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RESEARCH PAPER

Influence of Drug Lipophilicity on Terpenes as Transdermal Penetration Enhancers

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ABSTRACT

*Percutaneous absorption-enhancing effects on the skin of hairless mice of 11 monoterpenes [**1**, (+)-limonene; **2**, (−)-menthone; **3**, (+)-terpinen-4-ol; **4**, α-terpineol; **5**, 1,8-cineole; **6**, (+)-carvone; **7**, (−)-verbenone; **8**, (−)-fenchone; **9**, p-cymene; **10**, (+)-neomenthol; and **11**, geraniol] were investigated using three different model drugs (caffeine, hydrocortisone, triamcinolone acetonide [TA]) with varying lipophilicities. Terpenes were applied at 0.4 M in propylene glycol (PG) to mouse skin. The model drugs were applied as suspensions in PG 1 hr following enhancer pretreatment. The combination of terpenes in PG provided significant enhancement of the permeation of caffeine through mouse skin. The most active compounds **10** and **11** increased permeation by between 13-fold and 16-fold. The terpenes also enhanced the delivery of hydrocortisone, but not to as great an extent. The most active compounds **3** and **4** increased permeation between 3.9-fold and 5-fold. The compounds examined did not significantly increase the delivery of TA. The most active compound **4** only increased delivery 2.5-fold, while the next most active compound **6** only increased delivery 1.7-fold. Overall, these results indicate that the combination of terpenes with PG can significantly increase the transdermal penetration of the hydrophilic drug caffeine and the polar steroid hydrocortisone.*

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INTRODUCTION

Due to its accessibility, skin has long been considered as a potential route for systemic drug administration, and research into transdermal drug delivery has expanded greatly over the last two decades. Transdermal drug delivery possesses several advantages over more traditional methods. These include (*a*) avoidance of first-pass intestinal and hepatic metabolism; (*b*) avoidance of variable rates of absorption and metabolism inherent with oral administration; (*c*) continuous, noninvasive infusion of drugs having short biological half-lives; (*d*) avoidance of the risks and the inconvenience associated with parenteral treatment; and (*e*) elimination of gastrointestinal irritation resulting from pharmaceutically active and inactive ingredients. Unfortunately, only a few drugs possess the physicochemical properties necessary for delivery in quantities required for successful systemic therapy (1).

The main barrier to drug penetration through the skin is the topmost layer of the skin, the stratum corneum. The stratum corneum consists of protein-filled cells surrounded by lipid lamellar sheets in a brick and mortar wall-like configuration (2). Several methods have been employed to lessen the barrier function of the skin. One popular approach has been the use of transdermal penetration enhancers. These agents are chemical compounds that reversibly alter the barrier function of the skin and allow an increased rate of percutaneous permeation of coadministered drugs. Although the exact mechanism of action of enhancers has not been elucidated fully, it is known that they exert their effects partially by altering either the stratum corneum proteins or lipids or both (1).

A number of compounds, such as sulfoxides, pyrrolidinones, fatty acids, and alcohols, have been evaluated as penetration enhancers (3–6). The selection of an enhancer for a transdermal product should be based on its efficacy, lack of toxicity, and compatibility with other components of the transdermal system (7). An ideal enhancer should be pharmacologically inert, odorless, colorless, nontoxic, nonirritating, nonallergenic, and compatible with most drugs and excipients (8). In addition, the onset and duration of the enhancer effect should be predictable, reproducible, and reversible (7).

Many effective enhancers have not yet been adopted due to concerns regarding their systemic and localized toxicity (9). The use of natural products as enhancers may avoid some of these problems. Terpenes are constituents of essential oils, which are the volatile and fragrant substances found mainly in flowers, fruits, and the leaves of plants. These compounds have been used as flavorings, perfumes, and medicines. Recently, these compounds have been shown to be effective enhancers for a number of hydrophilic and lipophilic drugs, including diclofenac sodium, ketoprofen, indomethacin, and 5-fluorouracil (5-FU) (10–13). Terpenes are a series of naturally occurring compounds that consist of isoprene (C_5H_8) units that are highly lipophilic and have large partition coefficients between octanol and water (14). In addition to unsaturated hydrocarbons, oxygen-containing compounds such as alcohols, ketones, and oxides have also been found to occur naturally. Terpenes have been given the designation of generally recognized as safe (GRAS) by the FDA (15).

It has been suggested that the mechanism of action of terpenes involves disruption of the intercellular lipids of the stratum corneum. It appears that, for hydrophilic drugs, the primary effect of terpene enhancer treatment is to increase drug diffusivity in the stratum corneum (i.e., to reduce the barrier properties of the skin). For lipophilic drugs, terpenes seem to increase drug diffusivity, but also increase drug partitioning into the stratum corneum. Increases in partitioning are likely due to bulk solvent effects since many lipophilic drugs are moderately soluble in many of the terpenes (16,17).

In the present study, the enhancing effects of naturally occurring terpenes on the in vitro percutaneous absorption of the model drugs caffeine, hydrocortisone, and triamcinolone acetonide (TA) in propylene glycol (PG) were investigated. Terpenes were chosen from the broad chemical classes of hydrocarbons, alcohols, ketones, and oxides.

MATERIALS

The structures of the terpenes used in this study are shown in Fig. 1. All were extra pure reagent grade and were obtained from Aldrich Chemical Company (Milwaukee, WI). Sigma supplied the model drugs hydrocortisone, caffeine, and TA. Baxter Diagnostics, Incorporated (McGraw Park, IL) supplied reagent-grade solvents, except for methanol and acetonitrile, which were high-performance liquid chromatography (HPLC) grades. Male hairless mice, strain SKH1 (h/h), 8 weeks old, were supplied by Charles Rivers Laboratories (Wilmington, MA).

METHODS

In Vitro Permeability Studies

Permeability studies were performed using methods previously described (18). Full-thickness skins were ob-

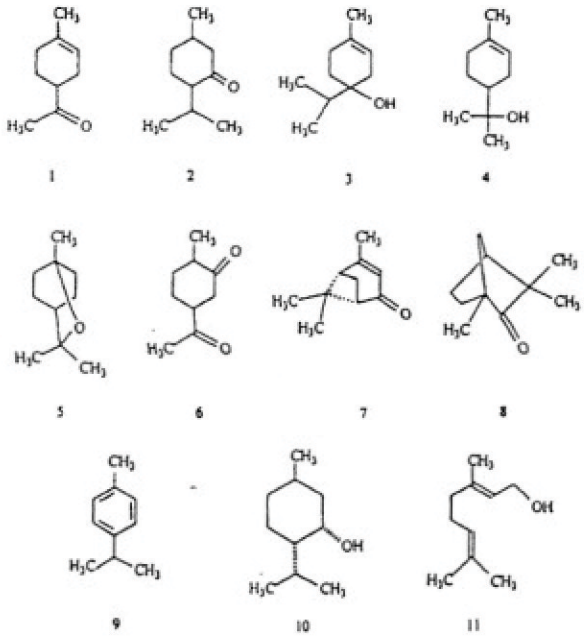

Figure 1. Structures of terpene enhancers: **1**, (+)-limonene; **2**, (−)-menthone; **3**, (+)-terpinen-4-ol; **4**, α-terpineol; **5**, 1,8-cineole; **6**, (+)-carvone; **7**, (−)-verbenone; **8**, (−)-fenchone; **9**, *p*-cymene; **10**, (+)-neomenthol; and **11**, geraniol.

tained from 8-week-old male hairless mice. Animals were sacrificed by CO_2 asphyxiation, and full-thickness abdominal and dorsal skin was excised. Any extraneous subcutaneous fat was removed from the dermal surface. The skins were stored at −80°C (Revco Scientific, Asheville, NC) until utilized. Skins then were thawed slowly, cut into small pieces, and mounted on modified Franz diffusion cells (Permegear, Riegelsville, PA).

Each diffusion cell (donor surface area 0.64 cm^2; receptor volume 5.1 ml) contained isotonic phosphate buffer solution (pH 7.2), 0.1% v/v 36% aqueous formaldehyde as a preservative, and 0.5% w/v polyoxyethylene 20 cetyl ether as a solubilizer (19). The receptors were maintained at 37°C ± 0.5°C by a circulating water bath. The receptors were stirred continuously at 600 rpm using magnetic stirring bars. Skins were allowed to hydrate for 1 hr prior to experimentation.

Following this hydration period, 1.6 µl of enhancer in PG was spread uniformly over each skin. All enhancers were liquids and were applied at a concentration of 0.4 M. The enhancer solution was left on the skin for 1 hr prior to drug application and was not washed off. Control experiments consisted of no pretreatment of the skin with enhancer solution, while PG pretreatment consisted of treating the skin with 1.6 µl of PG alone.

Following pretreatment, 16 µl of a suspension of drug (hydrocortisone, caffeine, or TA) in PG were placed on each skin (18). The drug suspensions were prepared by adding excess drug to PG to create a saturated solution. This solution was shaken gently, and the suspension of drug was administered to the skin. The drug suspension provided a high dose of drug to maintain a concentration gradient across the skin for the duration of the 24-hr experiment. The solubilities of the drugs in PG at 32°C ± 0.5°C were 0.03 M, 0.0043 M, and 0.015 M for hydrocortisone, caffeine, and TA, respectively (20). Following application of drug suspension, the donor compartment was occluded with Parafilm®. Samples (300 µl) of receptor phase were withdrawn at specified time points over 24 hr, with the samples being immediately replaced with fresh buffer. Analysis of samples was corrected for all previous samples removed.

Skin Homogenization

At 24 hr, the skins were removed from the receptor cells and washed three times in 100 ml of methanol for a total of 15 sec. Following room temperature drying, each skin was weighed, cut, placed in 4 ml of methanol, and homogenized using a tissue homogenizer (Biospec Products, Racine, WI). The homogenate was then gravity filtered and passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA); all samples were stored at −80°C until analysis (18).

Sample Analysis

Analysis of skin and receptor samples for drug content was performed using HPLC. All solvents were HPLC grade. The liquid chromatograph consisted of a binary pump solvent delivery system (Model P1500, Thermoseparations Products, Riviera Beach, FL), a 10-µl injection loop autosampler (Model AS 1000, Thermoseparations Products), and a variable-wavelength ultraviolet light absorbance detector (Model UV 1000, Thermoseparations Products). The analytical column was a 5-µm pore size, 4.6 mm × 150 mm Microsorb-MV C_{18} column (Rainin Instrument Company, Woburn, MA), with a guard column of the same material. The system was controlled and integrated by a personal computer running chromatography management software (PC 1000, Thermoseparations Products). Hydrocortisone was detected at 242 nm with a retention time of approximately 4.5 min using a mobile phase of 40:60 acetonitrile:water. Caf-

feine was detected at 270 nm with a retention time of approximately 3.5 min using a mobile phase of 60:40 methanol:water. TA was detected at 240 nm with a retention time of approximately 3.0 min using a mobile phase of 50:50 acetonitrile:water. All flow rates were 1 ml min^{-1}. Testing the linearity of the validation plot from 1.0 to 100 μg ml^{-1} revealed correlation coefficients of 0.9960, 0.9991, and 0.99992 for hydrocortisone, caffeine, and TA, respectively. Intraday and interday variability were also calculated and determined to be 6.6% and 11.4%, 4.4% and 11.5%, and 1.8% and 4.3%, for hydrocortisone, caffeine, and TA, respectively.

Data Analysis

Cumulative amounts of drug (μM) corrected for sample removal were plotted against time (hr). Permeation profiles were calculated from flux (F, μM cm^{-2} hr^{-1}), receptor concentrations at 24 hr (Q_{24}, μM), and skin content of drug (SC, μg g^{-1}). Enhancement ratios (ERs) were calculated from flux, 24-hr receptor concentration (Q_{24}), and skin content of drug (SC) after enhancer treatment of skin divided by the same parameter from control.

$$ER = \frac{\text{Permeation parameter after enhancer treatment}}{\text{Permeation parameter from control}}$$

Data treatment involved the use of analysis of variance (ANOVA), two-tailed Student t test, and a Tukey-Kramer post hoc multiple comparison analysis. The p value was set at .05, and the null hypothesis assumed the variances between enhancer and control(s) to be equal. Therefore, if $p < .05$, there was a significant difference between the enhancer and control(s).

RESULTS

Caffeine

The effects of the most active terpenes are shown in Fig. 2 (compounds **4**, **6**, **7**, and **10**). The flux, 24-hr receptor concentrations, and skin content, along with their enhancement ratios, are presented in Table 1. Control values for caffeine were determined to be 149.87 ± 49.43 μM for receptor concentrations at 24 hr (Q_{24}), 2.41 ± 1.04 μM cm^{-2} hr^{-1} for flux, and 252.3 ± 203.17 μg g^{-1} for skin concentrations. PG pretreatment significantly increased both Q_{24} (278.3 ± 76.5 μM) and flux (21.56 ± 4.12 μM cm^{-2} hr^{-1}). PG pretreatment decreased caffeine content in the skin, but not to a significant amount (146.6 ± 54.9 μg g^{-1}). All terpenes tested significantly

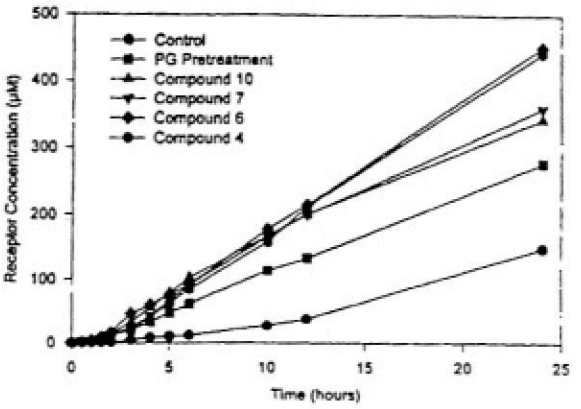

Figure 2. Cumulative amount of caffeine permeated across hairless mouse skin as a function of time. Each point is the mean of four determinations.

increased flux over the control, and all compounds, with the exception of **1**, **2**, and **10**, increased Q_{24} figures. However, only compounds **10** and **11** significantly increased flux over the PG pretreated skins. Fluxes for compounds **10** and **11** were 32.7 ± 4.51 and 37.8 ± 4.84 μM cm^{-2} hr^{-1}, respectively. It was also determined that only two compounds significantly increased Q_{24} for caffeine when compared to PG pretreatment. Compounds **4** and **6** increased receptor concentration to 442.9 ± 46.5 μM and 452.3 ± 68.3 μM, respectively. All other enhancers had no significant increase in Q_{24} values when compared to PG pretreatment. Compounds **1**, **2**, and **9** decreased 24-hr receptor concentrations to 192.5 ± 15.3, 205.0 ± 5.30, and 227.5 ± 57.1 μM, respectively. No enhancer changed the skin content of caffeine in hairless mouse skin to any significant extent versus the control. It was also observed that only one enhancer, compound **6** (374.4 ± 130.9 μg g^{-1}), increased skin content when compared to PG pretreatment despite the decrease in skin content associated with the pretreatment.

Hydrocortisone

The effects of the most active terpenes are shown in Fig. 3 (compounds **3**, **4**, **6**, and **10**). The flux, 24-hr receptor concentrations, and skin content, along with their enhancement ratios, are presented in Table 2. Control values for hydrocortisone were determined to be 9.82 ± 3.11 μM Q_{24}, 0.72 ± 0.24 μM cm^{-2} hr^{-1} for flux, and 44.6 ± 18.4 μg g^{-1} for skin concentrations. PG pretreatment had no significant effect on Q_{24} (9.83 ± 2.46 μM) or flux

Table I

Effect of Propylene Glycol Pretreatment and Enhancers 1–11 on Permeation and Skin Retention of Caffeine (Enhancement Ratios in Parentheses)

Enhancer in PG[a]	Flux ($\mu M\ cm^{-2} hr^{-1}$)	Q_{24} (μM)[b]	SC ($\mu g\ g^{-1}$)[c]
Control	2.41 ± 1.04 (1.0)	149.8 ± 49.43 (1.0)	252.3 ± 203.17 (1.0)
PG pretreatment	21.46 ± 4.12 (8.9)	278.3 ± 76.47 (1.9)	146.6 ± 54.9 (0.58)
1	7.44 ± 1.12 (3.1)	192.5 ± 15.32 (1.3)	465.1 ± 254.8 (1.8)
2	7.92 ± 2.73 (3.3)	205.0 ± 5.30 (1.4)	259.9 ± 86.41 (1.0)
3	21.13 ± 8.3 (8.8)	366.9 ± 62.64 (2.5)	342.3 ± 207.64 (1.4)
4	27.31 ± 2.54 (11.2)	442.9 ± 46.53 (2.9)	245.7 ± 124.9 (0.97)
5	22.88 ± 3.61 (9.5)	315.9 ± 30.37 (2.1)	554.3 ± 352.17 (2.2)
6	29.94 ± 10.61 (12.4)	452.3 ± 68.32 (3.1)	374.3 ± 130.9 (1.5)
7	30.94 ± 8.98 (12.8)	359.5 ± 62.93 (2.4)	124.9 ± 66.77 (0.50)
8	26.60 ± 4.99 (11.0)	394.1 ± 58.27 (2.7)	70.3 ± 26.08 (0.30)
9	20.50 ± 6.23 (8.5)	227.5 ± 57.11 (1.5)	73.71 ± 47.54 (0.28)
10	32.72 ± 4.51 (13.6)	343.4 ± 24.54 (2.3)	186.6 ± 127.5 (0.74)
11	37.77 ± 4.84 (15.7)	321.5 ± 14.71 (2.2)	126.5 ± 25.58 (0.50)

[a] PG, propylene glycol.
[b] Q_{24}, 24-hr receptor concentration.
[c] SC, skin content of caffeine.

($0.69 \pm 0.22\ \mu M\ cm^{-2}\ hr^{-1}$) and decreased skin content to $32.8 \pm 20.2\ \mu g\ g^{-1}$. Four compounds significantly increased the transdermal delivery of hydrocortisone over the control. Compounds **3**, **4**, **7**, and **11** increased Q_{24} to $26.8 \pm 7.97\ \mu M$, $27.7 \pm 9.9\ \mu M$, $25.5 \pm 4.9\ \mu M$, and $15.94 \pm 1.21\ \mu M$, respectively. All other compounds showed nonsignificant increases in 24-hr receptor content or decreased the amount of drug penetrating the skin. Only compounds **3** and **4** showed any significant increases in the flux of hydrocortisone, with values of $2.85 \pm 0.29\ \mu M\ cm^{-2}\ hr^{-1}$ and $4.79 \pm 2.93\ \mu M\ cm^{-2}\ hr^{-1}$, respectively. No terpene enhancers increased or decreased skin content of hydrocortisone to a statistically significant amount. Compounds **2**, **4**, and **9** had the highest skin concentrations of hydrocortisone, with concentrations of 80.9 ± 26.1, 71.4 ± 28.1, and $89.2 \pm 46.5\ \mu g\ g^{-1}$, respectively.

Triamcinolone Acetonide

The effects of the most active terpenes are shown in Fig. 4 (compounds **4**, **6**, **10**, and **11**). The flux, 24-hr receptor concentrations, and skin content, along with their enhancement ratios, are presented in Table 3. TA was generally not well absorbed through the skin. Control values for TA were determined to be only $2.02 \pm 0.33\ \mu M$ for Q_{24} and a flux of $0.23 \pm 0.16\ \mu M\ cm^{-2}\ hr^{-1}$. Skin concentration was $182.4 \pm 0.3\ \mu g\ g^{-1}$. PG pretreatment did increase both Q_{24} ($2.72 \pm 0.81\ \mu M$) and flux ($0.33 \pm 0.1 \mu M\ cm^{-2}\ hr^{-1}$), although neither was significantly higher than the control. PG did, however, significantly decrease the skin content of TA to $27.3 \pm 3.31\ \mu g\ g^{-1}$. All enhancers tested, with the exception of compound **4**, had no significant effects on the penetration of TA through hairless mouse skin. Only compound **4** had a sig-

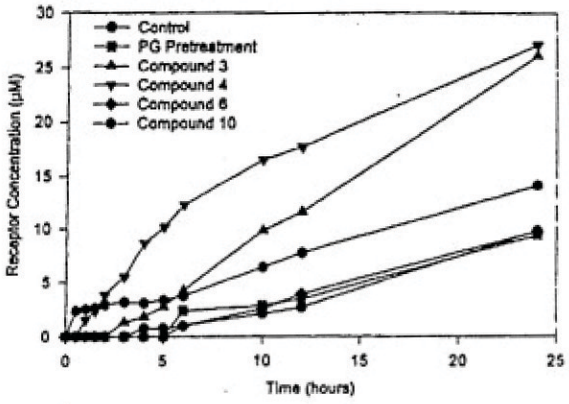

Figure 3. Cumulative amount of hydrocortisone permeated across hairless mouse skin as a function of time. Each point is the mean of four determinations.

nificant increase in flux (3.10 ± 0.28 μM cm^{-2} hr^{-1}) when compared to PG pretreatment control. This significance does not extend to Q_{24}. Terpene enhancers significantly decreased the amount of TA retained in the skin, with the exception of compound **1**. This enhancer decreased skin content from 182.4 ± 90.3 to 101.6 ± 66.7 μg g^{-1}, but this difference was not significant.

DISCUSSION

Terpene enhancers tested showed varying results for each of the model drugs tested. The enhancers exhibited the highest activity for caffeine. Terpenes in combination with PG as the vehicle demonstrated high permeation enhancement ratios for flux and Q_{24}. All compounds tested showed significant enhancement of flux when compared to the control, and all compounds, with the exceptions of compounds **1** and **2** (both hydrocarbon terpenes), showed significant 24-hr enhancement concentration. Compound **11** had the highest ER_{flux} with a value of 15.7. Several other compounds exhibited high ER_{flux} values, with compounds **10**, **7**, **6**, and **4** showing values of 13.6, 12.8, 12.4, and 11.2, respectively. It must be noted that both flux and 24-hr receptor concentrations are given in Table 1 since, for the more active enhancers, especially for caffeine, the donor was depleted faster, and the cumulative amount of drug permeated through the skin plateaued by 24 hr (Fig. 2). The use of this finite-dosing technique was chosen for its similarity to the clinical use of topical agents and because the authors have used it previously to examine enhancer compounds. Flux values were calculated using the early steady-state portion of the time-concentration graph.

Although the terpenes tested did exhibit high enhancement ratios, it must be noted that the use of PG pretreatment also significantly enhanced the percutaneous permeation of caffeine. PG pretreatment showed an ER_{flux} of 8.9 when compared to the control. When subtracting the effect of PG on the penetration of caffeine, only compounds **4**, **10**, and **11** exhibited significant enhancement of flux, and their enhancement values fell dramatically to 1.27, 1.52, and 1.76, respectively.

All of the effective compounds for the enhancement of caffeine penetration were capable of hydrogen bonding (Fig. 1). It has been well established that terpenes capable of hydrogen bonding are more effective penetration enhancers for hydrophilic drugs than for lipophilic drugs. Arellano and coworkers found that alcohol terpenes were most effective for the penetration enhancement of diclofenac sodium. Their most effective enhancer was geraniol (20-fold increase), followed by nerolidol (14-fold increase) and menthol (11-fold increase). *d*-Limonene (hydrocarbon) was shown to have a slight enhancing activity, while ketones were less effective (a decrease of 2-fold to 3-fold) (10).

In another study that looked at diclofenac sodium, Obata et al. (21) examined the effects of compounds *d*-limonene and l-menthol in combination with ethanol on the skin permeation of both un-ionized and ionized diclofenac sodium. The permeability coefficient on ionized and un-ionized drug was dramatically enhanced by a 1-hr pretreatment of *d*-limonene (100-fold and 10-fold, respectively). The present study also employed a 1-hr pretreatment of the skin, although the effects were not as pronounced. The observation that only a 1-hr pretreatment was needed for *d*-limonene to reach maximum enhancement suggests that *d*-limonene distributes or attacks the skin surface very quickly. In comparison, a relatively long pretreatment time was required to obtain a sufficient promoting activity with l-menthol. With this enhancer, permeability coefficients increased proportionally with the extension of the l-menthol pretreatment time.

Other hydrophilic drugs that have been studied in conjunction with terpenes include 5-FU, azidothymidine (AZT), and propranolol. With each of these drugs, it was found that hydrophilic terpenes enhanced their permeation to a greater extent than hydrocarbon terpenes. Gao and Singh reported 5-FU enhancement ratios of 91.62 for carvone, 153.72 for 1,8-cineole, and 273.75 for thymol when using the terpenes in conjunction with a 50% etha-

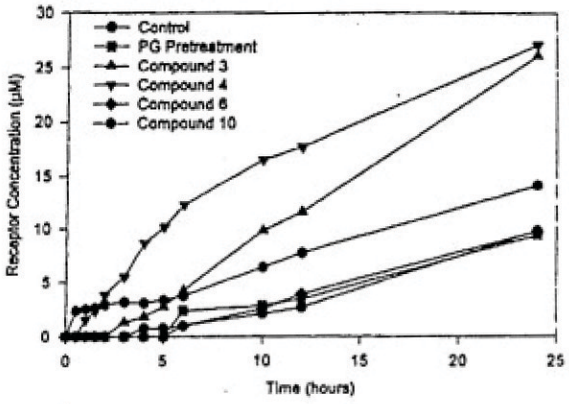

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Table 2

Effect of Propylene Glycol Pretreatment and Enhancers 1–11 on Permeation and Skin Retention of Hydrocortisone (Enhancement Ratios in Parentheses)

Enhancer in PG[a]	Flux ($\mu M\ cm^{-2} hr^{-1}$)	Q_{24} (μM)[b]	SC ($\mu g\ g^{-1}$)[c]
Control	0.72 ± 0.24 (1.0)	9.82 ± 3.11 (1.0)	44.59 ± 18.36 (1.0)
PG pretreatment	0.69 ± 0.22 (0.96)	8.93 ± 2.46 (0.74)	32.80 ± 20.24 (0.74)
1	0.68 ± 0.18 (0.94)	9.00 ± 2.39 (0.92)	30.47 ± 8.64 (0.68)
2	0.81 ± 0.15 (1.1)	12.5 ± 20.9 (1.3)	80.98 ± 26.12 (1.8)
3	2.85 ± 0.29 (3.9)	26.8 ± 7.97 (2.72)	29.76 ± 17.38 (0.67)
4	4.79 ± 2.39 (6.7)	27.5 ± 9.99 (2.8)	71.44 ± 28.06 (1.6)
5	0.30 ± 0.14 (0.42)	7.51 ± 1.78 (0.60)	26.63 ± 1.99 (0.60)
6	1.40 ± 0.59 (1.9)	9.78 ± 3.75 (0.95)	42.58 ± 3.87 (0.95)
7	1.73 ± 0.89 (2.4)	25.52 ± 4.92 (2.6)	42.36 ± 18.08 (0.95)
8	0.51 ± 0.18 (0.70)	6.14 ± 2.18 (0.46)	20.65 ± 4.97 (0.46)
9	0.92 ± 0.34 (1.4)	13.70 ± 4.10 (1.4)	89.19 ± 46.51 (2.0)
10	1.60 ± 0.41 (2.2)	14.10 ± 2.47 (1.4)	60.80 ± 11.77 (1.4)
11	1.82 ± 0.28 (1.0)	15.94 ± 1.21 (1.6)	44.35 ± 9.14 (0.99)

[a] PG, propylene glycol.
[b] Q_{24}, 24-hr receptor concentration.
[c] SC, skin content of hydrocortisone.

nolic vehicle (22). In another study involving the use of 5-FU, Cornwell and Barry found that sesquiterpene compounds with polar head groups were generally more potent enhancers than pure hydrocarbons. In addition, enhancers with the least-branched structures were the most active (23).

In a study of the transdermal penetration properties of AZT (a nonionic, relatively water-soluble compound with a log *P* of 0.02), it was found all enhancers tested (l-menthol, *t*-anethole, carvacol, thymol, and linalool) enhanced transport at least 25-fold (24). Finally, Kunta et al. reported that, at a 1% enhancer concentration, the permeability of propranolol was enhanced by all the terpenes studied (menthol, carvacol, limonene, and linalool) compared to the control. Menthol and carvacol were superior in enhancing the transdermal penetration of propranolol compared to limonene (25).

Terpenes showed less activity in the enhancement of hydrocortisone permeation. Only compounds **3**, **4**, **7**, and **11** showed significant enhancement of Q_{24}, and only compounds **3**, **4**, and **10** showed significant enhancement of flux. Like caffeine, the most active compounds for hydrocortisone show a plateauing effect on the time-concentration graph (Fig. 3). The ER_{flux} are 3.96, 6.65, and 2.2 for compounds **3**, **4**, and **10**, respectively. The pretreatment of the skin with PG alone had no significant effect on the amount of hydrocortisone that penetrated through the skin. Thus, the enhancement shown by the terpene/PG mixture can be attributed exclusively to the enhancer, unlike that of caffeine. When taking into account the en-

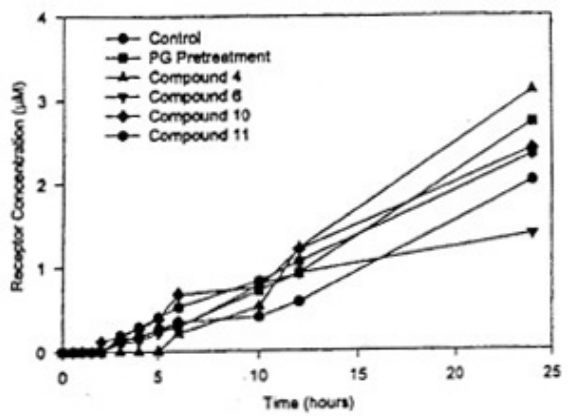

Figure 4. Cumulative amount of triamcinolone acetonide permeated across hairless mouse skin as a function of time. Each point is the mean of four determinations.

hancing effect of PG, the enhancement ratios for hydrocortisone actually exceed those of caffeine. While the highest ER_{flux} for caffeine when subtracting the effect of PG was 1.76; the highest ER_{flux} for hydrocortisone was 6.65.

Like caffeine, the compounds that significantly enhanced the transdermal penetration of hydrocortisone were also capable of hydrogen bonding. Hydrocortisone is considered a polar steroid with a log P of 1.43 (26). It is not unexpected that hydrophilic terpenes were more successful in enhancing the penetration of hydrocortisone than hydrocarbon terpenes.

Other studies have focused on examining the effect the lipophilicity of model drugs have on the penetration-enhancing properties of terpenes. One study investigated the effect of l-menthol on the penetration of a series of benzoic acid derivatives with varying degrees of lipophilicity. The addition of a solution of l-menthol in 15% ethanol increased the permeability coefficients of the relatively hydrophilic derivatives in excess of 10-fold, but there was no significant increase of the permeability coefficients for the lipophilic derivatives (27).

Another study compared the enhancing action of *d* limonene using mannitol (hydrophilic), 6-mercaptopurine (amphophilic), and butylparaben (lipophilic) as model drugs. Only the higher doses (192, 288, 384 μmol) of *d*-limonene could improve the penetration of 6-mercaptopurine through intact skin. *d*-Limonene showed little activity to improve mannitol permeation. *d*-Limonene enhanced butylparaben penetration through the intact skin depending on its pretreatment doses (28).

While the tested compounds showed enhancement of both caffeine and hydrocortisone penetration, the overall transdermal penetration of TA was very low. While caffeine penetrated in the range of hundreds of μM and hydrocortisone penetrated between 10 and 20 μM, the highest Q_{24} for TA was only 3.10 μM with compound **4**. PG pretreatment did increase TA delivery across the skin, but not to a significant extent. PG pretreatment's ER_{flux} and ER_{Q24} were only 1.43 and 1.35, respectively. Only two compounds (**4**, ER_{flux} 2.47; and **6**, ER_{flux} 1.57) had significant increases in flux for TA when compared to the control, and only compound **4** had a flux that was significantly greater when compared to PG pretreatment. In addition, compound **4** (ER_{Q24} 1.53) was also the only enhancer that significantly increased the 24-hr receptor concentration, and this enhancement was also found to be insignificant when compared to PG pretreatment.

The results of the TA study were unanticipated. The authors expected the hydrocarbon terpenes [e.g., (+)-limonene and (−)-menthone] to enhance the penetration of TA through the skin. Other studies have shown that hydrocarbon terpenes can be very effective in enhancing the penetration of lipophilic drugs. One study demonstrated that, in both skin and plasma, the concentration of indomethacin increased in a linear fashion with concentration (1–3%) of *d*-limonene in gel formulations. The plasma concentration was proportional to the amount of indomethacin in the skin, suggesting that the rate-limiting step of transdermal penetration was the penetration process from the gel to the skin rather than through the skin (29).

Another study reported the promotional effects of cyclic monoterpenes present in essential oils as percutaneous absorption of indomethacin, ketoprofen, and diclofenac sodium. Penetration of these drugs was remarkably enhanced on addition of hydrocarbons such as limonene, methane, terpinene, and terpinolene in the presence of ethanol. In this work, gel ointments containing ethanol were applied as test formulations. Previous reports noted an impressive enhancement of *d*-limonene on the percutaneous penetration of ketoprofen in gel ointment, although this effect disappears with the withdrawal of ethanol from the formulation. Ethanol and *d*-limonene are important additives and are important for enhancing the percutaneous absorption of ketoprofen (11). The vehicle used in the present study was PG. The results of this study showed that PG significantly increased the amount of caffeine penetrating the skin, but did not significantly increase the amount of hydrocortisone or TA through the skin.

Table 3

Effect of Propylene Glycol Pretreatment and Enhancers 1–11 on Permeation and Skin Retention of Triamcinolone Acetonide (Enhancement Ratios in Parentheses)

Enhancer in PG[a]	Flux ($\mu M\ cm^{-2}hr^{-1}$)	Q_{24} (μM)[b]	SC ($\mu g\ g^{-1}$)[c]
Control	0.23 ± 0.16 (1.0)	2.02 ± 0.33 (1.0)	182.4 ± 90.31 (1.0)
PG pretreatment	0.33 ± 0.1 (1.4)	2.72 ± 0.81 (1.4)	27.32 ± 3.31 (0.15)
1	0.29 ± 0.14 (1.3)	2.21 ± 0.85 (1.1)	101.6 ± 66.74 (0.56)
2	0.27 ± 0.05 (1.2)	1.59 ± 0.20 (0.79)	44.13 ± 26.09 (0.24)
3	0.22 ± 0.04 (0.96)	1.37 ± 0.27 (0.68)	54.21 ± 40.82 (0.30)
4	0.57 ± 0.04 (2.5)	3.10 ± 0.28 (1.5)	15.99 ± 4.74 (0.09)
5	0.25 ± 0.02 (1.1)	1.37 ± 0.08 (0.68)	23.40 ± 6.91 (0.13)
6	0.38 ± 0.13 (1.7)	1.38 ± 0.16 (0.63)	21.55 ± 7.18 (0.12)
7	0.31 ± 0.03 (1.4)	1.53 ± 0.11 (0.76)	33.39 ± 28.67 (0.18)
8	0.32 ± 0.03 (1.4)	1.95 ± 0.21 (0.97)	20.55 ± 14.14 (0.11)
9	0.31 ± 0.09 (1.4)	2.17 ± 0.20 (1.1)	22.41 ± 3.42 (0.12)
10	0.32 ± 0.07 (1.4)	1.20 ± 0.08 (0.60)	25.37 ± 12.26 (0.14)
11	0.36 ± 0.01 (1.4)	2.33 ± 0.51 (1.2)	14.16 ± 2.97 (0.08)

[a] PG, propylene glycol.
[b] Q_{24}, 24-hr receptor concentration.
[c] SC, skin content of triamcinolone acetonide.

Other lipophilic drugs that have been examined in conjunction with terpenes are testosterone and tamoxifen. One study examined the effect of l-menthol on the transdermal penetration of testosterone. It was discovered that testosterone and l-menthol form an eutectic mixture. Applying this mixture to the skin resulted in a flux about 8 times higher than that of testosterone alone. This result was explained by two observations: (*a*) the solubility of testosterone in the eutectic mixture was 2.8-fold higher in the presence of menthol, and (*b*) the permeability coefficient was increased 2.6-fold for the eutectic mixture. This dual mechanism of action has been termed a "push-pull" mechanism. The push results from the increase in drug solubility in the vehicle, and the pull results from the decreased barrier function of the stratum corneum, which results from a disordering of the lipid lamellae (30).

Another study examined the effects of terpenes on the enhancement of the lipophilic anticancer drug tamoxifen. It was reported that carvone was most efficient in enhancing the permeability of tamoxifen in comparison to a control. The rank order of the terpenes tested was carvone > 1,8-cineole > thymol > menthol. Once again, it was shown that hydrocarbon terpenes are more efficient at enhancing the permeation of lipophilic drugs (31).

In addition to a Student *t*-test analysis of the data, a Tukey-Kramer post hoc multiple comparison analysis was undertaken to group levels of enhancement activity by looking at significant differences between the enhancers at an α level of 0.05. For the enhancement of

caffeine flux, compound **11** was the most active enhancer, and this enhancement was significantly greater than that of any other compound. Compounds **10**, **7**, and **6** were the next most effective enhancers, and their flux enhancements were not significantly different from each other. Compounds **4** and **8** were also grouped together. The enhancements of compounds **5**, **3**, **9**, and PG pretreatment were not significant compared to each other. The final grouping of terpene enhancers with the lowest activities for flux enhancement included compounds **1** and **2**. The flux enhancements of these two compounds were significantly lower than that of the rest of the enhancers, although they were significantly greater than that of the control. A Tukey-Kramer analysis of Q_{24} enhancement showed that all terpene compounds had significant enhancement for this parameter, probably relating to its large values.

The same analysis was also performed on the enhancement of flux and Q_{24} for hydrocortisone. This analysis demonstrated that the enhancement of flux was not significantly different for the top five most active compounds (**4**, **3**, **10**, **6**, and **7**). The remaining enhancers, as well as the control and PG pretreatment, all have flux enhancements that are not significantly different from each other. Tukey-Kramer analysis of ER_{Q24} presents a slightly different result. The top two enhancers, compounds **3** and **4**, are not significantly different from each other. However, their enhancement activity is significantly higher than that of compound **7**, while the enhancement of compound **7** was significantly greater than that of compound **11** and **10**. The next grouping of compounds included **9** and **2**, and their ER_{Q24}'s were not significantly greater than each other. The ER_{Q24}'s of compounds **1** and **6**, as well as those of the control and PG pretreatment, can all be ranked together with no significant differences among them. Finally, the least-active compounds are **5** and **8**, with enhancement ratios less than 1 and no significant difference between them.

Finally, a Tukey-Kramer analysis of flux and Q_{24} enhancement ratios showed no significant differences among any of the enhancers and controls for TA. For ER_{flux}, comparing the most effective enhancer, compound **4**, and the least effective enhancer, compound **3**, gave a Tukey-Kramer Q value of only 0.75, while a value of 4.98 is required to show significant difference at an $\alpha = 0.05$. The same holds true for ER_{Q24}. Comparing compound **4** (most active) and compound **8** (least active) only gives a Q value of 3.77. It can be stated from these findings that each of the terpenes tested is equally effective (or ineffective) for the enhancement of TA.

The purpose of the Tukey-Kramer analysis was to group the terpene enhancers together in categories of effectiveness for each of the model drugs. In the future, pharmaceutical scientists may use these groupings to select a penetration enhancer capable of increasing, or perhaps decreasing, the amount of drug penetrating through the skin.

CONCLUSION

In conclusion, the results of the present study indicate that percutaneous penetration of hydrocortisone and caffeine can be significantly enhanced using a combination of terpene compounds and PG. The same cannot be said for increasing the penetration of TA. Final results of this study show that compound **11** was the most effective for the enhancement of caffeine penetration. Compound **4** was the most active enhancer for the percutaneous penetration of both hydrocortisone and TA, although the increase for TA was not significant. Further studies are required to investigate the effects of these compounds with other vehicles, such as ethanol, and the effects of vehicles on transdermal penetration of drugs.

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