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International Journal of Pharmaceutics 215 (2001) 229–240

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

The effects of terpene enhancers on the percutaneous permeation of drugs with different lipophilicities

Ayman F. El-Kattan [a,b], Charles S. Asbill [a,c], Nanhye Kim [a,d], Bozena B. Michniak [a,e,*]

[a] *College of Pharmacy, University of South Carolina, Columbia, SC 29208, USA*
[b] *Pfizer, Ann Arbor, MI, USA*
[c] *McWhorter School of Pharmacy, Samford University, Birmingham, AL, USA*
[d] *Watson Pharmaceuticals, Salt Lake City, UT, 84108, USA*
[e] *New Jersey Center for Biomaterials, Laboratory for Drug Delivery Center, 111 Lock Street, Newark, NJ 07103, USA*

Received 26 July 2000; received in revised form 7 December 2000; accepted 11 December 2000

Abstract

Four model drugs were selected based on their lipophilicity denoted as log P (nicardipine hydrochloride log P -0.99 ± 0.1, hydrocortisone log P 1.43 ± 0.47, carbamazepine log P 2.67 ± 0.38, and tamoxifen log P 7.87 ± 0.75). The enhancing activities of four terpene enhancers (fenchone log P 2.13 ± 0.30, thymol log P 3.28 ± 0.20, D-limonene log P 4.58 ± 0.23, and nerolidol log P 5.36 ± 0.38) were tested in vitro across full thickness hairless mouse skin with each of the evaluated drugs formulated in hydroxypropyl cellulose gel formulations. The relationships between lipophilicity (log P) of the terpene enhancers and model drugs and efficacy (represented by the enhancement ratio of flux ER_{flux}) of the drugs when coadministered with the enhancers were examined using linear regression. Terpene enhancers had significant effect on the percutaneous permeation of the model drugs. Nerolidol (highest lipophilicity) provided the highest increase in the flux of the evaluated model drugs. The flux of nicardipine hydrochloride increased by approximately 135-fold, hydrocortisone by 33-fold, carbamazepine 8-fold, and tamoxifen 2-fold. The lowest increase in the flux was observed with fenchone. Linear relationships were generated between the ER_{flux} of nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen and the log P of the terpene enhancers [$r = 0.951$, ($P = 0.049$), $r = 0.977$, ($P = 0.023$), $r = 0.942$, ($P = 0.057$), and $r = 0.874$, ($P = 0.126$), respectively]. Furthermore, the four terpene enhancers produced linear relationships, indicating that they were more effective at enhancing the penetration of hydrophilic drugs rather than lipophilic drugs $r = -0.824$ ($P = 0.176$) for fenchone, $r = -0.891$ ($P = 0.109$) for thymol, $r = -0.846$ ($P = 0.154$) for limonene, and $r = -0.769$ ($P = 0.232$) for nerolidol. © 2001 Published by Elsevier Science B.V.

1. Introduction

Skin, the largest human body organ, envelops and protects the body from the external environ-

* Corresponding author. Tel.: +1-973-9729720; fax: +1-973-3535336.
E-mail address: bmichniak@earthlink.net (B.B. Michniak).

0378-5173/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0378-5173(00)00699-2

ment while helping maintain the integrity and appropriate function of the complex inner body organs. The utilization of the transdermal drug delivery is associated with various advantages that have been extensively discussed in the literature. This route allows for controlled release of the drug at rates approaching zero-order simulating those provided by IV infusion. It also delivers the medication in a continuous input, which is important for drugs that have short biological half-lives and low therapeutic indices. Once the drug is absorbed through the skin, the first-pass intestinal and hepatic metabolism is bypassed. As a consequence of all these advantages, patient compliance is improved.

Despite the obvious advantages of transdermal drug delivery, this route presents unique challenges. The greatest obstacle is the stratum corneum, the outermost layer of the skin, which is considered to form the primary rate-limiting barrier to the permeation of the drugs across the skin (Zettersten et al., 1997). It consists of dead, flattened cells, filled with keratin, that are embedded in a lipid matrix (Bouwstra et al., 1999; Coderch et al., 1999). Lipids in the intercellular spaces are made of fatty acids, ceramides, and cholesterol (esters) and are arranged in bilayer structures, which run mainly parallel to the skin surface (Elias, 1996a,b). The stratum corneum has been described as hydrophilic protein 'bricks' in a hydrophobic mortar (Heisig et al., 1996). The search for solutions to this problem led investigators to employ several enhancement techniques such as iontophoresis, electroporation (Banga et al., 1999), or the application of drug eutectic mixtures (Kaplun-Frischoff and Touitou, 1997; Stott et al., 1998). However, the most widely implemented technique is the use of chemical penetration enhancers, which reversibly perturb the permeability barrier of the stratum corneum. Examples include many compounds such as Azone® and its analogues (Michniak, 1993; Michniak et al., 1993, 1994a,b), fatty acids (Aungst, 1989), alcohols (Takahashi et al., 1991), pyrrolidones (Southwell and Barry, 1983), sulfoxides (Choi et al., 1991), and terpenes (Williams and Barry, 1990; Cornwell and Barry, 1991; El-Kattan et al., 2000a,b). However, the skin has a special role as a major barrier in protecting a living body from cutaneous exposure to toxic chemicals, hence the safety of permeation enhancers is of primary consideration. Terpenes are of low cutaneous irritancy, possess good toxicological profile, provide excellent enhancement ability, and appear to be promising candidates for pharmaceutical formulations (Gao and Singh, 1998). A variety of terpenes have been shown to increase the percutaneous absorption of both hydrophilic (Zhao and Singh, 1999) and lipophilic drugs (Gao and Singh, 1998). Differential scanning calorimetery (DSC) studies showed that cyclic monoterpenes decreased the transition temperature associated with the stratum corneum lipids (Yamane et al., 1995). The DSC results support the speculation that terpene enhancers mainly increase drug diffusivity in the skin by disrupting the highly ordered intercellular lipid structure of the stratum corneum. Furthermore, this interaction with the stratum corneum lipids is reversible.

In this study, hydroxypropyl cellulose (HPC), which is a hydrophobic nonionic polymer, was incorporated in the gel formulations. HPC has several useful properties that render it widely used in topical and transdermal formulations. It is used as an emulsifier, suspending, and stabilizing agent for topical preparations (Wu et al., 1998). Furthermore, it can inhibit the formation of sediments (Wade and Weller, 1995).

The purpose of the present study was to investigate if a correlation exists between the efficacy of the terpene enhancers with different lipophilicity (denoted as log P) (fenchone log P 2.13 ± 0.30, thymol log P 3.28 ± 0.20, D-limonene log P 4.58 ± 0.23, and nerolidol log P 5.36 ± 0.38) and the log P of the model drugs (nicardipine hydrochloride log P -0.99 ± 0.1, hydrocortisone log P 1.43 ± 0.47, carbamazepine log P 2.67 ± 0.38, and tamoxifen log P 7.87 ± 0.75).

2. Methods

2.1. Materials

Fenchone, thymol, D-limonene, and nerolidol were all purchased from Aldrich Chemical Co.

(Milwaukee, WI). Nicardipine hydrochloride, hydrocortisone, carbamazepine, tamoxifen, potassium phosphate monobasic, and triethanolamine were supplied by Sigma Chemical Co. (St. Louis, MO). Acetonitrile, methanol, and water used were of HPLC grade and supplied by EM Science (New Briggs, NJ). Ethyl alcohol (USP) was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY). HPC and glycerol were provided by Spectrum Chemical Manufacturing Corp. (Gardena, CA). Female hairless mice strain SKH1 (h/h), 6–8 weeks old were obtained from Charles River Lab., Inc., (Wilmington, MA). All reagents were of analytical grades and used without further purification.

2.2. Preparation of gel formulations

HPC gels were prepared as described recently by El-Kattan et al. (El-Kattan et al., 2000a,b). The HPC gel composition is shown in Table 1. Briefly, a Sted Fast stirrer was used to mix ethanol with glycerol and distilled water. Then HPC powder was added to the obtained solution with continuous mixing until the gel was formed. The tested drug followed by a terpene enhancer were added to the gel and mixed. The gels were left overnight at ambient temperature. It is worth noting that tamoxifen, carbamazepine, and hydrocortisone were not completely soluble in the prepared gel formulations. Whereas, nicardipine hydrochloride was completely soluble in the gel formulations. Furthermore, all the evaluated terpene enhancers, but limonene were completely soluble in the gel formulations.

Table 1
Composition (% w/w) of drug HPC gel formulations [a]

Component	% w/w
Drug*	2
Terpene	2
HPC	2
Water	28
Glycerol	10
Ethanol	56

[a] * nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen.

2.3. In vitro skin permeability studies

Franz diffusion cells (effective diffusion area = 0.64 cm^2, receptor volume 5.1 ml) were used to evaluate the in vitro drug percutaneous permeation from HPC gel. The receptor compartment was filled with isotonic phosphate buffer (pH 7.2) and 0.1% formaldehyde (37%) as a preservative. The temperature was maintained at 37 ± 0.5°C and the receptor was constantly stirred at 600 rpm. Excised female full-thickness hairless mouse skin (strain SKH1 h/h, 8 weeks old, Charles River Laboratories, Wilmington, MA) was mounted between the donor and receptor compartments. Approximately 300 mg of HPC gel was placed on each skin. All cell donors were occluded with a Parafilm®. A 500-μl sample of the receptor was taken at predetermined time intervals over 24 h and replenished immediately with equal volume of the diffusion buffer. Samples were frozen at −70°C prior to HPLC analysis. After 24 h of sampling, the skins were removed from the cells and washed briefly in methanol (25 ml) for 15 s (Michniak et al., 1994a,b). Following drying at room temperature for 10 min, each skin was cut up and then homogenized in 4-ml methanol. The samples were then centrifuged and the supernatant layer was taken and stored at −70°C before HPLC analysis.

Control gel formulations for each model drug were developed. Control formulations consisted of all the formulation components except the terpene enhancer. All experiments were $n = 5$.

2.4. HPLC analysis of model drugs

All drugs were analyzed using a reverse phase C_{18} column (MICROSORB-MV™, 15 cm, 5 μm) and at a flow rate 1 ml/min. HPLC analysis was performed using Hewlett Packard 1100 with an autosampler equipped with a quaternary pump, a variable-wavelength detector. Nicardipine hydrochloride was detected at 240 nm with a mobile phase composition of 60:40 acetonitrile:0.02 M potassium phosphate monobasic and injection volume of 20 μl (Kobayashi et al., 1993). Hydrocortisone was detected at 242 nm with a mobile phase composition of 40:60 acetonitrile:water and

Table 2
Effect of terpene enhancers on the percutaneous parameters of nicardipine hydrochloride formulated in HPC gels [a]

Terpene	Flux (μg/cm^2 per h)	ER_{flux}	Q_{24} (μg/cm^2)	SC (μg/g)
Control	2.5 ± 1.0	1.0	45 ± 5	4305 ± 400
Fenchone	44.8 ± 5.0	17.9	793 ± 19	13 359 ± 1023
Thymol	45.5 ± 6.2	18.2	811 ± 62	9803 ± 845
Limonene	150.0 ± 20.0	60.0	3304 ± 350	21 483 ± 4532
Nerolidol	337.2 ± 31.2	134.8	6105 ± 590	18 046 ± 3425

[a] Mean ± S.D., $n = 5$. ER_{flux}, Enhancement ratio of nicardipine HCl flux; Q_{24}, cumulative amount of nicardipine HCl in the receptor after 24 h; SC, skin content of nicardipine HCl after 24 h.

injection volume of 40 μl (Michniak, 1993). Carbamazepine was detected at 210 nm with a mobile phase composition of acetonitrile:water (30:70) and injection volume of 20 μl (Psillakis et al., 1999). Tamoxifen was detected at 275 nm with a mobile phase composition of water:triethanolamine:methanol and injection volume of 100 μl (70:1.8:928) (Herrlinger et al., 1992).

2.5. Data analysis

The in vitro skin permeation data obtained was graphically plotted as the cumulative corrected amount of drug penetrated into the receptor phase as a function of time. The permeation profiles provided the following parameters: The slope of the straight line portions of this plot (at steady state) yielded the values of flux (μg/cm^2 per h) and the cumulative corrected receptor concentrations at 24 h Q_{24} (μg/cm^2). Skin content values were expressed as μg of drug per gram of hydrated full-thickness mouse skin.

Log P values of terpenes enhancers and drugs were determined using ACD software program (Advanced Chemistry Incorporated, Ontario, Canada).

Enhancement ratios for flux (ER_{flux}) were calculated using the following equation

$$ER_{flux} = \frac{\text{Model drug flux with terpene in gel}}{\text{Model drug flux without terpene in gel (control)}} \quad (1)$$

Controls were assigned the value of 1.00 and the data was presented as mean ± S.D. of five experiments. Statistical analyses were performed using one-way analysis of variance (one way ANOVA). 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.

3. Results

3.1. Percutaneous absorption of nicardipine hydrochloride in vitro

The effects of terpene enhancers on the percutaneous permeation parameters of nicardipine hydrochloride (flux, ER_{flux}, cumulative amount of nicardipine hydrochloride after 24 h (Q_{24}), and skin content) from HPC gel formulations are shown in Table 2. Controls consisted of gel formulations with no terpene enhancers. The values for the percutaneous permeation parameters of nicardipine hydrochloride control gels are as follows 2.5 ± 1.0 μg/cm^2 per h for flux, 45 ± 5 μg/cm^2 for Q_{24}, and 4305 ± 400 μg/g for nicardipine hydrochloride skin content. All the evaluated terpene enhancers had significant effects on the percutaneous permeation of nicardipine hydrochloride relative to the control. Nerolidol provided the best enhancement activity for nicardipine hydrochloride. It increased nicardipine hydrochloride flux 134.8-fold relative to the control ($P = 0.0000042$) followed by limonene with 60.0-fold ($P = 0.00021$), thymol 18.2 fold ($P = 0.000244$), and fenchone 17.9-fold ($P = 0.00013$). The highest increase in the Q_{24} was also provided by nerolidol (6105 ± 590 μg/cm^2) fol-

lowed by limonene (3304 ± 350 μg/cm^2), thymol (811 ± 62 μg/cm^2), and fenchone (793 ± 19 μg/cm^2). It should be emphasized that the increase in the Q_{24} provided by nerolidol, limonene, thymol, and fenchone was significantly higher than that recorded with the control ($P = 4 \times 10^{-6}$, 4×10^{-6}, 0.00011, and 0.000063, respectively). The highest nicardipine hydrochloride skin content was observed with limonene (21 483 ± 4532 μg/g) followed by nerolidol (18 046 ± 3425 μg/g), fenchone (13 359 ± 1023 μg/g), and thymol (9803 ± 845 μg/g), which were significantly higher than that provided by the control ($P = 0.0027$, 0.0025, 0.0014, and 0.00090, respectively).

3.2. Percutaneous absorption of hydrocortisone in vitro

The effects of terpene enhancers on the percutaneous permeation parameters of hydrocortisone (flux, ER_{flux}, cumulative amount of hydrocortisone after 24 h (Q_{24}), and skin content) from HPC gel formulations are presented in Table 3. Control values for hydrocortisone were determined to be 6.0 ± 1.5 μg/cm^2 per h for flux, 145 ± 6 μg/cm^2 for Q_{24}, and 8052 ± 290 μg/g for hydrocortisone skin content. All tested terpene enhancers had significant effects on both hydrocortisone flux and Q_{24} relative to the control. The highest increase in the flux was observed with nerolidol, which increased the flux by 32.7-fold relative to the control ($P = 0.0011$) followed by limonene with 28.0-fold ($P = 0.00071$), thymol 10.5-fold ($P = 0.00088$), and fenchone with 7.8 fold ($P = 0.000169$). Similar to the ER_{flux} results, nerolidol provided the highest increase in the Q_{24} (1837 ± 106 μg/cm^2) that was significantly higher than that observed with the control ($P = 8 \times 10^{-6}$) followed by limonene (1569 ± 232 μg/cm^2) ($P = 0.00095$) and thymol (1158 ± 168 μg/cm^2) ($P = 0.00059$). The lowest increase in the Q_{24} was recorded with fenchone (1017 ± 148 μg/cm^2) yet it was significantly higher than that observed with the control ($P = 0.00094$). Terpene enhancers did not increase the hydrocortisone skin content values significantly relative to the control. All the terpene enhancers, with the exception of fenchone (10 611 ± 3139 μg/g) had lower skin content values relative to the control.

3.3. Percutaneous absorption of carbamazepine in vitro

The effects of terpene enhancers on the percutaneous permeation parameters of carbamazepine (flux, ER_{flux}, cumulative amount of carbamazepine after 24 h (Q_{24}), and skin content) from HPC gel formulations are presented in Table 4. The control percutaneous permeation parameters for carbamazepine were 24.6 ± 4.5 μg/cm^2 per h for flux, 680 ± 123 μg/cm^2 for Q_{24}, and 3873 ± 644 μg/g for carbamazepine skin content. It is interesting to note that all the terpene enhancers, with the exception of fenchone had major effects on the carbamazepine flux and Q_{24} relative to the control. Nerolidol provided the highest increase in the carbamazepine flux relative to the control. It increased the flux by 7.5-fold ($P = 0.00041$) followed by limonene with 6.6-fold ($P = 0.00011$), thymol 4.2-fold ($P = 0.00077$), and fenchone with 1.5-fold ($P = 0.049$). Similar to the ER_{flux} results, nerolidol provided the highest increase in the Q_{24} (1283 ± 251 μg/cm^2) relative to the control ($P =$

Table 3
Effect of terpene enhancers on the percutaneous parameters of hydrocortisone formulated in HPC gels [a]

Terpene	Flux (μg/cm^2 per h)	ER_{flux}	Q_{24} (μg/cm^2)	SC (μg/g)
Control	6.0 ± 1.5	1.0	145 ± 8	8052 ± 290
Fenchone	47.2 ± 4.9	7.8	1017 ± 148	10 611 ± 3139
Thymol	63.4 ± 10.6	10.5	1158 ± 168	6640 ± 2015
Limonene	168.9 ± 29.3	28.0	1569 ± 232	8342 ± 1250
Nerolidol	196.5 ± 39.0	32.7	1837 ± 106	4603 ± 1421

[a] Mean ± S.D., $n = 5$. ER_{flux}, enhancement ratio of hydrocortisone flux; Q_{24}, cumulative amount of hydrocortisone in the receptor after 24 h; SC, skin content of hydrocortisone after 24 h.

Table 4
Effect of terpene enhancers on the percutaneous parameters of carbamazepine formulated in HPC gels [a]

Terpene	Flux (μg/cm^2 per h)	ER_{flux}	Q_{24} (μg/cm^2)	SC (μg/g)
Control	24.6 ± 4.5	1.0	680 ± 123	3873 ± 644
Fenchone	37.8 ± 6.4	1.5	773 ± 123	1686 ± 525
Thymol	103.9 ± 13.2	4.2	994 ± 79	7840 ± 2012
Limonene	162.5 ± 16.97	6.6	1121 ± 48	16 742 ± 1531
Nerolidol	185.1 ± 25.0	7.5	1283 ± 251	34 910 ± 9924

[a] Mean ± S.D., $n = 5$. ER_{flux}, enhancement ratio of carbamazepine flux; Q_{24}, cumulative amount of carbamazepine in the receptor after 24 h; SC, skin content of carbamazepine after 24 h.

0.020) followed by limonene (1121 + 48 μg/cm^2) ($P = 0.0041$) and thymol (994 ± 79 μg/cm^2) ($P = 0.020$). The lowest increase in the Q_{24} was provided by fenchone (773 ± 123 μg/cm^2), which was not significantly different from the control ($P = 0.41$). It is interesting to note that nerolidol, limonene, and thymol significantly increased the carbamazepine skin contents relative to the control ($P = 0.0058$, 0.00016, and 0.032, respectively). However, the carbamazepine skin content recorded with fenchone (1686 ± 525 μg/g) was significantly lower than that provided by the control ($P = 0.0081$).

3.4. Percutaneous absorption of tamoxifen in vitro

The effects of terpene enhancers on the percutaneous permeation parameters of tamoxifen (flux, ER_{flux}, cumulative amount of tamoxifen after 24 h (Q_{24}), and skin content) from HPC gel formulations are shown in Table 5. Controls consisted of gel formulations with no terpene enhancer and the values for the percutaneous permeation of tamoxifen control gel were found to be 2.8 ± 1.0 μg/cm^2 per h for flux, 52 ± 25 μg/cm^2 for Q_{24}, and 3584 ± 1642 μg/g for tamoxifen skin content. Unlike the results found with other model drugs, terpene enhancers did not have major effects on the percutaneous permeation parameters of tamoxifen relative to the control. However, nerolidol provided the best enhancement activity for tamoxifen. It increased the flux 1.7-fold relative to the control followed by limonene with 1.6-fold, and thymol 1.4-fold. Unlike other terpenes, fenchone decreased the flux 0.6-fold relative to the control. Nerolidol also provided the highest increase in the Q_{24}. It increased Q_{24} to 82 ± 13 μg/cm^2 followed by limonene (81 ± 25 μg/cm^2), thymol (74 ± 55 μg/cm^2), and fenchone (57 ± 15 μg/cm^2). It should be emphasized that the obtained Q_{24} results with nerolidol, limonene, thymol, and fenchone were not significantly higher than that provided by the control ($P = 0.12$, 0.21, 0.51, and 0.78, respectively). The highest tamoxifen skin content was observed with limonene (7140 ± 1932 μg/g) followed by thymol (7115 ± 3600 μg/g), fenchone (5773 ± 1000 μg/g), and nerolidol (5532 ± 1431 μg/g). It should be emphasized that limonene, thymol, fenchone, and nerolidol did not increase tamoxifen skin contents significantly relative to the control ($P = 0.065$, 0.196, 0.117, and 0.208, respectively).

3.5. Correlation of terpene efficacy with log P values of model drugs

In this study, the effect of terpene enhancers on the percutaneous permeation of model drugs with different lipophilicity as denoted by their log P values were investigated. Correlation coefficients were established between the lipophilicties of the model drugs and the ER_{flux} and between the terpene enhancers lipophilicities and the ER_{flux}.

Fig. 1 presents the relationships between the lipophilicities of the model drugs (nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen) and their ER_{flux} with the terpene enhancers. The findings suggest that the increase in the lipophilicities of the model drug is associated with a decrease in the percutaneous permeation of the drug. The relationships for the four terpene enhancers were found to be linear [$r = -0.824$,

($P = 0.176$) for fenchone, $r = -0.891$, ($P = 0.109$) for thymol, $r = -0.846$, ($P = 0.154$) for limonene, and $r = -0.769$, ($P = 0.232$) for nerolidol].

Fig. 2 represents the relationships between the lipophilicities of the terpene enhancers (fenchone, thymol, limonene, and nerolidol) and the ER_{flux} of the tested model drugs. It can be suggested that the increase in the lipophilicities of the terpene enhancers is associated with an increase in the percutaneous permeation of the drug. The relationships for the five model drugs were found to be linear [$r = 0.951$, ($P = 0.049$) for nicardipine hydrochloride, $r = 0.977$, ($P = 0.023$) for hydrocortisone, $r = 0.942$, ($P = 0.057$) for carbamazepine, and $r = 0.874$, ($P = 0.126$) for tamoxifen].

4. Discussion

Terpenes were reported to be effective penetration enhancers for both hydrophilic and lipophilic drugs (Cornwell and Barry, 1991; Hori et al., 1991; Gao and Singh, 1998; Zhao and Singh, 1998). In this study, the tested terpene enhancers provided significant enhancements for the flux and cumulative amounts of the evaluated model drugs at 24 h (Q_{24}). Nerolidol in particular was the most effective terpene enhancer in promoting the permeation of all the model drugs followed by limonene and thymol. Fenchone provided the lowest increase in the flux for all the tested model drugs. The obtained results are similar to those reported with other research groups. In 1991, Cornwell et al. evaluated the effect of terpene enhancers on the percutaneous permeation of 5-fluorouracil across the skin. They reported that nerolidol provided the highest enhancement for the permeation of 5-fluorouracil (Cornwell and Barry, 1991). Furthermore, the high enhancement activity of nerolidol was reported by Arellano et al., who found that nerolidol was an effective enhancer for the permeation of diclofenac sodium across the rat skin (Arellano et al., 1996). The effective promoting activity of nerolidol was attributed to its amphiphilic structure that was suitable for alignment within the lipid lamellae of the stratum corneum and disrupting its highly organized packing (Cornwell and Barry, 1994).

It is worth noting that limonene provided higher enhancing activity for the permeation of nicardipine hydrochloride (hydrophilic calcium channel blocker log P -0.99 ± 0.1) and hydrocortisone (a polar steroid with a log P of 1.43 ± 0.25) relative to fenchone and thymol. These findings conflict with the results observed by other authors. It has been recognized that hydrophilic terpenes capable of hydrogen binding (such as fenchone and thymol) are more active towards promoting the permeation of hydrophilic drugs, whereas, hydrocarbon terpenes (such as limonene) provide higher enhancing activity for lipophilic drugs (Hori et al., 1991; Moghimi et al., 1997). In 1992, Katayama et al. studied the effect of L-menthol on the skin permeability of mannitol, cortisone or indomethacin using hairless mouse skin in vitro (Katayama et al., 1992). They observed that when the donor solution contained L-menthol in an aqueous ethanol vehicle at pH 7.4 the permeability coefficients of mannitol and indomethacin were increased by 100-fold relative to that recorded with the control (an aqueous vehicle)

Table 5
Effect of terpene enhancers on the percutaneous parameters of tamoxifen formulated in HPC gels [a]

Terpene	Flux (μg/cm^2 per h)	ER_{flux}	Q_{24} (μg/cm^2)	SC (μg/g)
Control	2.8 ± 1.0	1.0	52 ± 25	3584 ± 1642
Fenchone	1.8 ± 0.8	0.6	57 ± 15	5773 ± 1000
Thymol	3.9 ± 1.5	1.4	74 ± 55	7115 ± 3600
Limonene	4.5 ± 1.3	1.6	81 ± 25	7140 ± 1935
Nerolidol	4.8 ± 0.7	1.7	82 ± 13	5532 ± 1431

[a] Mean ± S.D., $n = 5$. ER_{flux}, enhancement ratio of tamoxifen flux; Q_{24}, cumulative amount of tamoxifen in the receptor after 24 h; SC, skin content of tamoxifen after 24 h.

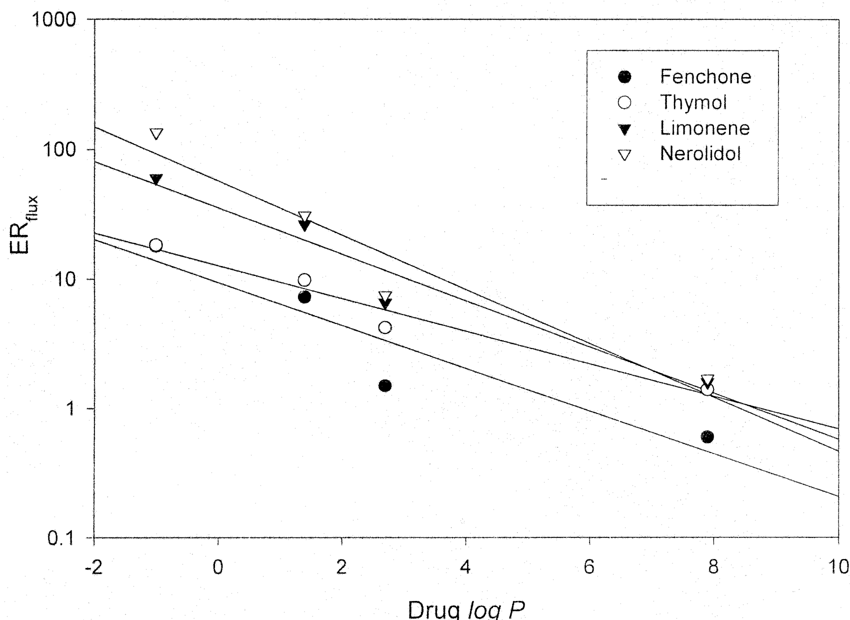

Fig. 1. The relationship between the log P of model drugs and the ER_{flux} of the evaluated model drugs (nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen), using fenchone, thymol, limonene, and nerolidol as penetration enhancers.

and increased those of cortisone by about 10-fold. However, L-menthol did not increase the permeation of indomethacin at pH 3.0, since the majority of the species would be in the unionized form.

The effects of terpenes on the permeation of propranolol hydrochloride (hydrophilic drug) and diazepam (lipophilic drug) were evaluated by Hori et al. The purely hydrocarbon terpenes promoted both propranolol and diazepam permeation (Hori et al., 1991). Whereas, the terpenes with hydrogen-bonding ability only enhanced the flux of propranolol.

The data in our study can be explained by the following information. The higher enhancement activity of limonene relative to thymol and fenchone can be attributed to its higher thermodynamic activity in the gel since limonene was not completely soluble in the gel at 2% concentration. On the contrary, thymol and fenchone were found to be completely soluble in the gel at the evaluated concentration. Similar results were also reported by Obata et al., who attributed the higher enhancing activity of the permeation of diclofenac by limonene to its higher thermodynamic activity in the 40% ethanol-buffer solution at the evaluated concentration relative to L-menthol (Obata et al., 1993).

The lipophilicity of the permeant, as well as the enhancer molecule is thought to play an important role in determining the enhancers promoting activity on the permeation of the drug across the skin (Okabe et al., 1990; Hori et al., 1991; Lee et al., 1993, 1994; Phillips and Michniak, 1995; Borras-Blasco et al., 1997; Godwin and Michniak, 1999; El-Kattan et al., 2000a,b; Sung et al., 2000).

In the present study, terpene enhancers in combination with the gel solvent mixtures exhibited high enhancement for flux and Q_{24} of the tested model drugs. The enhancers produced the highest activity for the most hydrophilic drug i.e. nicardipine hydrochloride followed by hydrocortisone, and carbamazepine. The lowest activity of the enhancers was recorded with the most lipophilic compound i.e. tamoxifen. Terpene enhancers did not increase tamoxifen flux and Q_{24} significantly relative to the control (Table 5). The results were anticipated, since other studies have demonstrated the significant effect of model drug

lipophilicity on the penetration enhancing properties of terpenes (Kitagawa et al., 1998). Guy et al. reported that compounds with log P of more than 2 may have potential problems in achieving steady plasma concentrations in reasonable time spans due to the fact that the drug is delayed in the stratum corneum where a reservoir may be established (Guy and Hadgraft, 1989). Kitagawa et al. evaluated the effect of L-menthol in 15% ethanol on the permeation of paraben analogues through excised guinea pig dorsal skin. They found that the permeability coefficients of the parabens correlated with their lipophilicity (log P). Addition of 1% L-menthol in 15% ethanol increased the permeability coefficient of methyl paraben 16-fold, whereas this enhancer decreased that of butyl paraben to about one fifth of the control value (Kitagawa et al., 1997).

In our study, significant linear correlations were established between the ER_{flux} and the log P of the tested model drugs for the four tested terpene enhancers [$r = -0.824$, ($P = 0.176$) for fenchone, $r = -0.891$, ($P = 0.109$) for thymol, $r = -0.846$, ($P = 0.154$) for limonene, and $r = -0.769$, ($P = 0.232$) for nerolidol (Fig. 1)].

In the present investigation, the lipophilicity denoted as log P of the terpene enhancers greatly influenced the enhancement ratios of the evaluated model drugs at log P ranges of 2.13–5.36 and strong correlation coefficients were established between the ER_{flux} of nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen and the log P of the terpene enhancers [$r = 0.951$, ($P = 0.049$), $r = 0.977$, ($P = 0.023$), $r = 0.942$, ($P = 0.057$), and $r = 0.874$, ($P = 0.126$), respectively, (Fig. 2)]. These results are in a good agreement with earlier findings reported by Williams et al., who found linear relationships between the lipophilicity of the epoxide and ketone terpenes and the enhancement ratio for 5-fluorouracil permeation (Williams and Barry, 1991). The mechanism of permeation enhancement of the terpene enhancers has been evaluated using differential scanning calorimetery, Fourier transform infrared, and X-ray diffraction. These studies suggested that terpenes enhance the permeation of the drug across the skin mainly by disrupting the highly ordered intercellular packing of the stratum corneum lipids and increasing drug diffusivity (Williams and Barry, 1991; Cornwell et

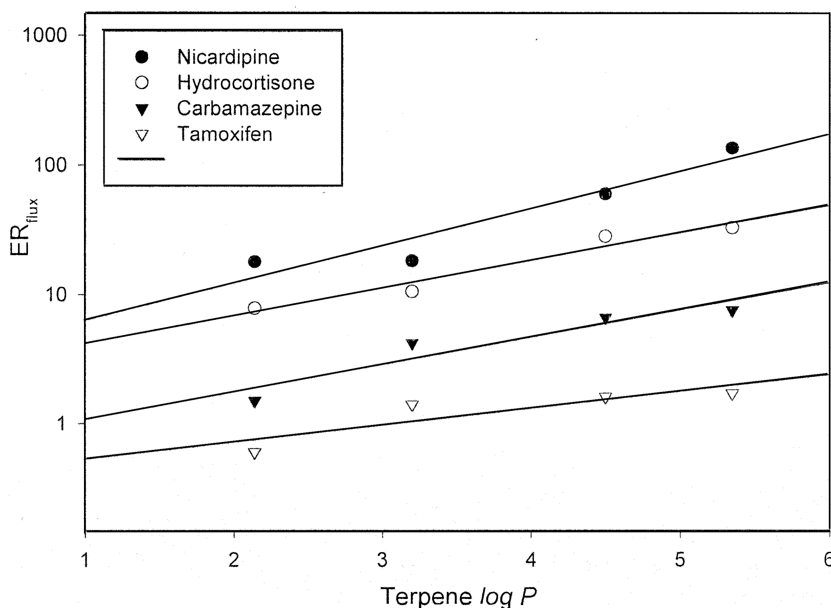

Fig. 2. The relationship between the log P of the terpene enhancers and the ER_{flux} of the evaluated model drugs (nicardipine hydrochloride, hydrocortisone, carbamazepine, and tamoxifen), using female hairless mouse skin.

al., 1994). Taking these finding into consideration, it can be speculated that the increase in the lipophilicity of the terpenes may increase their ability to disrupt the highly hydrophobic lipid lamella of the stratum corneum.

5. Conclusions

In conclusion, terpene enhancer lipophilicity had a significant effect on the percutaneous permeation of the model drugs tested. Increasing the log *P* of the terpene enhancers was associated with an increase in drug permeation. Nerolidol was the most effective terpene enhancer for the permeation of the drugs followed by limonene and thymol. Fenchone was the least effective terpene enhancer. However, the higher enhancing activity of limonene relative to fenchone and thymol was unexpected and attributed to its higher thermodynamic activity in the gel. Unlike fenchone and thymol, limonene was not completely soluble in the gel at 2% concentration. This may suggest the importance of the gel formulation vehicle compositions on the enhancing activity of the terpene enhancers. Furthermore, model drug lipophilicity had a significant impact on the terpene enhancers promoting activity. Increasing the lipophilicity of the model, drug was associated with a decrease in the enhancement activity of the terpenes. Terpene enhancers provided the highest enhancement activity for nicardipine hydrochloride, which was the most hydrophilic compound. The lowest enhancement activity was provided for tamoxifen, which was the most lipophilic compound. It should be stressed that the obtained results were in agreement with earlier findings of Kitagawa et al., who reported that the addition of 1% L-menthol in 15% ethanol increased the permeability coefficient of methyl paraben 16-fold, whereas this enhancer decreased that of butyl paraben to about one fifth of the control value (Kitagawa et al., 1997).

Further work needs to be conducted using additional drugs and other series of enhancer compounds.

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