou et al. (1998). The gel formulation was composed of hydrocortisone (2%), HPMC (2%), ethanol (56%), water (28%), glycerol (10%), and terpene (2%). For the preparation of the gels, ethanol was mixed with distilled water and glycerol using a StedFast™ mixer (Fisher Scientific). HPMC was added slowly until a clear gel was formed. Then, terpene enhancer followed by hydrocortisone powder was added to the vortex of agitated gel. The hydrocortisone was not completely soluble in the gel formulation, it was in the form of suspension.

2.3. Solubility measurements

The solubility measurements was conducted using previously described method by Viegas et al. (1997) and Ohara et al. (1995). Briefly, HC was added in excess to 10 ml of ethanol/water/glycerin: 60/30/10 including the appropriate terpene enhancer. The suspension was shaken at 25°C for 24 h, then centrifuged at 3000 rpm for 15 min. The supernatant was diluted with methanol and used for the determination of HC using HPLC. All experiments were performed in triplicate ($n = 3$).

2.4. Diffusion cell apparatus

Modified Franz diffusion cells (Permegear, Riegelsville, PA) were used for the evaluation of the gel formulation (diffusional area of 0.64 cm^2, the receptor volume 5.1 ml). The receptor was filled with isotonic phosphate buffer (pH 7.2) containing no preservative, maintained at 37 ± 0.5°C, and continuously stirred at 600 rpm.

2.5. Skin permeation studies

Female hairless SKH1 mice, 6–8 weeks old, (Charles River Laboratory) were euthanized, using carbon dioxide asphyxiation, and full thickness abdominal skin was excised. Any extraneous subcutaneous fat was removed from the dermal surface. The skins were sealed in double evacuated polyethylene bags at -20°C and used within 2 weeks (Harrison et al., 1984). Frozen skins were slowly thawed, and mounted between the donor and receptor compartments in vertical Franz diffusion cells. Skins were allowed to equilibrate for 1 h before experimentation. After equilibration,

300 mg of HPMC gel was placed on each skin and all donor cells were occluded with triple layers of Parafilm®. Receptor samples (300 μl) were taken at predetermined times over 24 h and frozen at −70°C prior to HPLC analysis. Following each receptor sample withdrawal, the volume was replenished immediately with diffusion buffer. The amount of drug withdrawn was corrected in the subsequent calculations of cumulative amount of the drug penetrated.

2.6. Skin content studies

After 24 h of sampling, the skins were removed from the cells and washed briefly in methanol, using a previously reported procedure (Michniak et al., 1994). The skins were then homogenized in 4 ml methanol, using a Kinematica GmbH tissue homogenizer (Switzerland). The homogenate was filtered to remove skin debris, centrifuged, if required, then passed through a Sep Pak C_{18} cartridge (Waters, MA), and the samples were frozen at −70°C (Revco Scientific, Asheville, NC) prior to HPLC analysis.

This extraction method produced more than 97% recovery of the originally applied donor concentration of HC (Godwin et al., 1997). Quantities of HC were calculated as μg/g of partially hydrated skin. All samples were analyzed using HPLC.

Table 1
Log *P* of terpenes used as enhancers

Terpene	Log *P*
Terpinen-4-ol	1.06 ± 0.20
Verbenone	1.97 ± 0.23
Fenchone	2.13 ± 0.30
Carvone	2.23 ± 0.25
Menthone	2.63 ± 0.30
α-Terpineol	2.73 ± 0.28
Cineole	2.82 ± 0.25
Geraniol	3.18 ± 0.30
Thymol	3.28 ± 0.20
Cymene	4.05 ± 0.25
D-Limonene	4.58 ± 0.23
Nerolidol	5.36 ± 0.38

2.7. HPLC analysis of hydrocortisone

Analysis of samples was performed using a Hewlett–Packard 1100 with an autosampler equipped with a quaternary pump, a variable-wavelength detector (242 nm), and a C_{18}-Microsorb column (15 × 0.46 cm; 5 μm) at ambient temperature. The injection volume was 40 μl. The mobile phase was acetonitirile:water (4:6) with a flow rate of 1 ml/min. The retention time of HC was approximately 3.4 min. HC was quantitated using an external standard technique.

2.8. Data analysis:

The permeation of HC from HPMC gels was measured over 24 h, and plots were constructed of the cumulative corrected amounts of hydrocortisone (μg/cm^2) against time (h). The *x*-intercept of the extrapolated linear region gave the lag time. The slope of this linear portion of the graph provided maximum flux values at steady state (μg/cm^2 per h). All parameters in Table 1 are expressed as means ± S.D. Log *P* values of terpenes enhancers were determined using ACD software program (Advanced Chemistry Incorporated, Ontario, Canada). The permeation-enhancing activities were expressed as enhancement ratios of flux (ER_{flux}), i.e. the ratio of the flux value with enhancer to that obtained with control:

$$ER_{flux} = \frac{\text{HC flux with enhancer in gel}}{\text{HC flux with no enhancer}}$$

The apparent solubility enhancement ratio of HC in gel solvent mixture was calculated using the following equation: Solubility enhancement ratio = $C_o/C_s$where C_o is the HC concentration in enhancer gel solvent mixture and C_s is the saturation solubility of HC in control gel solvent mixture (no terpene).

Statistical analysis was made using analysis of variance (ANOVA). The level of significance was taken as $P < 0.05$. Correlation analyses were performed by the least squares linear regression method. Correlation coefficients were examined for significance ($P < 0.05$) by Student's *t*-test.

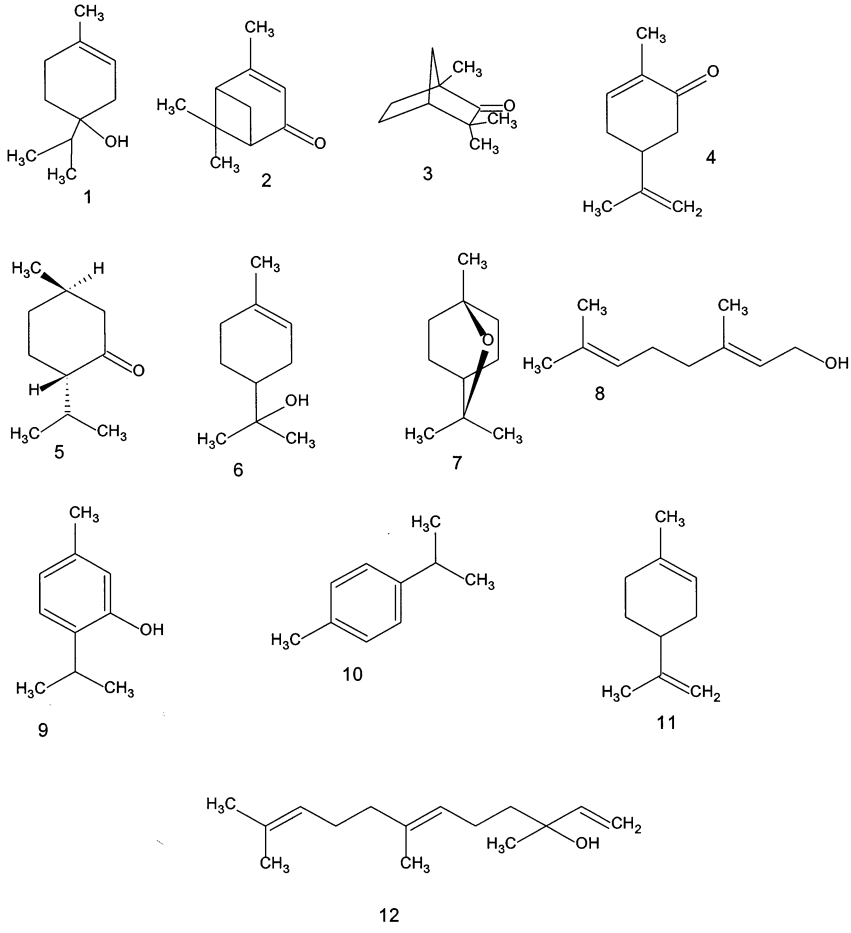

Fig. 1. The chemical structures of the evaluated terpene enhancers: (1) Terpinen-4-ol; (2) Verbenone; (3) Fenchone; (4) Carvone; (5) Menthone; (6) α-Terpineol; (7) Cineole; (8) Geraniol; (9) Thymol; (10) Cymene; (11) D-Limonene; (12) Nerolidol.

3. Results and discussion

The log P values and structures of terpenes are shown in Table 1 and Fig. 1, respectively. Table 2 summarizes the data on the flux, cumulative amount in the receptor after 24 h, skin content, and ER_{flux} of HC for the permeation experiment. The absorption of HC was enhanced significantly by the addition of terpenes to the gel formulation ($P < 0.05$) and in particular the use of nerolidol in the gel resulted in a significant increase in the permeation of HC (Fig. 2a and b). Nerolidol provided an approximately 35.3-fold increase in HC flux followed by limonene, with a 28.4-fold increase, and cymene with a 22.9-fold increase. ER_{flux}, for both cymene and D-limonene were significantly different from each other ($P < 0.05$) and also significantly higher than that of other terpenes ($P < 0.05$). Menthone produced a moderate enhancing activity with an 18.7-fold increase. Geraniol, cineole, α-terpineol, and carvone showed only mild enhancement activity. Verbenone, terpinen-4-ol, and thymol were less effective enhancers. Of all terpenes tested, fenchone exhibited the lowest ER_{flux} of only 10.1.

In addition to providing the highest ER_{flux}, nerolidol also provided the highest Q_{24} (1733 ± 93 μg/cm^2), followed by cymene (1451 ± 108 μg/cm^2), geraniol (1134 ± 19 μg/cm^2), carvone (1104 ± 185 μg/cm^2), and limonene (1089 ± 190 μg/cm^2).

Cymene produced Q_{24} values which were not significantly different from nerolidol ($P < 0.05$). On the contrary, geraniol, carveone, and limonene Q_{24} values were significantly lower than nerolidol ($P < 0.01$). Cineole, fenchone, α-terpineol, menthone, thymol, terpinen-4-ol, and verbenone produced comparable Q_{24} values. However, verbenone exhibited the lowest Q_{24} (653 ± 105 μg/cm^2).

In this series of terpenes, nerolidol provided the best enhancement activity for HC permeation. This conclusion is supported by several other publishers. According to Cornwell and Barry (1991b), nerolidol was the most effective terpene enhancer in promoting the permeation of 5-fluorouracil through the skin among series of studied terpene enhancers. Furthermore, Arellano et al. (1996) reported that nerolidol was an effective enhancer for the permeation of diclofenac sodium through the rat skin. This was attributed to nerolidol possessing an amphiphile-like structure that was appropriate for the disruption of the lipid packing of the SC.

The results of this study indicate that hydrophilic terpenes, capable of hydrogen bonding, were not as effective in promoting the permeation of HC (a polar steroid with a log P of 1.43 ± 0.25), compared to hydrocarbon terpenes (D-limonene and cymene). These results are different from those obtained by other researchers. Most studies suggest that hydrophilic terpenes (alcohol, ketone, and oxide terpenes) are more effective in enhancing the permeation of hydrophilic drugs, whereas, hydrocarbon terpenes (such as limonene and cymene) are more active towards lipophilic drugs (Hori et al. 1991; Moghimi et al., 1997). In this study, the higher enhancement activity of hydrocarbon terpenes can be attributed to their higher thermodynamic activity in the gel. D-Limonene and cymene were not completely dissolved at the 2% concentration in the gel as compared to the hydrophilic terpenes that were completely soluble in the gel containing 56% ethanol. Similar findings were reported by Obata et al. (1993) that demonstrated that D-limonene had higher enhancement activity for diclofenac at 1% concentration compared to L-menthol at the same concentration in 40% ethanol-buffer solution. The solubility of D-limonene in 40% ethanol-buffer was lower than that of L-menthol, resulting in a higher D-limonene thermodynamic activity.

For topical formulations, drug skin content is considered an important parameter and in the present study HC skin content was determined at 24 h (Tojo et al., 1987). Cineole provided the highest HC skin content (18999 ± 5666 μg/g)

Table 2
Effect of terpene enhancers on the percutaneous parameters of hydrocortisone formulated in HPMC gels[a]

Terpene	Flux μg/(cm^2 h)[b]	ER_{flux}	Q_{24} μg/cm^2[b]	SC μg/g[b]	Lag time (h) [b]
Control	4.5 ± 0.6	1.0	117 ± 19	2553 ± 557	2.6 ± 0.8
Terpinen-4-ol	51.0 ± 14.3	11.3	709 ± 91	4749 ± 1672	3.4 ± 0.5
Verbenone	51.6 ± 10.6	11.5	653 ± 105	1417 ± 420	3.8 ± 0.1
Fenchone	45.6 ± 6.6	10.1	953 ± 184	3578 ± 361	4.5 ± 0.7
Carvone	59.1 ± 7.9	13.1	1104 ± 185	2483 ± 375	2.1 ± 1.2
Menthone	84.0 ± 13.4	18.7	817 ± 181	5755 ± 1706	1.4 ± 0.8
α-Terpineol	60.0 ± 4.4	13.3	829 ± 43	6886 ± 258	3.1 ± 0.2
Cineole	65.1 ± 17.6	14.5	969 ± 153	18999 ± 5666	3.0 ± 1.0
Geraniol	76.2 ± 4.4	16.9	1134 ± 19	16682 ± 5390	3.0 ± 0.6
Thymol	49.6 ± 9.7	11.0	727 ± 154	1151 ± 293	3.2 ± 0.5
Cymene	103.0 ± 10.8	22.9	1451 ± 108	3498 ± 1118	0
D-Limonene	128.0 ± 9.7	28.4	1089 ± 190	10299 ± 4564	0.5 ± 0.4
Nerolidol	159.0 ± 23.7	35.3	1733 ± 93	1384 ± 168	1.2 ± 0.3

[a] Female hairless mouse skin was used. SC, skin content of hydrocortisone after 24 h; Q_{24}, cumulative amount of hydrocortisone in the receptor after 24 h; ER_{flux}, Enhancement ratio of hydrocortisone flux.

[b] Means ± S.D, $n = 4$.

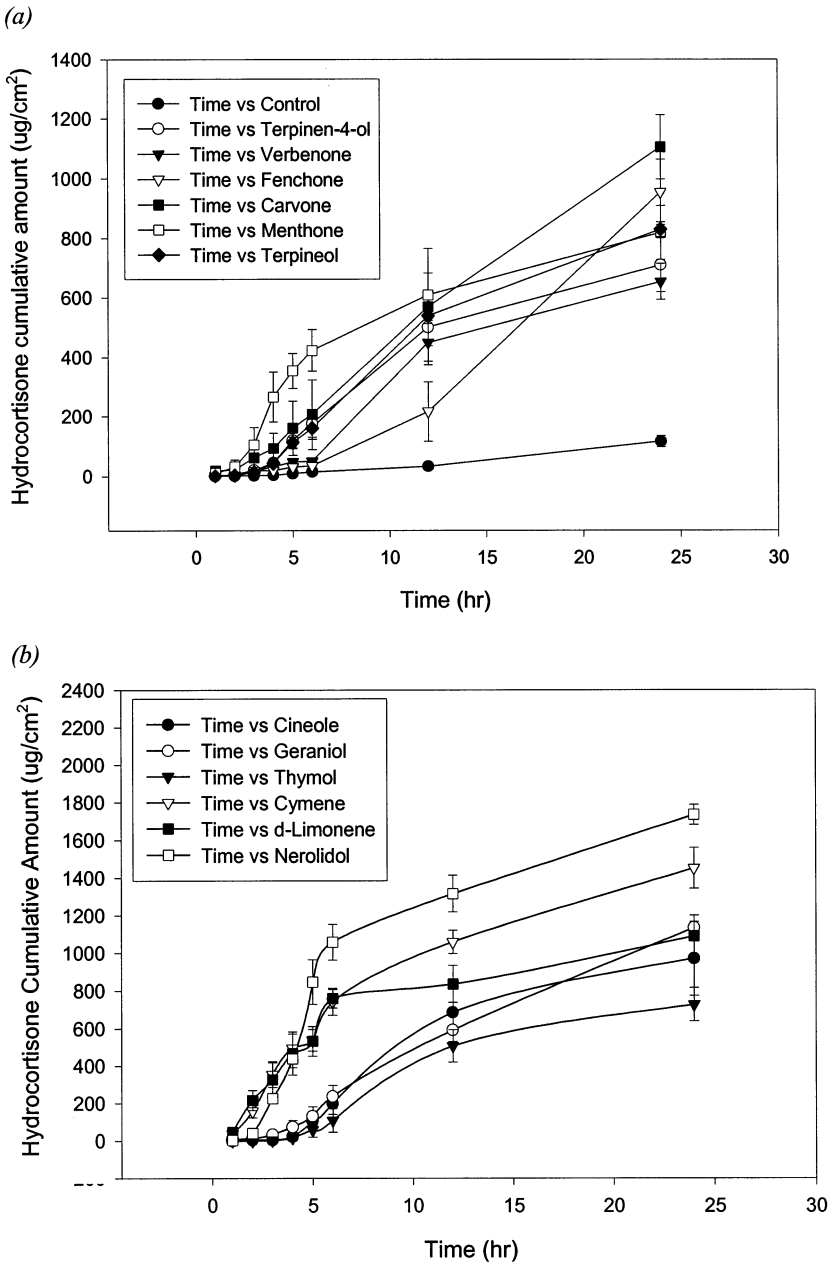

Fig. 2. The permeation profile for hydrocortisone from the HPMC gel system using terpene enhancers. Means ± S.D., $n = 4$.

which was not significantly different from geraniol (16682 ± 5390 μg/g), and D-limonene (10299 ± 4564 μg/g) ($P > 0.05$). However, cineole, geraniol, and D-limonene provided HC skin contents that were significantly higher than those of the other terpenes ($P < 0.05$). It is of interest to note that Ogiso et al. (1995) found that cineole and D-limonene significantly increased indomethacin skin content compared to controls. Thymol gave the lowest HC skin content (1151 ± 293 μg/g)

which was lower than control (2553 ± 557 μg/g). However, it was not significantly different from verbenone (1417 ± 420 μg/g), and nerolidol (1384 ± 168 μg/g) ($P > 0.05$). There was no correlation between HC skin contents and the flux values. This may suggest that the enhancers localized in the stratum corneum may retain the drug and partially prevent its diffusion and final penetration (Ogiso et al., 1992).

In this study, fenchone produced the longest HC lag time (4.5 ± 0.7 h) which was significantly higher than that of control ($P < 0.05$). Verbenone, terpinen-4-ol, thymol, α-terpineol, geraniol, and cineole had lower lag times than fenchone, which were not significantly different from fenchone lag time ($P > 0.05$). Other terpenes had a moderate effect on HC lag time.

Table 3
Effect of terpene enhancers on the hydrocortisone solubility in the hydrogel solvent mixture at 25°C ($n = 3$), (Means ± S.D.)

Enhancer	Solubility (g/ml)	ER_{solty} [a]
Control	0.0210 ± 0.0007	1.00
Terpinen-4-ol	0.0220 ± 0.0005	1.04
α-Terpineol	0.0220 ± 0.0030	1.04
Fenchone	0.0230 ± 0.0020	1.09
Carvone	0.0220 ± 0.0010	1.04
Menthone	0.0230 ± 0.0005	1.09
Verbenone	0.0200 ± 0.0001	0.95
Cineole	0.0230 ± 0.0050	1.10
Geraniol	0.0170 ± 0.0010	0.81
Thymol	0.0260 ± 0.0010	1.23
Cymene	0.0270 ± 0.0005	1.29
D-Limonene	0.0290 ± 0.0009	1.38
Nerolidol	0.0260 ± 0.0010	1.23

[a] ER_{solty}, enhancement ratio of hydrocortisone solubility

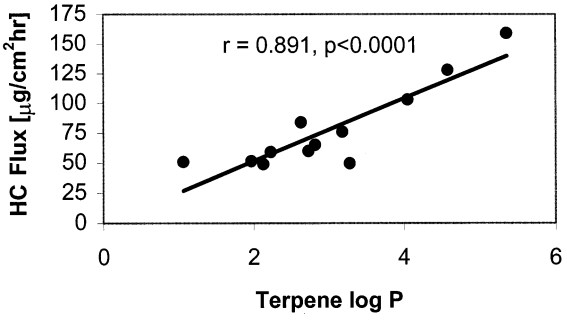

Fig. 3. The relationship between the log P of the terpene enhancers and hydrocortisone flux across female hairless mouse skin ($n = 4$) [$r = 0.891$, $P < 0.0001$].

The solubility of HC in the enhancer, ethanol, water, glycerin solvent system was determined after equilibration for 24 h at 25 °C. The ratio of drug concentration in presence of enhancer (C_o) to its solubility (C_s) in the gel solution gave an approximation of drug thermodynamic activity (Coldman et al., 1969). The control gel solvent mixture was represented by a ratio of unity. Solubility enhancement ratios of HC are listed in Table 3. Most enhancers, except for verbenone and geraniol, increased (C_o/C_s) ratio. However, the increase in the ratio was not significant, suggesting that the main contribution to the enhancement of HC permeation could be attributed to other factors, such as increase in the fluidity of the SC lipids and increased drug partitioning into skin.

In this study, an attempt was made to correlate the log P of the terpene enhancers with percutaneous parameters of HC. The effect of enhancers on the permeation of a drug usually depends upon the physicochemical characteristics of both permeant as well as the enhancer molecule. The lipophilicity of the enhancer is thought to play a major factor in its activity. Williams and Barry (1991) found a linear relationship between the log P of a group of epoxide and ketone terpenes and the enhancement ratio for 5-fluorouracil permeation. Furthermore, Takayama et al. (1993) studied the effect of terpene lipophilicity on their enhancing activity. These authors reported that terpenes with relatively high lipophilic index values provided significant enhancing activities, whereas further increase in their lipophilicity resulted in lower enhancing activity. In addition, Obata et al. (1993) attributed this phenomenon to differences in the thermodynamic activity of the enhancers in the vehicle.

In the present study, the log P of terpene enhancers greatly influenced HC flux and Q_{24} values at log P ranges of 1.06–5.36 (Figs. 3 and 4). The correlation coefficients for the two relations were $r = 0.891$, $P < 0.0001$, and $r = 0.772$, $P < 0.005$, respectively. In addition, a significant correlation existed between the lag time of HC and the log P

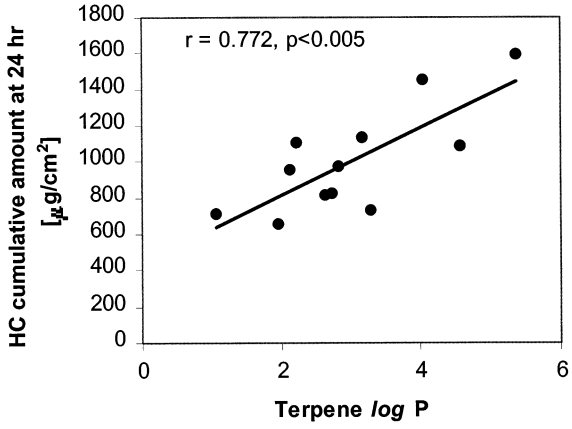

Fig. 4. The relationship between the log P of the terpene enhancers and the cumulative amount of hydrocortisone after 24 h found in the receptor of diffusion cells containing female hairless mouse skin ($n=4$) [$r=0.772,\ P<0.005$].

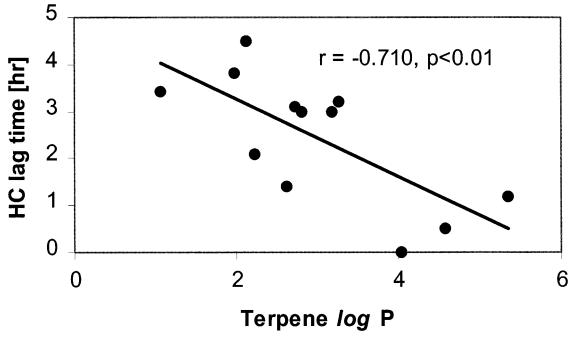

Fig. 5. The relationship between the terpene enhancer log P and hydrocortisone lag time using female hairless mouse skin ($n=4$)[$r=-0.710,\ P<0.01$].

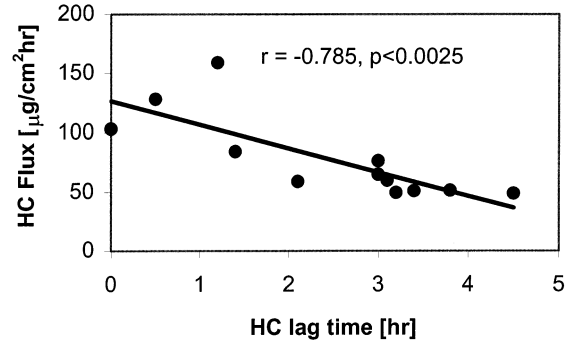

Fig. 6. The relationship between the lag time for hydrocortisone and hydrocortisone flux using female hairless mouse skin ($n=4$) [$r=-0.785,\ P<0.0025$].

of terpene enhancers ($r=-0.710,\ P<0.01$) (Fig. 5). In contrast, there was no correlation between the HC skin content and the log P of the enhancers ($r=0.01$). However, there was a correlation between the flux of HC and the lag time ($r=-0.785,\ P<0.0025$) (Fig. 6). Williams and Barry (1990) evaluated the effect of terpene enhancers on the SC using differential scanning calorimetery (DSC). They found that terpene enhancers promote the permeation of the drug and this is accompanied by disruption of the intercellular lipid packing. The results of this study may suggest that the increase in the lipophilicity of the terpene enhancers increase their ability to disrupt the hydrophobic lipid packing of the SC thereby increasing drug diffusion through the skin.

In conclusion, a strong relationship was established between the log P of the terpenes and the flux of HC and Q_{24}. Significantly high flux values were obtained using nerolidol which possessed the highest log P compared to other terpenes. In addition, a correlation was found between the log P of terpene enhancers and HC lag time ($r=-0.710$, $P<0.01$). However, no correlation was found between the log P of the terpenes and HC skin content. The effect of terpenes on the C_o/C_s ratio was not significant suggesting that the enhancement activity can be attributed to increase in the drug diffusion and partitioning into the skin.

References

Akimoto, T., Aoyagi, T., Minoshima, J., Nagase, Y., 1997. Polymeric percutaneous drug penetration enhancer synthesis and enhancing property of PEG/PDMS block copolymer with a cationic end group. J. Control Release 49, 229–241.

Arellano, A., Santoyo, S., Martin, C., Ygartua, P., 1996. Enhancing effect of terpenes on the in vitro percutaneous absorption of diclofenac sodium. Int. J. Pharm. 130, 141–145.

Asano, J., Suisha, F., Takada, M., Kawasaki, N., Miyazaki, S., 1997. Effect of pulsed output ultrasound on the transdermal absorption of indomethacin from an ointment in rats. Biol. Pharm. Bull. 20, 288–291.

Barry, B., 1983. Dermatological Formulations Percutaneous Absorption. Marcel Dekker, New York.

Bando, H., Sahashi, M., Yamashita, F., Takakura, Y., Hashida, M., 1997. In vivo evaluation of acyclovir prodrug penetration and metabolism through rat skin using a diffusion/bioconversion model. Pharm. Res. 14, 56–62.

Cevc, G., 1996. Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit. Rev. Ther. Drug Carr. Syst. 13, 257–388.

Coldman, M., Poulsen, B., Higuchi, T., 1969. Enhancement of percutaneous absorption by the use of volatile, nonvolatile systems as vehicles. J. Pharm. Sci. 58, 1098–1102.

Cornwell, P., 1993. Mechanism of action of terpene penetration enhancers in human skin. Ph.D. thesis, University of Bradford, UK.

Cornwell, P., Barry, B., 1991a. Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-flurouracil. J. Pharm. Pharmacol. 46, 261–269.

Cornwell, P., Barry, B., 1991b. The effect of a series of homologous terpene alcohols on the lipid structure of human stratum corneum as assessed by differential scanning calorimetry. In: Scott, R.C., Guy, R.H., Hadgraft, J., Bodde, H.E. (Eds.), Prediction of Percutaneous Penetration: Methods, Measurements, Modeling. IBC Technical Services, London, pp. 394–400.

Cornwell, P., Barry, B., 1994. Wide-angle X-ray diffraction of human stratum corneum effect of hydration and terpene enhancer treatment. J. Pharm. Pharmacol. 46, 938–950.

Davis, A., Hadgraft, J., 1991. Effect of supersaturation on membrane transport. 1. Hydrocortisone acetate. Int. J. Pharm. 76, 1–8.

Elias, P.M., 1996. The stratum corneum revisited. J. Derm. 23, 756–758.

Gao, S., Singh, J., 1998. In vitro percutaneous absorption enhancement of lipophilic drug tamoxifien by terpenes. J. Control Release 51, 193–199.

Giannakou, S., Dallas, P., Rekkas, D., Choulis, N., 1998. Development and in vitro evaluation of nimodipine transdermal formulations using factorial design. Pharm. Dev. Tech. 3, 517–525.

Godwin, A., Michniak, B., Creek, K., 1997. Evaluation of transdermal penetration enhancers using a novel skin alternative. J. Pharm. Sci. 86, 1001–1005.

Harrison, S., Barry, B., Dugard, P., 1984. Effects of freezing on human skin permeability. J. Pharm. Pharmacol. 36, 261–262.

Hori, M., Satoh, S., Maibach, H.I., Gug, R.H., 1991. Enhancement of propranolol hydrochloride and diazepam skin absorption in vitro: effect of enhancer lipophilicity. J. Pharm. Sci. 80, 32–35.

Iwasa, A., Irimoto, K., Kasai, S., Okuyama, H., Nagai, T., 1991. Effect of non-ionic surfactant on percutaneous absorption of diclofenac sodium. Yakuzaigaku 51, 16–21.

Kim, Y., Ghanem, A., Higuchi, W., 1992. Model studies of epidermal permeability. Semin. Dermatol. 11, 145–156.

Kuratomi, Y., Miyauchi, K., 1988. Methods and instruments of moxibustion. US Patent ,747,4,841.

Michniak, B., Player, M.R., Chapman, J.M., Sowell, J.W., 1994. Azone analogues as penetration enhancers: effect of different vehicles on hydrocortisone acetate skin permeation and retention. J. Control Release 32, 147–154.

Mao-Qiang, M., Feingold, K.R., Elias, P.M., 1993. Inhibition of cholesterol and sphingolipid synthesis causes paradoxical effects on permeability barrier homeostasis. J. Invest. Dermatol. 1011, 85–90.

Moghimi, H., Williams, A., Barry, B., 1996. A lamellar matrix for stratum corneum intercellular lipids. IV. Effects of terpene penetration enhancers on the permeation of 5-fluorouracil and oestradiol through the matrix. Int. J. Pharm. 145, 49–59.

Moghimi, H., Williams, A., Barry, B., 1997. A lamellar matrix model for stratum corneum intercellular lipids. V. Effects of terpene penetration enhancers on the structure and thermal behavior of the matrix. Int. J. Pharm. 146, 41–54.

Morimoto, Y., Sugibayashi, K., Kobayashi, D., Shoji, H., Yamazaki, J., Kimura, J., 1993. A new enhancer-coenhancer system to increase skin permeation of morphine hydrochloride in vitro. Int. J. Pharm. 91, 9–14.

Obata, Y., Takayama, K., Machida, Y., Nagai, T., 1991. Combined effect of cyclic monoterpenes and ethanol on percutaneous absorption of diclofenac sodium. Drug Des. Deliv. 8, 137–144.

Obata, Y., Takayama, K., Maitani, Y., Machida, Y., Nagai, T., 1993. Effect of pretreatment of skin with cyclic monoterpenes on permeation of diclofenac in hairless rat. Biol. Pharm. Bull. 16, 312–314.

Ogiso, T., Iwaki, M., Bechako, K., Tsutsumi, Y., 1992. Enhancement of percutaneous absorption by laurocapram. J. Pharm. Sci. 81, 762–767.

Ogiso, T., Iwaki, M., Tsuyoshi, P., 1995. Effects of enhancers on transdermal penetration of indomethacin and urea, and relationship between penetration parameters and enhancement factors. J. Pharm. Sci. 84, 482–488.

Ohara, N., Takayama, K., Nagai, T., 1995. Combined effect of D-limonene pretreatment and temperature on the rat skin permeation of lipophilic and hydrophilic drugs. Biol. Pharm. Bull. 18, 439–442.

Okabe, H., Obata, Y., Takayama, K., Nagai, T., 1990. Percutaneous absorption enhancing effect and skin irritation of monocyclic monoterpenes. Drug Des. Deliv. 6, 229.

Opdyke, D., 1979. Monographs on fragrance raw materials. Food Cosmet. Toxicol. 1, 11–17 (Suppl.).

Pfister, W., Dean, S., Hsieh, S., 1990. Permeation enhancers compatible with transdermal drug delivery systems. I. Selection and formulation considerations. Pharm. Tech. 8, 132–140.

Roy, S., 1999. Preformulation aspects of transdermal drug delivery systems. In: Ghosh, T., Pfister, W., Yum, S. (Eds.), Transdermal and Topical Drug Delivery Systems. Interpharm, Buffalo Grove, pp. 139–166.

Sato, S., Hirotani, Y., Ogura, N., Sasaki, E., Kitagawa, S., 1998. Enhancing effect of *N*-dodecyl-2-pyrrolidone on the percutaneous absorption of 5-fluorouracil derivatives. Chem. Pharm. Bull. 46, 831–836.

Takayama, K.., Kikuchi, K., Obata, Y., Okabe, H., Machida, Y., Nagai, T., 1993. Terpenes as percutaneous absorption promoters. STP Pharm. Sci. 183, 25–30.

Tanojo, H., Bouwstra, J., Junginger, H., Boddé, H., 1997. In vitro human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies. Pharm. Res. 14, 42–49.

Tojo, K., Chiang, C., Chien, Y., 1987. Drug permeation across the skin: effect of penetrant hydrophilicity. J. Pharm. Sci. 76, 123–126.

Valpato, N., Nicoli, S., Laureri, C., Colombo, P., Santi, P., 1998. In vitro acyclovir delivery in human skin layers after transdermal iontophoresis. J. Control Release 50, 291–296.

Viegas, T., Hikal, A., Jones, A., 1997. Percutaneous absorption of bendroflumethiazide from gel and membrane-controlled gel systems: an in vitro and in vivo study. Int. J. Pharm. 152, 165–178.

Wade, A., Weller, P., 1995. Handbook of Pharmaceutical Excipients, 2nd ed. American Pharmaceutical Association, Washington DC.

Wang, Su., Kara, M., Krishnan, T., 1998. Transdermal delivery of cyclosporin-A using electroporation. J. Contr. Rel. 50, 61–70.

Wester, R., Maibach, H., 1992. Percutaneous absorption of drugs. Clin. Pharmacokinet. 23, 235–266.

Williams, A., Barry, B., 1990. Differential scanning calorimetery does not predict the activity of terpene penetration enhancers in human skin. J. Pharm. Pharmacol. 42, 156P.

Williams, A., Barry, B., 1991. Terpenes and the lipid-protein-partitioning theory of skin penetration enhancement. Pharm. Res. 8, 17–24.

Wu, P., Huang, Y., Fang, J., Tsai, Y., 1998. Percutaneous absorption of captopril from hydrophilic cellulose derivatives through excised rabbit skin and human skin. Drug Devel. Ind. Pharm. 24, 179–182.

Yamane, M., Williams, A., Barry, B., 1995. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol. 47, 978–989.