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A lamellar matrix model for stratum corneum intercellular lipids IV. Effects of terpene penetration enhancers on the permeation of 5-fluorouracil and oestradiol through the matrix

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Abstract

It is widely accepted that the intercellular lipid domain of the stratum corneum (SC) is the main barrier to transdermal permeation of most drugs. Previously, we reported a lamellar lipid matrix capable of modelling the structural properties of the SC intercellular lipids and its barrier performance toward oestradiol (OE) and 5-fluorouracil (5-FU). To investigate the ability of the matrix in modelling the effects of terpene penetration enhancers on the barrier performance of the SC, permeation of model drugs (OE and 5-FU) through cineole or limonene treated matrices were investigated here. Results revealed that the matrix is able to model the effects of cineole and limonene on the permeability coefficients of OE through the SC. The effects of cineole on the permeability coefficient of 5-FU through the SC seem to be underestimated by the model matrix; the difference was attributed to the effects of cineole on the SC intracellular proteins or to differences in the hydrophilicities of the SC lipids and the matrix. The matrix failed to model the enhancement effect of limonene toward permeation of 5-FU through the SC which might indicate that limonene increases the permeation of 5-FU in part through interactions with SC proteins which are not modelled in the matrix. Copyright © 1996 Elsevier Science B.V.

Keywords: 1,8-Cineole; 5-Fluorouracil; (+)-Limonene; Model human stratum corneum lipids; Oestradiol; Terpene penetration enhancers

1. Introduction

The stratum corneum (SC) provides the main barrier to transdermal delivery of most drugs (Berenson and Burch, 1951). It is widely accepted that the intercellular lipid domain is the main

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pathway for permeation of most drugs through the SC (Elias and Friend, 1975; Albery and Hadgraft, 1979; Boddé et al., 1991; Moghimi et al., 1996a).

Intercellular lipids of the SC arrange into bilayers (Elias and Friend, 1975; Elias et al., 1977, 1979). Preparation of a model for the intercellular lipids provides opportunities to investigate the barrier nature of the SC and the effects of accelerants on the permeation of molecules through this membrane. We formed a simple lamellar mesomorphic structure (matrix) consisting of 20% cholesterol, 25% water and 55% free fatty acids and their soaps as an analogue for the intercellular lipid region of the SC. X-ray diffraction, hot-stage polarised light microscopy and differential scanning calorimetry studies characterised the matrix, showing good structural correlation with SC intercellular lipids (Moghimi et al., 1996b). Release and permeation studies using oestradiol (OE)—a model lipophilic drug—and 5-fluorouracil (5-FU)—a model hydrophilic drug—revealed that the matrix models well the SC intercellular pathway and that both drugs permeate the SC through the intercellular channels; we predicted the permeability coefficients of the drugs through human epidermis from model matrix data (Moghimi et al., 1996a).

To investigate the ability of the matrix in modelling the effects of skin penetration enhancers on the barrier performance of the SC, release of model drugs (OE and 5-FU) from, and their permeation through, terpene treated matrices were investigated. The enhancing abilities of a series of monoterpenes toward permeation of OE and 5-FU through human epidermis have been studied and it was shown that they increase the permeation of both OE and 5-FU (Williams and Barry, 1991a,b, 1992). Among different chemical classes of terpenes studied, 1,8-cineole (abbreviated to cineole here), a cyclic monoterpene from the ether class, and (+)-limonene (limonene), a cyclic hydrocarbon monoterpene, provided a wide range of accelerant activity toward permeation of these drugs across epidermal membrane (see Williams and Barry, 1991a,b for more details). Therefore, these two terpenes were selected for release and permeation experiments. The release results are presented in our companion paper (Moghimi et al., 1996c) and the studies considered in the present paper deal with permeation experiments.

In permeation studies the effects of different concentrations of cineole and limonene on the permeability coefficients of OE and 5-FU across a model matrix membrane and partitioning of these drugs from their aqueous solutions into the matrix were investigated. Results were then compared with the effects of the same terpenes on the barrier performance of human SC toward OE and 5-FU. Because of difficulties with handling of the fragile SC, most permeability studies use epidermal membranes. Permeation studies, performed at our laboratories (Williams and Barry, 1991b), indicated that the SC is the main barrier to transdermal permeation of 5-FU and OE. Therefore, the permeability coefficients of 5-FU and OE through epidermal membranes were used here as estimates of the SC values.

2. Materials and methods

2.1. Materials

All materials were used as received. Tritium-labelled 5-fluorouracil (5-[6-^{3}H]-FU) and oestradiol ([2,4,6,7-^{3}H(N)]-OE), both with radiochemical purity of 99%, were supplied by NEN (Dupont) Research Products (Dreiech, Germany). Unlabelled 5-FU (99%) was supplied by Aldrich Chemical Company (Dorset, England). Unlabelled OE (99.6%), 1,8-cineole (99.5%) and (+)-limonene (99.2%) were supplied by Sigma (Dorset, England). PTFE filters (pore size 200 nm, thickness 60 μm and 80% porosity) were purchased from Sartorius (Germany). All other solvents and reagents were of analytical grade.

2.2. Preparation of drug and terpene loaded matrices

Preparation of the model matrix, performed in two steps of lipid mixture and hydration, was described previously (Moghimi et al., 1996b). Radiolabelled drugs, OE and 5-FU, were added as

solutions in methanol during the preparation of the lipid mixture (before hydration) to give final concentrations of 0.1% (w/w) in the model matrix. Cineole and limonene were added to matrices containing 0.1% radiolabelled OE or 5-FU to yield final concentrations of 5, 10, 15, 20 and 25% (w/w) terpenes in the model matrix.

2.3. Preparation of matrix membranes

For preparation of matrix membranes, plain or terpene-treated matrix samples were placed in a circular hole (4 mm in diameter) of a PTFE matrix spacer and covered from both sides with PTFE filters to provide membranes of approximately 1.35 mm in thickness. The PTFE filters were wetted with methanol and stored in distilled water overnight prior to use. All membranes were prepared immediately before use.

2.4. Permeation experiments

Permeation studies of OE and 5-FU through matrices employed untreated matrix and matrices containing 5, 10, 15, 20 and 25% (w/w) cineole or limonene and were performed at 32°C. These experiments employed an automated diffusion system equipped with 24 stainless-steel diffusion cells with flow-through receptor compartments as described by Akhter et al. (1984). The cells provided a diffusional area of 0.126 cm^2 and were mounted on four copper arms of the diffusion apparatus through which temperature controlled water was circulated to maintain a desired temperature on the surface of the matrix membranes. The matrix membranes were placed between the receptor and donor chambers of the diffusion cells and 150 ml donor solutions were dispensed into each of the cell donor compartments. Drug donor solutions were saturated aqueous solutions of radiolabelled OE and 5-FU at 32°C. Aqueous solubilities of OE and 5-FU at 32°C are reported to be 3.6 $\mu g\ ml^{-1}$ and 14.3 $mg\ ml^{-1}$ respectively (Cornwell, 1993). Since the solubility of OE in water is very low, donor depletion was expected. To prevent such a change, OE donor solutions were replenished every 8 h. HCl solution (pH 1) was used as the receptor fluid to minimise the extraction of the matrix lipids (Lange-Lieckfeldt and Lee, 1992). The receptor solution was pumped through the cells at 2 $ml\ h^{-1}$ and samples collected in scintillation vials over 1–2.5 h intervals for a minimum of 30 h. The activity of the permeated radiolabelled drugs was determined as counts per minute using a TRI-CARB liquid scintillation analyser (model 1600 TR, Packard, USA). The fluxes and permeability coefficients of OE and 5-FU through terpene-treated and untreated matrix membranes were then calculated and the enhancers' activities expressed as the enhancement ratios (ER) as used in the skin studies:

$$\mathrm{ER} = \frac{\text{Permeability coefficient after terpene treatment}}{\text{Permeability coefficient before terpene treatment}} \quad (1)$$

The partitioning of drug molecules from the applied vehicle into a membrane or between different parts of the barrier is one of the important steps governing transmembrane permeation. The permeability coefficient (K_p) and diffusion coefficient (D) of a drug through a membrane and its membrane/donor partition coefficient (K_m) are related to each other through Fick's equation (Eq. (2)). Therefore, assuming that the enhancers do not change significantly the thickness of the membrane (h), the effects of enhancers on the membrane/donor partition coefficients of drugs (expressed as partitioning ratio, PR) can be estimated from ER and diffusivity ratio (DR, Eq. (3)) using Eq. (4).

$$K_p = \frac{DK_m}{h} \quad (2)$$

$$\mathrm{DR} = \frac{\text{Diffusion coefficient after terpene treatment}}{\text{Diffusion coefficient before terpene treatment}} \quad (3)$$

$$\mathrm{PR} = \frac{\text{Membrane/donor partition coefficient after enhancer treatment}}{\text{Membrane/donor partition coefficient before enhancer treatment}} = \frac{\mathrm{ER}}{\mathrm{DR}} \quad (4)$$

Table 1
Effect of cineole on the permeation of oestradiol from its saturated aqueous solution through the matrix at 32°C

Cineole concentration (% w/w)	Flux (ng cm^{-2} h^{-1})	Permeability coefficient (cm $h^{-1} \times 10^3$)	Enhancement ratio[a]	Partitioning ratio[b]
0 (Control)	8.48 ± 1.13	2.36 ± 3.14	1.00	1.00
5	25.7 ± 6.3	7.15 ± 1.75	3.03 ± 0.74	1.71
10	45.1 ± 11.0	12.5 ± 3.0	5.31 ± 1.29	3.25
15	33.3 ± 17.0	9.24 ± 4.71	3.92 ± 2.00	2.48
20	39.6 ± 21.4	11.0 ± 5.9	4.67 ± 2.53	2.36
25	69.0 ± 24.8	19.2 ± 6.9	8.14 ± 2.93	3.40

Data are mean ± S.D., $n = 3–4$.
[a]Cineole-treated/control permeability coefficients.
[b]Enhancement ratio/diffusivity ratio. Diffusivity ratios are from Moghimi et al. (1996c).

Note that the matrix is not a homogeneous system and consists of aqueous regions and lipid bilayers. Therefore, the calculated PR from diffusion parameters may be related to the effect of enhancers on the partition coefficient between two different phases inside the membrane which are related to the barrier property of the matrix and not necessarily to the uptake of drugs by the whole matrix.

If the results of our experiments are to be compared with those of human SC, the amount of enhancer in both systems should ideally be the same. In our laboratories, most permeation and uptake studies related to the effects of enhancers used 12 h enhancer treatment (the duration of time for which the membrane had been in contact with the enhancer before drug application). Therefore, it was necessary to prepare matrices with the same amount of enhancer which is taken up by the SC intercellular lipids after 12 h enhancer treatment. As discussed in our companion paper (Moghimi et al., 1996c) the uptake of limonene and cineole by SC intercellular domain after 12 h terpene treatment is 49 and 85% (w/w) of the original dry lipid weight respectively. These values equate approximately to 25% limonene and 40% cineole (w/w) in a mixture of terpenes and intercellular lipids containing 25% (w/w) water (water content of the model matrix). Because of liquefaction of the model matrix, we were not able to perform permeation experiments with matrices containing more than 25% terpenes. Therefore, the permeability coefficients of OE and 5-FU in the model matrix containing 40% cineole were estimated by extrapolation of permeability data versus cineole concentration profiles.

The changes in the permeation parameters of drugs in the model matrix due to terpenes were analysed statistically using a two-tailed t-test, assuming that data are distributed normally and the populations have equal variances.

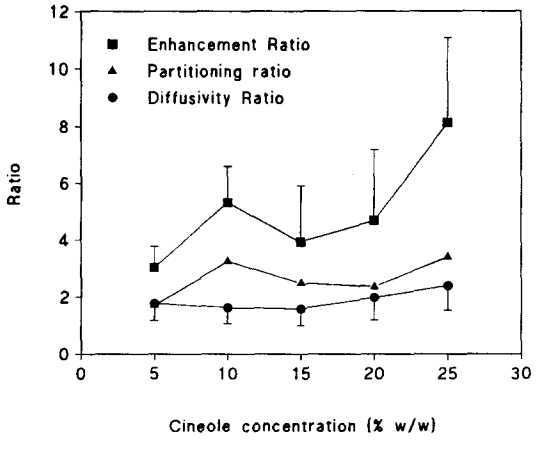

Fig. 1. Effects of cineole on the permeation of oestradiol through the model matrix at 32°C. Enhancement and diffusivity ratios are mean ± S.D., $n = 4$. Partitioning ratios are mean enhancement ratios/mean diffusivity ratios. Diffusivity ratios are from Moghimi et al. (1996c).

Table 2
Effect of limonene on the permeation of oestradiol from its saturated aqueous solution through the matrix at 32°C

Limonene concentration (% w/w)	Flux (ng cm^{-2} h^{-1})	Permeability coefficient (cm $h^{-1} \times 10^3$)	Enhancement ratio[a]	Partitioning ratio[b]
0 (Control)	17.4 ± 5.0	4.85 ± 1.40	1.00	1.00
5	24.3 ± 8.7	6.74 ± 2.43	1.39 ± 0.50	0.880
10	15.2 ± 5.1	4.21 ± 1.43	0.869 ± 0.295	1.00
15	22.2 ± 10.9	6.16 ± 3.02	1.27 ± 0.62	1.06
20	28.0 ± 11.0	7.77 ± 3.04	1.60 ± 0.63	1.18
25	156 ± 62	43.2 ± 17.3	8.91 ± 3.57	2.23

Data are mean ± S.D., $n = 3–4$.
[a]Limonene-treated/control permeability coefficients.
[b]Enhancement ratio/diffusivity ratio. Diffusivity ratios are from Moghimi et al. (1996c).

3. Results and discussion

3.1. Effects of cineole on the barrier performance of the model matrix toward oestradiol

Table 1 provides the effects of cineole on the permeation of OE through the model matrix at 32°C. Cineole increased the permeability coefficient of oestradiol significantly (t-test, $P = 0.05$) between 3.0 and 8.1 times through the matrices containing 5–25% enhancer relative to the untreated matrix (Table 1). The same terpene showed an enhancement ratio of 4.4 for the permeation of OE through human epidermis at 32°C after 12 h enhancer treatment (Williams and Barry, 1991b). As discussed earlier, if the results of the cineole-treated matrix are to be compared with the human epidermis data for 12 h enhancer treatment, the enhancement ratio for a matrix containing 40% cineole should be calculated by extrapolation. Extrapolation of the plot of enhancement ratio versus cineole concentration to 40% cineole, based on assumed linear relationship in the cineole concentration range of 15–25% (Fig. 1), resulted in an enhancement ratio of 14. This value is three times greater than that of human epidermis, but still acceptable taking the variability of the SC into account; SC lipid content is reported to vary between 3 and 50% (w/w) (Raykar et al., 1988). However, a better correlation exists between the effect of 12 h cineole treatment on the permeation of OE through human epidermis (ER = 4.4) and the model matrix results without extrapolation (ER = 8.1 for cineole concentration of 25%). As was shown in our companion paper (Moghimi et al., 1996c) although there was a very good correlation between diffusivity ratio of OE in the cineole-treated human epidermis (2.1) and the model matrix after extrapolation of the matrix data to a cineole concentration of 40% (DR = 3.6), a better correlation existed without extrapolation (DR = 2.4 for matrix cineole content of 25%). This might suggest that the enhancement efficiency of cineole toward OE through the model matrix and human epidermis reaches a plateau at an equivalent of

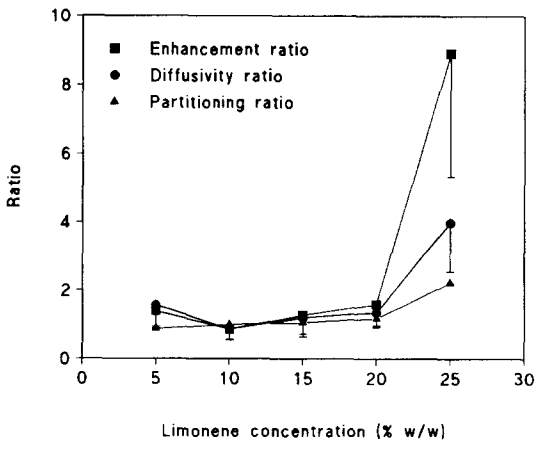

Fig. 2. Effects of limonene on the permeation of oestradiol through the model matrix at 32°C. Enhancement and diffusivity ratios are mean ± S.D., $n = 3–4$. Partitioning ratios are mean enhancement ratios/mean diffusivity ratios. Diffusivity ratios are from Moghimi et al. (1996c).

Table 3
Effect of cineole concentration on the permeation of 5-fluorouracil from its saturated aqueous solution through the model matrix at 32°C

Cineole concentration (% w/w)	Flux (μg cm^{-2} h^{-1})	Permeability coefficient (cm h^{-1} × 10^3)	Enhancement ratio[a]	Partitioning ratio[b]
0 (Control)	46.3 ± 2.1	3.24 ± 1.49	1.00	1.00
5	59.6 ± 5.6	4.17 ± 0.39	1.00 ± 0.51	0.680
10	59.5 ± 24.4	4.16 ± 1.70	1.29 ± 0.53	0.616
15	55.7 ± 5.4	3.89 ± 0.37	1.20 ± 0.12	0.346
20	119 ± 45	8.32 ± 3.11	2.57 ± 0.96	0.384
25[c]	122 ± 45	8.54 ± 3.12	6.15 ± 2.25	0.475

Data are mean ± S.D., $n = 3$–5.
[a]Cineole-treated/control permeability coefficients.
[b]Enhancement ratio/diffusivity ratio. Diffusivity ratios are from Moghimi et al. (1996c).
[c]Control values: flux = 19.8 ± 5.9 μg cm^{-2} h^{-1}, permeability coefficient = 1.39 ± 0.41 × 10^{-3} cm h^{-1}, data are mean ± S.D., $n = 4$.

25% cineole in the model matrix. It is interesting in this context to note that the enhancement efficiency of azone toward permeation of diazepam across human SC was reported to reach a plateau at azone loading of 12% (Schückler and Lee, 1992).

The enhancement ratio is the product of partitioning and diffusivity ratios (see Eq. (4)). Fig. 1 illustrates the profiles of enhancement, partitioning and diffusivity ratios vs cineole concentration for the permeation of OE across the model matrix and reveals that cineole increases the permeation of oestradiol across the model matrix by increasing both the diffusion and partition coefficients, in good agreement with the literature data (Williams and Barry, 1991b). Cineole increased the partitioning of OE from its aqueous solution into the model matrix 1.7–3.4-fold in the concentration range of 5–25% (Table 1) which is in good agreement with the effect of cineole on the SC/water partition coefficient of OE (PR = 2.1, Williams and Barry, 1991b).

3.2. Effects of limonene on the barrier performance of the model matrix toward oestradiol

Table 2 and Fig. 2 summarise the effects of limonene on the permeation of oestradiol through the model matrix at 32°C. The enhancement ratios of limonene toward oestradiol permeating the model matrix was very low (0.87–1.6) and not significant (t-test, $P = 0.05$) in matrices containing 5–20% terpene. On increasing the concentration of limonene to 25% (which resulted in liquefaction of the model matrix), the ER increased significantly ($P = 0.05$) to 8.9 which is in reasonable agreement with the human epidermis data (ER = 3.8) at 32°C after 12 h enhancer treatment (Williams and Barry, 1991b). The partitioning ratios of OE into the limonene-treated matrices were calculated to be 0.88–2.2 (Table 2 and Fig. 2). Limonene did not change the partition coeffi-

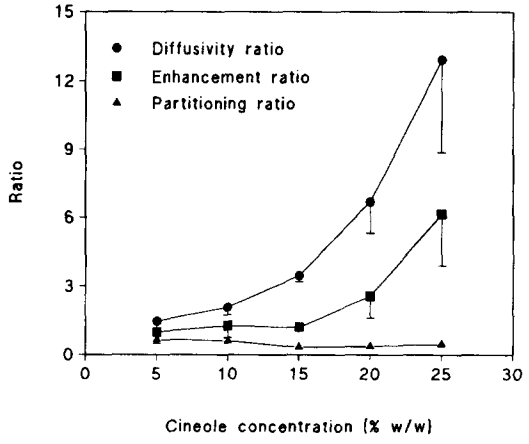

Fig. 3. Effects of cineole on the permeation of 5-fluorouracil through the model matrix at 32°C. Enhancement and diffusivity ratios are mean ± S.D., $n = 3$–4. Partitioning ratios are mean enhancement ratios/mean diffusivity ratios. Diffusivity ratios are from Moghimi et al. (1996c).

Table 4
Effect of limonene on the permeation of 5-fluorouracil from its saturated aqueous solution through the matrix at 32°C

Limonene concentration (% w/w)	Flux (μg cm^{-2} h^{-1})	Permeability coefficient (cm h^{-1} × 10^3)	Enhancement ratio[a]	Partitioning ratio[b]
0 (Control)	43.8 ± 24.0	3.07 ± 1.69	1.00	1.00
5[c]	16.1 ± 1.9	1.13 ± 0.14	0.455 ± 0.052	0.469
10[c]	9.23 ± 1.69	0.646 ± 0.118	0.246 ± 0.045	0.397
15	8.44 ± 2.81	0.590 ± 0.197	0.192 ± 0.064	1.13
20	1.08 ± 1.31	0.075 ± 0.091	0.025 ± 0.030	1.60
25	0.966 ± 0.616	0.068 ± 0.043	0.022 ± 0.014	0.335

Data are mean ± S.D., $n = 3$–4.
[a]Limonene-treated/control permeability coefficients.
[b]Enhancementratio/diffusivity ratio. Diffusivity ratios are from Moghimi et al. (1996c).
[c]Control values: flux = 37.6 ± 16.8 μg cm^{-2} h^{-1}, permeability coefficient = 2.63 ± 1.17 × 10^{-3} cm h^{-1}, data are mean ± S.D., $n = 4$.

cient of OE between SC and water (PR = 0.96) after 12 h enhancer treatment (Williams and Barry, 1991b).

As shown in Table 2 and Fig. 2, up to limonene concentration of 20%, the partitioning and diffusivity ratios and, therefore, the enhancement ratios are very low (1–2). As the limonene concentration increases to 25%, the permeability coefficient of OE in the model matrix increases 8.9 times relative to the untreated matrix and the improved diffusion coefficient (DR = 4.0, Moghimi et al., 1996c) is the major mechanism. However, the increased partition coefficient also plays a role (PR = 2.2).

Both cineole and limonene increased the matrix/donor partitioning of oestradiol. At terpene concentration of 25%, cineole and limonene produced partitioning ratios of 3.4 and 2.2 respectively. Improved partitioning would be expected considering the solubility of oestradiol in water and terpenes. Solubilities of oestradiol in water, cineole and limonene are reported to be 0.003, 8.02 and 0.025 mg ml^{-1} at 21°C (Williams, 1990; Williams and Barry, 1991b). Although a trend is apparent with limonene having less effect on the partitioning and providing a lower drug solubility compared with cineole which improved the partitioning more than limonene and provided a greater drug solubility, no mathematical relationship exists between the partitioning ratios and the solubility of OE in terpenes. The same phenomenon was reported for the effects of terpenes on the partitioning of OE into human SC (Williams and Barry, 1991b).

3.3. Effects of cineole on the barrier performance of the model matrix toward 5-fluorouracil

Table 3 summarises the effects of cineole on the permeation of 5-FU across the model matrix at 32°C. Cineole showed a low enhancement efficiency toward the permeation of 5-FU through the model matrix at low enhancer concentrations (Table 3 and Fig. 3). The permeability coefficients of 5-FU through matrices containing 5, 10 and

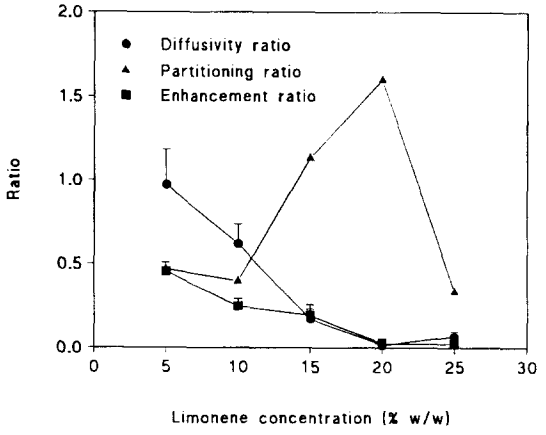

Fig. 4. Effects of limonene on the permeation of 5-fluorouracil through the model matrix at 32°C. Enhancement and diffusivity ratios are mean ± S.D., $n = 3$–4. Partitioning ratios are mean enhancement ratios/mean diffusivity ratios. Diffusivity ratios are from Moghimi et al. (1996c).

15% cineole (ER = 1.0–1.3) were not significantly different (t-test, $P = 0.05$) from that of untreated matrix. When the cineole concentration in the model matrix increased to 20 and 25%, the enhancement ratio increased significantly ($P = 0.05$) to 2.6 and 6.1 respectively.

The enhancement ratio of 5-FU through human epidermis at 32°C after 12 h cineole treatment is reported to be 95 (Williams and Barry, 1991a; Yamane, 1994). As discussed above, the enhancement ratio of 5-FU in a matrix containing 40% cineole should be calculated by extrapolation of the ER versus cineole concentration profile if the results are to be compared with 12 h cineole-treated human epidermis data. For such an extrapolation, the relationship between ER and cineole concentration is needed. However, using differential scanning calorimetry, Yamane (1994) showed that cineole affects different lipids of human SC at different treatment times (the time for which the enhancer had been in contact with epidermal membrane before drug application). This observation may indicate that the mechanism of action of cineole on the permeation of drugs through the SC and the model matrix is different at different treatment times or concentrations. If we consider that different mechanisms may operate for the lower and higher concentrations of cineole in the matrix, we may divide the profile of enhancement ratio vs cineole concentration into lower and higher concentration regions (5–15% and 15–25% respectively, Fig. 3) and use the data of the 15–25% concentration region for extrapolations. As shown in our companion paper (Moghimi et al., 1996c), the relationship between diffusivity ratio of 5-FU in the model matrix and cineole concentration seems to be exponential. However, the profile of enhancement ratio vs cineole treatment time for permeation of 5-FU through human epidermal membrane studied by Yamane (1994) shows a linear relationship between logER and treatment time ($r = 0.99$) as analysed here. If we similarly consider an exponential relationship between enhancement ratio and cineole concentration (i.e. a linear relationship between log ER and concentration) in the range of 15–25% ($r = 0.999$), the extrapolation gives an enhancement ratio of almost 68 for matrices containing 40% cineole which although slightly lower than human epidermis data (ER = 95) is well acceptable within the limits of skin variability. However, if we suppose that the relationship between the enhancement ratio and the cineole concentration is linear in the range of 15–25% cineole in the model matrix ($r = 0.97$) and then extrapolate this plot to a cineole concentration of 40%, an enhancement ratio of almost 13 is calculated. This is almost seven times less than that of human epidermis. The same quantitative disagreement between model matrix data and skin was reported for the effects of enhancers on the permeation of a hydrophilic drug (cyclobarbital) by Miyajima et al. (1994). Their results showed that the enhancement efficiencies of Azone, decylmethylsulphoxide, oleic acid, lauric acid and capric acid toward permeation of cyclobarbital through the model matrix membrane were up to seven times less than the effects of same enhancers toward the permeation of cyclobarbital through the skin. However, the difference between the effects of same enhancers toward a lipophilic drug (ibuprofen) in the model matrix and skin were around 2-fold (Miyajima et al., 1994). These results are comparable with our matrix and skin data which indicate that the matrix models the effects of cineole toward permeation of OE (a lipophilic drug) through the SC better than that of 5-FU (a hydrophilic drug). Plausible reasons for these differences might include; (i) the importance of the transcellular route for the permeation of hydrophilic drugs through enhancer-treated SC and (ii) difference between the hydrophilicity and therefore partitioning behaviour of the models and SC intercellular lipids as discussed below.

In spite of recent concentration on the intercellular route of the SC as the rate-determining pathway for drug permeation, the transcellular route should not be dismissed and the diffusivity in lipids and proteins and the partitioning of drugs between these domains may play important roles in the permeation of drugs and the effects of enhancers on this process (Michaels et al., 1975; Albery and Hadgraft, 1979; Tojo, 1987; Barry, 1991). For a hydrophilic drug, partitioning into the corneocytes should not be a rate limiting step

and if such drugs do not permeate the SC through the transcellular route, the limiting step should be a diffusional barrier. Suppose that an enhancer decreases the diffusivity barrier of the corneocytes and/or their envelopes. This reduction in the diffusivity barrier of the transcellular pathway will improve the permeation of hydrophilic drugs but may not be useful for lipophilic drugs as they find the intercellular domain favourable and do not, therefore, partition easily into the more hydrophilic corneocytes. Such a mechanism may explain why the model lipid systems underestimate the effects of enhancers on the barrier performance of the SC toward hydrophilic (and not lipophilic) drugs.

As shown in our companion paper (Moghimi et al., 1996c), the matrix is able to model the effect of cineole on the diffusion coefficient of 5-FU through the SC. Therefore, as enhancement ratio is the product of diffusivity and partitioning ratios, the underestimation of enhancement ratio by the model matrix should be a partitioning phenomenon. Our results showed that cineole decreases the partition coefficient of 5-FU into the model matrix at all concentrations studied (Table 3 and Fig. 3) and even with the extrapolation of the partitioning ratio versus cineole concentration profile to a cineole concentration of 40% based on linear relationships between PR or log PR versus cineole concentration in the range of 15–25% cineole, the partitioning ratio still remained below one (approximately 0.7). Our results are opposite to those of Yamane (1994) who reported a partitioning ratio of 2.5 for 5-FU into 12 h cineole-treated SC samples as calculated from uptake studies. This difference can be related to the increased equilibrium uptake of 5-FU into the SC corneocytes (which are absent in our model matrix) or to differences between the hydrophilicities of the model matrix and the SC lipids, both of which require further investigation.

3.4. Effects of limonene on the barrier performance of the model matrix toward 5-fluorouracil

Table 4 and Fig. 4 illustrate the effects of limonene on the permeation of 5-FU through the model matrix at 32°C. Limonene decreased the permeability coefficient of 5-FU through the model matrix at 32°C and its retardation effect increased with increasing concentration. The enhancement ratio of 5-FU was calculated to be 0.45 for matrices containing 5% limonene and further decreased as the concentration of terpene increased to reach 0.02 in matrices containing 25% limonene (Table 4). Hypothesis testing using t-test ($P = 0.05$) revealed that the decrease in the permeability coefficient of 5-FU in the model matrix due to limonene is not significant at limonene concentration of 5% but is significant for higher concentrations used here.

Permeability studies at 32°C have shown that limonene increases the permeation of 5-FU through human epidermis only 2.1 (Williams and Barry, 1991a) and 3.5-fold (Yamane, 1994) after 12 h enhancer treatment. Our matrix data show that limonene acts as retardant toward 5-FU and indicates that the matrix does not model the effect of limonene on the permeation of 5-FU through human epidermis. Differential scanning calorimetry studies suggested that limonene does not interact with the SC lipids to disorganise them at skin temperature, but it may interact with the SC proteins (Yamane, 1994; Cornwell et al., 1996). But, small-angle X-ray diffraction studies showed that limonene reduces lipid bilayer periodicity in the SC (Cornwell et al., 1996). This agrees with our matrix differential scanning calorimetry and polarised light microscopy studies which showed that limonene causes phase transformation in the model matrix around physiologic temperature (Moghimi et al., 1996d). These data show that limonene affects both lipids and proteins of the SC and may increase the diffusivity of 5-FU in the SC through protein interactions, which are not, of course, modelled in the simple lipid matrix.

In summary, a lamellar mesomorphic matrix was used as a model for the intercellular lipids of human stratum corneum. Release and permeability experiments suggested that model drugs, OE and 5-FU, permeate the SC through the intercellular pathway (Moghimi et al., 1996a). In this paper the effects of established skin penetration enhancers (cineole and limonene) toward perme-

ation of OE and 5-FU through the model matrix were investigated. The effects of cineole and limonene on the structure of the model matrix and the mechanism of action of these terpenes on the permeation of model drugs through the matrix are discussed in our companion paper (Moghimi et al., 1996d). Briefly, cineole creates a liquid pool in the model matrix and limonene first increases the consistency of the matrix up to a terpene concentration of 20% and then fluidises the model matrix when limonene concentration increases to 25%. Limonene also introduces a continuous lipophilic phase into the system.

The effects of limonene and cineole on the permeability coefficient of oestradiol through the model matrix were in good correlation with human epidermal membrane data and revealed that the matrix is able to model the effects of enhancers on the diffusion of OE and possibly other lipophilic drugs through human epidermis.

The matrix was able to model the effects of cineole toward permeation of 5-FU through human epidermis only when an exponential relationship was considered between permeability coefficient and cineole concentration. When a linear relationship was assumed, the effect of cineole on the permeation of 5-FU through human epidermis was underestimated by almost seven times by the model matrix. If the actual relationship between ER and cineole concentration is linear, this underestimation might be due to the effects of cineole on the SC proteins or to differences in the polarity and hence partitioning behaviour of the model matrix and SC lipids. Both of these suggestions require further investigation. The matrix is unable to model the effects of limonene on the permeation of 5-FU through human epidermis and this might indicate that limonene slightly increases the permeability coefficient of 5-FU through protein interactions in human SC, a mechanism which is supported by differential scanning calorimetry data (Yamane, 1994; Cornwell et al., 1996).

Our results show that except for the limonene-oestradiol system which shows a gradual increase in the partitioning ratio with terpene concentration, no clear correlation exists between the partition coefficient and terpene concentration in terpene-treated matrices. A similar phenomenon was reported by Schückler and Lee (1992) for the effect of azone on the partition coefficient of diazepam between SC and buffer, calculated from permeation data.

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