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Review Article

Percutaneous Permeation Enhancement by Terpenes: Mechanistic View

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Abstract. A popular approach for improving transdermal drug delivery involves the use of penetration enhancers (sorption promoters or accelerants) which penetrate into skin to reversibly reduce the barrier resistance. The potential mechanisms of action of penetration enhancers include disruption of intercellular lipid and/or keratin domains and tight junctions. This results in enhanced drug partitioning into tissue, altered thermodynamic activity/solubility of drug etc. Synthetic chemicals (solvents, azones, pyrrolidones, surfactants etc.) generally used for this purpose are rapidly losing their value in transdermal patches due to reports of their absorption into the systemic circulation and subsequent possible toxic effect upon long term application. Terpenes are included in the list of Generally Recognized As Safe (GRAS) substances and have low irritancy potential. Their mechanism of percutaneous permeation enhancement involves increasing the solubility of drugs in skin lipids, disruption of lipid/protein organization and/or extraction of skin micro constituents that are responsible for maintenance of barrier status. Hence, they appear to offer great promise for use in transdermal formulations. This article is aimed at reviewing the mechanisms responsible for percutaneous permeation enhancement activity of terpenes, which shall foster their rational use in transdermal formulations.

KEY WORDS: DSC; FTIR; percutaneous permeation enhancement; terpene; transdermal.

INTRODUCTION

The skin acts as a barrier for diffusion of substances into the body. The main barrier for most substances is located in upper layer of skin, the stratum corneum (SC). The SC consists of keratin enriched dead cells, surrounded by crystalline intercellular lipid domains. These domains are continuous structures in the SC and are required for competent skin barrier function (1). To achieve and to maintain effective therapeutic plasma drug concentrations, the barrier properties of skin sometimes have to be overcome to enable successful delivery. Both physical approaches (stratum corneum stripping, stratum corneum hydration, electrically assisted transdermal drug delivery) and chemical approaches (synthesis of lipophilic analogue, delipidization of SC, coadministration of penetration enhancers) have been investigated for accomplishing this goal. Extensive research during the past two decades has revealed considerable information on several classes of penetration enhancers, including surfactants (e.g. tween; 2), fatty acids/esters (e.g. oleic acid; 3), solvents (e.g. dimethylsulfoxide, ethanol; 4) and terpenes (e.g. limonene; 5). Despite their fairly satisfactory performance in enhancing the permeation of drug molecule across the skin, chemical enhancers are viewed with suspicion in transdermal formulations due to their irritancy potential when employed at concentrations necessary for achieving useful levels of penetration enhancement (6).

Efforts have been directed at identifying safe and effective enhancers from both natural products and synthetic chemicals. In particular, terpenes from natural sources and laboratory designed terpenoids have attracted great interest (7–9). Terpenes are generally considered to be less toxic with low irritancy potential compared to surfactants and other synthetic skin penetration enhancers. Further, quite a few terpenes are included in the list of Generally Recognized As Safe (GRAS) agents issued by US FDA (9,10).

Terpenes can increase skin permeation by one or more of the mechanisms (Table I): interacting with SC lipids and/or keratin, and increasing the solubility of drug into SC lipids (9). However, the interaction of terpenes with SC in presence of various solvents may not be similar due to differences in the physico-chemical properties of these solvents and their interactions with SC. These interactions can be determined by instrumental methods, such as, differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). Table II summarizes the effect of several terpenes on thermotropic behaviour of skin. DSC thermograms of skin treated with permeation enhancers can be evaluated by comparing the endotherms and exotherms for mean transition temperature (T_m), cooperativity and their enthalpies (ΔH). Shifting of T_m of both lipid transitions T2 and T3 to lower temperature is generally ascribed to the disruption of lipid bilayer while the reduction in ΔH is associated with fluidization of lipid bilayers of SC (28,29). Table III summa-

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Table I. Influence of Terpene Treatment on Percutaneous Permeation Enhancement of Drugs

Terpene	Vehicle	Skin	Study Duration (h)	Drug	ER	Proposed Mechanism	Reference
d-limonene	Neat liquid	–	12	5-FU	4	Lipid disruption	(11)
	PG: Water (80:20)	Human		5-FU	4.3	Freezing point depression	(12)
	Hydroxy propyl cellulose gel	Hairless mice	24	Nicardipine HCl	60	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	CPZ	6.6	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	Hydrocortisone	28	–	(13)
	PG	Human	60	Haloperidol	26.52	–	(14)
Limonene oxide	Ethanol (50% *v/v*)	Human	48	Haloperidol	14.3	Lipid disruption and extraction	(17)
	PG	Human	48	Haloperidol	9.18	Lipid disruption	(17)
	Ethanol (50% *v/v*)	Human	48	Haloperidol	40.25	Lipid disruption, extraction and fluidization	(17)
	PG	Human	48	Haloperidol	10.4	Lipid disruption	(17)
1,8-cineole	Neat liquid	–	12	5-FU	95.3	Lipid disruption	(11)
	PG:water (80:20)	Human		5-FU	24	Lipid disruption	(12)
	Ethanol (47% *v/v*)	Human	40	Thyrotropin releasing hormone	3.39	Modification of lipid bilayer structure with terpene and lipid extraction with ethanol	(18)
	Ethanol (66.6% *v/v*)	Rat	24	Zidovudine	55.8	Intercellular lipoidal pathway	(19)
	Ethanol (66.6% *v/v*)	Human	24	Zidovudine	18	Lipid fluidization	(20)
	PG	Human	60	Haloperidol	6.88	–	(14)
Nerolidol	Neat liquid	–	12	5-FU	25	Lipid disruption	(11)
	PG:water (80:20)	Human		5-FU	18	Lipid disruption	(12)
	Hydroxy propyl cellulose gel	Hairless mice	24	Nicardipine HCl	134.8	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	CPZ	7.5	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	Hydrocortisone	32.7	–	(13)
Menthone	Neat liquid	–	12	5-FU	42	Lipid disruption	(11)
	PG:water (80:20)	Human		5-FU	20	Lipid disruption	(12)
	Ethanol (47% *v/v*)	Human	40	Thyrotropin releasing hormone	3.33	Modification of lipid bilayer structure with terpene and lipid extraction with ethanol	(18)
	Ethanol (66.6% *v/v*)	Rat	24	Zidovudine	46.09	Through intercellular lipoidal pathway	(19)
Menthol	Ethanol (70% *v/v*)	–	24	Nicardipine HCl	7.12	Lipid extraction	(21)
	IPA/PG/water (30:30:40)	Rat	24	Zidovudine	87	Increased diffusion of drug in SC	(22)
	Ethanol (66.6% *v/v*)	Rat	24	Zidovudine	49.97	Through intercellular lipoidal pathway	(19)
	Hydroxy propyl cellulose gel	Rat	24	Nicardipine HCl	7.12	SC lipid extraction	(21)
	Poloxamer 407 gel	Mice	24	Ibuprofen	2.08	–	(15)
	Poloxamer 407 liquid formulation	Mice	24	Ibuprofen		–	(15)
	Carboxy methyl cellulose sodium gel	Rat	24	Etodolac	1.06	Increased hydrophilicity of SC resulted in lesser permeation of lipophilic drug.	(16)
Carvone	Ethanol (66.6% *v/v*)	Human	24	Zidovudine	11.4	Lipid fluidization	(20)
	Ethanol (70% *v/v*)		24	Nicardipine HCl	7.89	Lipid extraction	(23)
	Ethanol (60% *v/v*)	Rat	24	Nimodipine	4.56	Lipid bilayer disruption and partial extraction of SC lipids	(24)
	Ethanol (66.6% *v/v*)	Rat	24	Zidovudine	32.02	Through intercellular lipoidal pathway	(19)
	Ethanol:glycerine:PB (60:10:30)	Rat	24	Diclofenac sodium		Increase in drug diffusion and partitioning into skin	(25)

Carvacrol	Ethanol (50% *v/v*)		48	Haloperidol	12.1	Lipid extraction and disruption	(26)
	IPA:water (60:40)	Rat	24	Zidovudine	8.52	Increased diffusion of drug in SC	(22)
	PG		48	Haloperidol	4.4	Lipid extraction and disruption	(27)
	Carboxy methyl cellulose sodium gel	Rat	24	Etodolac	0.02	Increased hydrophilicity of SC resulted in lesser permeation of lipophilic drug.	(15)
Linalool	Ethanol (50% *v/v*)		48	Haloperidol	6.33	Lipid extraction and disruption	(26)
	IPA:water (60:40)	Rat	24	Zidovudine	24.6	Increased diffusion of drug in SC	(22)
	PG		48	Haloperidol	85.7	Lipid extraction and disruption	(27)
	PG	Human	60	Haloperidol	8.05	–	(14)
α-terpineol	Ethanol (50% *v/v*)		48	Haloperidol	8.86	Lipid extraction and disruption	(26)
	PG		48	Haloperidol	1.73	Lipid extraction and disruption	(27)
	Ethanol (66.6% *v/v*)	Rat	24	Zidovudine	44.55	Through intercellular lipoidal pathway	(19)
Fenchone	Hydroxypropyl cellulose gel	Hairless mice	24	Nicardipine HCl	17.9	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	CPZ	1.5	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	Hydrocortisone	7.8	–	(13)
Thymol	Hydroxypropyl cellulose gel	Hairless mice	24	Nicardipine HCl	18.2	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	CPZ	4.2	–	(13)
	Hydroxy propyl cellulose gel	Hairless mice	24	Hydrocortisone	10.5	–	(13)
Pinene oxide	Ethanol (50% *v/v*)		48	Haloperidol	14.3	Lipid disruption and extraction	(17)
	PG		48	Haloperidol	9.18	Lipid disruption and extraction	(17)
	Ethanol (50% *v/v*)	Human	48	Haloperidol	24.23	Lipid fluidization within the ordered SC environment	(17)
	PG	Human	48	Haloperidol	6.53	Lipid fluidization within the ordered SC environment	(17)
Pulegeon	Ethanol (66.6% *v/v*)	Rat	24	Zidovudine	45.34	Through intercellular lipoidal pathway	(19)

rizes the effects of several terpenes on the FTIR spectrum of skin. FTIR analysis provides information about the molecular and conformational changes in SC lipids and proteins. The asymmetric and symmetric C–H vibrations obtained at 2,920 and 2,850 cm^{-1}, respectively, have been ascribed to hydrocarbon lipid chains of SC. The height and area of these two peaks have been found to be proportional to the amount of lipids present in SC (32). The C=O stretching vibration of lipid polar head groups produce a band near 1,740 cm^{-1}. In addition, strong amide and water absorbance bands are found in region of 1,500–1,700 and 3,000–3,600 cm^{-1}, respectively. The bands at 1,650 and 1,550 cm^{-1} have been suggested to arise from amide I and amide II stretching vibrations, respectively, of the SC proteins. The frequencies of these two bands, especially amide I band, are sensitive and shift to higher or lower frequencies according to the change in protein conformation (33). Therefore, both methods provide independent but complementary data about the interaction of terpenes with SC in the presence of different solvents.

These techniques can further be complemented with microscopic studies in order to assess the effect of terpenes on various domains of skin. Further, application of quantitative correlation between permeation effects and physicochemical descriptors of terpenes by QSAR models can provide insights into the possible mechanisms responsible for skin permeation enhancement activity.

In view of the renewed interest in herbal components, this article is aimed at reviewing the use of terpenes as drug penetration enhancers in transdermal drug delivery and to discuss the possible mechanisms for this activity.

TERPENES AND TERPENOIDS

Terpenes and terpenoids are usually the constituents of volatile oil. Several natural sources and their major terpene content are summarized in Table IV. The basic chemical structure consists of a number of repeated isoprene (C_5H_8) units, which is used to classify terpenes. Thus, monoterpenes have two isoprene units (C_{10}), sesquiterpenes have three (C_{15}), and diterpenes have four (C_{20}), etc. Terpenes may also be classified as acyclic/linear, monocyclic and bicyclic.

Numerous terpenes have been used as antispasmodics, carminatives, flavoring agents, perfumes etc. For example, menthol is traditionally used in inhalation pharmaceuticals and has a mild antipruritic effect when incorporated into emollient preparations. However, the potential of terpenes as percutaneous absorption enhancer was suggested (5). Several cyclic terpenes like cineole, *d*-limonene, and α-pinene have been intensively investigated as penetration enhancers.

d-Limonene

Okabe *et al.* reported the effect of ethanolic solution of *d*-limonene on percutaneous absorption of indomethacin and ketoprofen. Limonene was found to be essential for increasing the penetration of poorly permeable drugs. However, enhanced permeability of drugs cannot be easily maintained due to difficulty in sustaining high activity of enhancers during the applied period (36,37). In order to maintain the

Table II. Influence of Terpene Treatment on Thermotropic Attributes of Drugs

				Thermotropic Attributes					
				T2		T3	T4		
Terpene	Vehicle	Drug	T_m (°C)	% ΔH (J/g)	T_m (°C)	% ΔH (J/g)	T_m (°C)	Proposed Mechanism	Ref.
d-limonene	Neat liquid	5-FU	−23	NS	−16	NS	Increased	Lipid disruption	(11)
	PG/W (20:80)	5-FU	−22		−13			Freezing point depression	(12)
	PG/W (50:50)	5-FU	−23		−15			Freezing point depression	(12)
	PG/W (80:20)	5-FU	−24		−15			Freezing point depression	(12)
1,8-cineole	Neat liquid	5-FU	−23	50	−16	50	Increased	Lipid disruption	(11)
	PG/W (20:80)	5-FU	−24	18	−18	NS	NS	Lipid disruption	(12)
	PG/W (50:50)	5-FU	−24	37	−17	NS	NS	Lipid disruption	(12)
	PG/W (80:20)	5-FU	−28	60	−17	22	NS	Lipid disruption	(12)
Nerolidol	Neat liquid	5-FU	−4.2	Increased	−4	Increased	Absent	Lipid disruption	(11)
	PG/W (20:80)	5-FU	−3	6	−4	NS	NS	Lipid disruption	(11)
	PG/W (50:50)	5-FU	−4	15	−4	NS	NS	Lipid disruption	(12)
	PG/W (80:20)	5-FU	−6	30	−8	NS	NS	Lipid disruption	(12)
Menthone	Neat liquid	5-FU	−19.4	Increased	−10	Increased	Absent	Lipid disruption	(11)
	PG/W (20:80)	5-FU	−15	8	−14	NS	NS	Lipid disruption	(11)
	PG/W (50:50)	5-FU	−15	17	−15	NS	NS	Lipid disruption	(12)
	PG/W (80:20)	5-FU	−16	42	−15	NS	NS	Lipid disruption	(12)
Menthol	EtOHl (70% *v/v*)	Nicardipine HCl	NS	Decreased	Absent	Absent	Absent	Lipid extraction	(21)
Carvone	EtOH (70% *v/v*)	Nicardipine HCl	NS	Decreased	Absent	Absent	Absent	Lipid extraction	(21)
Carvacrol	EtOH (50% *v/v*)	Haloperidol	−16	Increased	−12	Decreased	+20	Lipid disruption and extraction	(26)
	PG		−5	NS	−7	Decreased	Absent	Lipid disruption and extraction	(27)
Linalool	EtOH (50% *v/v*)	Haloperidol	−32	Decreased	−15	Decreased	−5	Lipid disruption and extraction	(26)
	PG		−5	NS	−3.5	Decreased	Absent	Lipid disruption and extraction	(27)
α-terpeniol	EtOH (50% *v/v*)	Haloperidol	−32	Decreased	−23	NS	−12	Lipid disruption and extraction	(26)
	PG		−5	NS	−3.5	Decreased	+10	Lipid disruption and extraction	(27)
Limonene oxide	EtOH (50% *v/v*)	Haloperidol		Coalescence		Coalescence	Absent	Lipid disruption and extraction	(17)
	PG		−2	NS	−3		Absent	Lipid disruption	(17)
Pinene oxide	EtOH (50% v/v)	Haloperidol	−26	Decreased	−24	NS	Absent	Lipid disruption and extraction	(17)
	PG		−5.5	Decreased	−6	Decreased	Absent	Lipid fluidization	(17)

NS Not significant, *5-FU* 5-fluorouracil, *PG* propylene glycol, *EtOH* Ethanol, *w* water

activity of the enhancer, Okabe *et al.* (5) employed a rate limiting membrane to control the permeation of ethanol used along with *d*-limonene. The cooperative effect of ethanol and *d*-limonene was reported to be an effective binary enhancer system (5,37,38). The enhancement of ethanol released on addition of *d*-limonene was found to be due to a change in structure of the adhesive layer of the transdermal patch because of its high affinity for *d*-limonene. The formulated system yielded high steady state permeation of drugs for a long period. The enhancement action was mainly attributed to the improved diffusivity of indomethacin in skin because the lag time for permeation was greatly shortened (5).

Ohara *et al.* evaluated the combined effect of *d*-limonene and temperature on the skin permeation of ketoprofen across rat skin. The apparent penetration rate (R_p) increased sigmoidally with increase in the temperature, and at lower temperature R_p was almost constant. A linear relationship was observed when the skin was pretreated with 30% *v/v* ethanol without *d*-limonene. However, Arrhenius plots of permeability coefficient value obtained after skin pretreatment with 1.5% *w/v* *d*-limonene in 30% *v/v* ethanol exhibited a convex curvature, suggesting that skin structure was altered with increase in temperature (39).

Krishnaiah *et al.* evaluated a limonene-based transdermal therapeutic system for its ability to provide the desired steady state plasma concentration of nicorandil in human volunteers. The flux of nicorandil from the limonene-based hydroxy propyl methyl cellulose drug reservoir across EVA 2825 (Ethylene vinyl acetate coated with 28% copolymers) membrane decreased to 216±10 μg/cm^2/h as compared to control (371±4 μg/cm^2/h) indicating that EVA 2825 effectively functioned as a rate controlling membrane. The

limonene-based drug reservoir was sandwiched between an adhesive coated EVA 2825-release liner composite and a backing membrane, and was heat-sealed to form a circular patch (20 cm^2). This patch yielded a steady state plasma concentration of 21.3 ng/ml through 24 h in healthy human volunteers (40). Hydrocarbon limonene was found to be more effective as penetration enhancer as compared to oxygenated linalool and cineole because it produced 26.5-fold greater permeation than control. Incorporation of limonene into an organogel-containing transdermal patch was observed to successfully deliver haloperidol at a sustained rate (14).

Limonene was reported to enhance the permeation of nicardipine HCl (hydrophilic) and hydrocortisone (polar steroid) significantly higher as compared to fenchone and thymol. These findings conflicted with the results observed by other researchers. It has been recognized that hydrophilic terpenes capable of hydrogen bonding (such as fenchone and thymol) actively promoted the permeation of hydrophilic drugs, whereas, hydrocarbon terpenes (e.g. limonene) provided higher permeation for lipophilic drugs (41,42). It was revealed that the lipophilicity of the permeant, as well as the enhancer molecule played an important role in determining the penetration promoting activity (13,41,43–50).

Cineole

The effects of propylene glycol (PG)/water co-solvent systems and terpene penetration enhancers [1,8-cineole, menthone, nerolidol and (+)-limonene] on the absorption rate of 5-FU (a hydrophilic permeant) were investigated using excised human skin. Co-application of each terpene in PG co-solvent system significantly enhanced the permeation of 5-FU. The penetration-enhancement activity of terpene depended on the propylene glycol content in the vehicle. Maximum permeation of 5-FU was obtained from formulations containing terpenes dispersed in 80% PG, which when normalized to the flux obtained using respective PG-water mixture yielded enhancement ratios ranging from 24 to 4-fold, respectively, for 1,8-cineole and (+)-limonene. The data obtained from DSC analysis was analyzed to calculate the entropy change in skin lipids after treatment with different terpenes. The treatment with 1,8-cineole, menthone and nerolidol was found to produce less change in the entropy (as compared to control) of skin lipids than the treatment with (+)-limonene. This indicated fluidization or reduction in lipid order at normal skin temperature. However, the observed higher entropy change of skin lipids after treatment with (+)-limonene than the control was suggested to be predominantly indicative of freezing point depression effect. Therefore, the mild effect of freezing point depression of skin lipids after treatment with (+)-limonene was able to enhance the permeation of 5-FU only by 4-fold as compared to 24-fold enhancement produced by 1,8-cineole (12).

The addition of cineole (3% *w*/*v*) to ethanol (47% *v*/*v*) increased the penetration of thyrotropin releasing hormone (TRH) across human epidermis to 0.92±0.03 μg/cm^2/h from 0.27±0.01 μg/cm^2/h. Although, carveol (3% *w*/*v*) and menthone (3% *w*/*v*) in combination with ethanol (47% *v*/*v*) increased the permeation of TRH to 1.07±0.02 μg/cm^2/h and 1.05±0.03 μg/cm^2/h, respectively, cineole showed most rapid attainment of steady state flux. It was interesting to note that neither ethanol alone nor the combination of any of the terpene and ethanol had any significant effect on the flux of TRH during a 2–4 h period. This suggested that the initial binding and saturation of the epidermal membrane by TRH was not affected by these materials (18).

Various oxygen-containing monoterpenes enhanced the transdermal flux of zidovudine in the following order: cineole > menthol > menthone ~ pulgeone ~ α-terpeniol > carvone. SC or vehicle partition-coefficient of zidovudine was not altered significantly ($p>0.05$) but the lag time required for zidovudine permeation was significantly reduced by all these terpenes. No significant difference in flux between vehicles, 1% or 2.5% *w*/*v* cineole was observed. As concentration of cineole was further increased to 5% and 10% *w*/*v* in vehicle, the flux was significantly enhanced by 56-fold and 65-fold, respectively. The observed difference in permeation enhancement activity was attributed to different thermodynamic activity of terpenes in the vehicle (19). In another investigation, 1,8-cineole was found to increase the flux of zidovudine 18-fold but lag time also increased from 4.9 to 8.5 h (20). Recent studies conducted by Heard revealed that enhancement of mefenamic acid using 1,8-cineole was due to "drag" or "pull" effect (51). However, another study suggested intercalation of cineole into lipids and proteins by forming hydrogen bonds thus, altering the organization of SC (52).

Carvone

The permeation of nicardipine HCl was observed to increase markedly by incorporating carvone in hydroxypropylcellulose gel and a maximum flux of 244±2 μg/cm^2/h was observed with an enhancement ratio of 7.9 when 12% *w*/*w* carvone was used as enhancer. The enhancement was attributed to partial lipid extraction of the SC (23). The transdermal permeation of nimodipine across rat abdominal skin was found to increase from 1.67±0.03 mg/cm^2/24 h to 3.68±0.06 mg/cm^2/24 h when carvone (2% to 12% *w*/*w*) was used with HPMC gel. However, no significant difference in the permeability coefficient of nimodipine was observed by incorporating 12% *w*/*w* carvone (10.9±0.08) as compared to that obtained with 10% *w*/*w* of carvone (10.73±0.5 cm/h×10^3; 24).

Menthol

Menthol, a monocyclic monoterpene free from significant toxic effects, has been approved as a penetration enhancer in the transdermal delivery of several drugs (53–55). Kabayashi *et al.* evaluated the combined effects of 1-menthol and ethanol as skin permeation enhancer, and derived two equations describing the permeability coefficient through full-thickness skin (P_{FT}) and the full-thickness skin/vehicle concentration ratio (C_{FT}/C_V) of drugs as a function of their octanol/vehicle partition coefficient (K_{OV}). A two-layer model was applied for skin. A nonlinear least-squares method was employed to determine six coefficients (three diffusion coefficients, the porosity of the SC, and two terms of the linear free energy relationship) using the two equations and experimentally obtained P_{FT} and C_{FT}/C_V values. Addition of *l*-menthol to water or ethanol (40%) improved the diffusion coefficient of morphine hydrochloride, atenolol, nifedipine

Table III. Influence of Terpene Treatment on Spectroscopic Attributes of Drugs

			C–H Stretching									
			Asymmetric				Symmetric					
Terpene	Drug	Skin	Peak Height	Percent Decrease	Peak Area	Percent Decrease	Peak Height	Percent Decrease	Peak Area	Percent Decrease	Proposed Mechanism	Ref.
Control (50% v/v EtOH)	Haloperidol	Human	0.44±0.0	9.55±0.55	14.02±1.1	9.75±1.44	0.25±0.01	6.35±0.94	2.49±0.16	5.45±0.68	NS	(17)
Limonene oxide	Haloperidol	Human	0.31±0.01	21.04±1.19	9.65±0.2	20.89±0.97	0.18±0.01	20.99±1.46	1.71±0.17	23.39±2.39	NS	
Pinene oxide	Haloperidol	Human	0.34±0.01	13.08±1.02	10.25±0.4	15.38±0.36	0.2±0.01	11.94±0.15	1.78 v 0.06	14.84±0.51	NS	
Control (PG)	Haloperidol	Human	0.43±0.05	12.91±2.6	14.04±1.41	13.59±2.08	0.27±0.06	12.85±1.89	2.71±0.56	11.68±2.59	NS	(17)
Limonene oxide	Haloperidol	Human	0.36±0.02	12.22±1.95	11.54±0.65	12.68±1.18	0.21±0.01	11.87±1.17	2.07±0.1	11.07±1.21	NS	
Pinene oxide	Haloperidol	Human	0.38±0.01	9.58±1.41	12.13±0.59	8.88±0.89	0.23±0.01	9.57±1.36	2.14±0.04	10.81±1.96	NS	
Control	Haloperidol	Human	0.43±0.05	12.91±2.6	14.04±1.41	13.59±2.08	0.27±0.06	12.85±1.89	2.71±0.56	11.68±2.59		(27)
Carvacrol	Haloperidol	Human	0.35±0.08	28.417±1.83	11.31±1.48	29.81±1.71	0.2±0.04	2.71±0.56	2.06±0.35	29.43±1.85	Lipid disruption and extraction	
Linalool	Haloperidol	Human	0.33±0.06	25.73±2.25	11.44±1.81	26.33±4.18	0.18±0.04	2.01±0.38	2.01±0.38	27.94±5.44	Lipid disruption and extraction	
Terpineol	Haloperidol	Human	0.27±0.05	26.11±0.27	10.65±1.36	29.07±1.85	0.15±0.02	1.62±0.18	1.62±0.18	28.24±2.78	Lipid disruption and extraction	
Control	Nicardipine hydrochloride	Rat	0.25±0.01	21.8±0.01	NS	NS	0.19±0.0	24±0.01	NS	NS		(21)
Menthol (1%)	Nicardipine hydrochloride	Rat	0.21±0.01	16±0.01	NS	NS	0.18±0.01	5.26±0.01	NS	NS	Lipid extraction	
Menthol (2%)	Nicardipine hydrochloride	Rat	0.2±0.01	20±0.01	NS	NS	0.16±0.01	15.79±0.01	NS	NS	Lipid extraction	
Menthol (5%)	Nicardipine hydrochloride	Rat	0.16±0.01	36±0.01	NS	NS	0.09±0.01	52.63±0.01	NS	NS	Lipid extraction	
Menthol (8%)	Nicardipine hydrochloride	Rat	0.11±0.01	56±0.01	NS	NS	0.07±0.0	63.15±0.0	NS	NS	Lipid extraction	
Menthol (10%)	Nicardipine hydrochloride	Rat	0.1±0.02	60±0.01	NS	NS	0.06±0.0	68.42±0.0	NS	NS	Lipid extraction	
Menthol (12%)	Nicardipine hydrochloride	Rat	0.1±0.01	60±0.01	NS	NS	0.06±0.01	68.42±0.01	NS	NS	Lipid extraction	
Water	Nicardipine hydrochloride	Rat	0.32±0.01	–	NS	NS	0.25±0.01	–	NS	NS		(23)
70% EtOH		Rat	0.25±0.01	21.8±0.08	NS	NS	0.19±0.01	24.0±0.01	NS	NS	Lipid extraction	
8% Carvone	Nicardipine hydrochloride	Rat	0.1±0.01	64±0.01	NS	NS	0.07±0.07	63.16±0.01	NS	NS	Lipid extraction	
Control (60% *v/v* EtOH)	Nimodipine	Rat	0.26±0.01	23.52±0.01	NS	NS	0.19±0.01	24±0.01	NS	NS		(24)
Carvone (2%)	Nimodipine	Rat	0.21±0.01	19.23±0.01	NS	NS	0.16±0.01	15.799±0.01	NS	NS	Lipid bilayer disruption and partial extraction of SC lipids	
Carvone (8%)	Nimodipine	Rat	0.13±0.01	50±0.01	NS	NS	0.08±0.01	57.83±0.01	NS	NS	Lipid bilayer disruption and partial extraction of SC lipids	

Carvone (10%)	Nimodipine	Rat	0.11±0.01	57.69±0.01	NS	NS	0.07±0.01	63.15±0.01	NS	NS	Lipid bilayer disruption and partial extraction of SC lipids	(30)
Control	Tamoxifen	Porcine	0.34±0.01	–	0.72±0.1	–	0.23±0.01	–	2.4±0.1	–	–	
EtOH	Tamoxifen	Porcine	0.26±0.01	22.09	5.25±0.0	21.86	0.16±0.01	32.76	1.78±0.02	25.96	–	
Eugenol-EtOH	Tamoxifen	Porcine	0.17±0.01	49.25	3.80±0.0	42.54	0.12±0.0	48.28	1.77±0.01	26.33	Lipid extraction and improvement in partitioning of drug in SC	
d-limonene-EtOH	Tamoxifen	Porcine	0.15±0.01	54.03	3.01±0.1	55.16	0.09±0.0	62.5	0.86±0.06	64.23	Lipid extraction and improvement in partitioning of drug in SC	
Menthone-EtOH		Porcine	0.15±0.01	56.12	3.13±0.43	53.39	0.08±0.0	64.66	0.86±0.06	66.72	Lipid extraction	
EtOH	Insulin	Rat	NS	NS	0.88±0.05	81.39	NS	NS	NS	NS		(31)
Cineole/EtOH	Insulin	Rat	NS	NS	2.8±1.11	62.31	NS	NS	NS	NS	Lipid extraction	
Pulgeon/	Insulin	Rat	NS	NS	3.61±0.01	59.84	NS	NS	NS	NS	Lipid extraction	
Menthol /	Insulin	Rat	NS	NS	3.57±1.26	49.5	NS	NS	NS	NS	Lipid extraction	
Neat menthone	Insulin	Rat	NS	NS	2.58±0.86	47.13	NS	NS	NS	NS	Lipid extraction	

EtOH Ethanol, *PG* propylene glycol, *NS* not specified, *5- FU* 5-Fluorouracil

and vinpocetine in lipid and pore pathways of the SC, whereas addition of ethanol to water and *l*-menthol (5%) improved the solubility of drugs in the vehicle and increased the contribution of the pore pathway towards whole skin permeation (56).

The mechanism of *l*-menthol as an enhancer was examined using diclofenac as a hydrophobic drug and diclofenac sodium as a hydrophilic drug through ethanol-treated and untreated silicone membranes. Results indicated that *l*-menthol enhanced the permeation of both salts of drug through both lipid as well as pore pathways (57).

Menthol has been shown to increase the skin absorption of testosterone by forming a eutectic mixture, thereby lowering its melting point drastically from 153.7°C to 39.9° C, as reflected by DSC studies. Hence, the skin permeation enhancement of testosterone by menthol was suggested to be due to increase in the solubility of testosterone accompanied with altered barrier properties of SC (53).

Hydrogel-based patches of propranolol hydrochloride were formulated with and without (1%, 5%, 10% *w*/*v*) menthol as an enhancer. Permeation of propranolol hydrochloride across hairless mouse skin was significantly higher ($p < 0.05$) from patches containing menthol. This observation might be due to the preferential distribution of menthol into the intercellular spaces of SC, which resulted in reversible disruption of SC lipid domains (54).

In combination with 15% *v*/*v* ethanol, *l*-menthol (1% *w*/*v*) increased the permeability coefficient of methyl paraben about 16-fold, while it decreased the permeability coefficient of butyl paraben to 20% of the control value through guinea pig dorsal skin. A spin label study with SC showed that these enhancers increased the lipid bilayer fluidity and led to enhanced permeation of hydrophilic substances (55).

Krishnaiah *et al.* observed marked enhancement in permeation of nicardipine hydrochloride from gel systems containing menthol (1–12% *w*/*w*) through excised rat epidermis. The cumulative amounts of nicardipine hydrochloride that permeated over 24 h increased from 1.74±0.13 to 5.03±0.03 mg/cm^2/24 h from the hydroxypropyl cellulose gels containing menthol. However, a lag period of 2–3 h in the permeation of the drug was observed. The flux remained constant when menthol was employed at 2% *w*/*w* concentration. However, when concentrations greater than 5% *w*/*w* were used, there was a rapid increase in flux (21). In addition, the study revealed that enhancement remained unaltered from the gel containing more than 8% *w*/*w* of menthol. There was a statistically insignificant ($p > 0.05$) decrease in enhancement of drug permeation with 10 or 12% w/w of menthol. Menthol is reported to increase both the moisture uptake capacity as well as release rate of propranolol hydrochloride from polymeric films (21). Further *l*-menthol (5% *w*/*v*) was found to significantly ($p < 0.05$) enhance the pseudo steady state flux of zidovudine across human cadaver skin with an enhancement factor of 53. However, the lag time was observed to increase. Partitioning of *l*-menthol into skin was found to be poor thereby indicating that it might have lipid fluidizing activity on SC lipids (20). Chang *et al.* evaluated a series of terpenes as enhancers for meloxicam gel. Maximum flux was obtained when menthol was used as permeation enhancer and its enhancing activity was attributed to its hydrogen bonding ability (58). However, the study conducted

Table IV. Major Components of Terpenes From Natural Sources

Source	Botanical Name	Main Terpene Component	Ref.
Angelica root	*Angelica archngelica*	β-Phellandrene, α-phellandrene, α-pinene	(34)
Anisi stellati fructus	*Illicium verum*	Monoterpenoid hydrocarbon (limonene, α-pinene)	(34)
Apti fructus	*Apium graveolens*	Limonene	(34)
Basilici herba	*Osimum basilicum*	Linalool eugenol, scimene, cineole	(34)
Cajuput	*Melaleuca leucadendron*	1,8-cineole, α-terpineol, *d*-limonene	(35)
Cardamom	*Elettaria cardamomum*	1,8-Cineole, α-terpineol, α-terpinyl acetate	(35)
Carvi fructus	*Carum carvi*	(*S*)-(+)-Carvone, ®-(+)-limonene, α-pinene, cpinene, dihydrocarvone, dihydrocaveol	(34)
Caryophylli flos	*Eugenia caryophyllus, Caryophyllus aromaticus*	Eugenol, eugenol acetate, α-pinene, β-caryophyllene and its oxide	(34)
Coriandri fructus	*Coriandrum sativum*	D-(+)-linalool, monoterpene hydrocarbons (α-pinene, *d*-limonene, γ-terpinene, ρ-cymene	(34)
Eucaluptus folium	*Eucalyptus globulus*	1,8-Cineole, eucalyptol, moderate amounts of monoterpenes (ρ-cymene, α-pinene	(34)
Foeniculi fructus	*Foeniculum vulgare*	*Trans*-anethol, some terpenoid hydrocarbons (α-pinene, α-phellandrene	(34)
Juniperi fructus	*Juniperus communis*	α-Pinene, β-pinene, limonene, terpinen-4-ol, α-terpineol, borneol, geraniol	(34)
Melissa	*Melissa officinalis*	Geranial, neral	(35)
Melissae folium	*Melissa officinalis*	Monoterpenes (citronellal, citral A, citral B),sesquiterpene (β-caryophyllene, germacrene D)	(34)
Myrtle	*Myrtus communis*	1,8-Cineole, α-pinene, myrtenyl acetate	(35)
Niaouli	*Melaleuca virdiflora*	1,8-Cineole, α-pinene, α-terpineol, *d*-limonene	(35)
Orange	*Citrus aurantium*	*d*-Limonene	(35)

by Anjos and coworkers revealed that *l*-menthol stabilized mainly in the central region of stratum corneum membranes, which interacted with the membrane lipids and resulted in disruption of hydrogen bonds in the polar membrane interface (59). The combination of menthol and iontophoresis was suggested to enhance the *in vitro* permeation of methotrexate due to alteration of lipid-protein domains of mice skin (60).

Few studies aimed at synthesizing *o*-alkyl and *o*-acylmenthol derivatives for increasing the permeation of drugs through skin. Among the synthesized compounds *o*-ethylmenthol (MET) showed greatest drug penetration activity and caused relatively little skin irritation (61). The flux of ketoprofen was markedly increased by using small amount of MET. It was concluded that MET increased retention of drugs in the skin surface (62). Obata *et al.* (63) evaluated MET for their structural activity relationships employing an artificial neural network. Confocal scanning laser microscopy revealed that MET contributed to enhancement by lipid perturbation, thus enhancing drug diffusivity in skin lipids (64).

Table V. Log *p* Values of Few Terpenes (85)

Terpene	Log *p* Value	Terpene	Log *p* Value
Carveol	2.68	(*S*)-(−)-citronellal	3.48
(−) dihydro carveol	2.92	Carvacrol	3.28
(+)-dihydro carveol	2.92	Citral	3.17
(+)-dihydrocarvone	2.47	Menthone	2.63
(±)-linalool	3.28	Nerol	3.28
(*R*)-(−)-carvone	2.27	Thymol	3.28
(*R*)-(+)-pulgeone	2.56	β-cittronellal	3.38
Phytol retinol	8.66	β-carotene	15.51
Squalene	13.09	(±)-nerolidol	5.31

Nerolidol

The effects of fenchone, thymol, *d*-limonene and nerolidol on the percutaneous permeation of gel formulations of nicardipine hydrochloride, hydrocortisone, carbamazepine and tamoxifen were studied across hairless mouse skin. Nerolidol was found to be most effective in promoting permeation of all drugs (13). The results were in accordance with other studies conducted for 5-FU (65) and diclofenac sodium (66). According to Cornwell and Barry, the effective permeation promoting activity of nerolidol was due to its amphiphilic structure that was suitable for alignment within the lipid lamellae and also for disruption of the highly organized packing of SC (67). Nerolidol, at 2% *w/v* concentration was found to produce a 2-fold and 20-fold increase in the permeation of enoxaparin sodium (68) and 5-FU (65), respectively. An unsaturated sesquiterpene, α-bisabolol was reported to enhance permeation of 5-FU and triamcinolone acetonide by 17- and 73-fold across excised human skin due to lipid fluidization of SC (69).

QUANTITATIVE STRUCTURAL ACTIVITY RELATIONSHIP (QSAR)

QSAR has been explored for relating the skin permeation of compounds to their physicochemical properties (70). In 1990, Flynn compiled skin permeability coefficients across human skin from different literature sources and identified Log P as the most important factor for determining the

permeability coefficients (71). The subsequent QSAR studies raised interest in using QSAR for modeling skin permeation (70,72–82). These QSAR models provide an insight into the mechanism of skin penetration and guidance for predicting permeability of new compounds.

Ghafourian *et al.* constructed QSAR models for 34 terpenes, 16 pyrrolidinone derivatives and seven *N*-acetylprolinate esters with respect to several drugs (83). These QSAR models were based on data of different sources and skin types, including human, rats and hairless mouse skins. However, the data needs more careful analysis when combined into a single dataset as it is difficult to explain the large variations, primarily due to inter-laboratory differences such as skin sample types and sources, solvent systems for enhancers and experimental protocols.

The Log *p* value of terpene (Table V) appears as a predictor in many QSAR models (70,84). Kang *et al.* determined permeability coefficient of different terpenes experimentally using human skin and employed non-linear regression model. Their results suggested that (1) liquid terpenes tend to produce better enhancing effects than solid terpenes; (2) triterpenes and tetraterpenes generally had poor penetration effect than other terpenes; (3) terpenes with larger Log *p* values were more effective enhancers than those with smaller Log P as it was easier for lipophilic terpenes to get mixed with SC intercellular lipids (for extraction or for lipid phase transition; 19,83,85,86); (4) the liquid terpenes could form more number of hydrogen bonds with intercellular lipids of SC (20,25,85); (5) terpenes with aldehyde and ester functional groups were found to be better enhancers (85). In addition, the size of a terpene also determined the penetration ability. Smaller terpenes tended to be more active than the larger terpenes (86). Furthermore, smaller alcoholic terpenes with a higher degree of unsaturation appeared to be good candidates for enhancing the permeation of hydrophilic drugs (83). Cal and his coworkers investigated the absorption and elimination of different terpenes from human skin layers and observed terpinen-4-ol to accumulate in the skin to a greater extent when compared to pinenes (α and β pinene) and eucalyptol. This property was ascribed to the presence of polar groups, which increased its affinity towards polar region of SC and hydrophilic dermis. Almost negligible penetration was observed for pinenes in spite of the same log *p* values (87). Fang *et al.* concluded that oxygen containing terpenes were more effective than hydrocarbon terpenes. In addition, oxygen containing terpenes with a bicyclic structure displayed a lesser permeation enhancing effect (88).

IN VITRO/*IN VIVO* CORRELATIONS

Though the ultimate goal of enhancing percutaneous absorption relates to humans, *in vivo* studies are not always possible, for various reasons. Although comparative studies of *in vitro* and *in vivo* absorption through animal models or human skin are limited, the existing data strongly support the relevance of in vitro data.

The investigations of Karali *et al.* revealed azidothymidine to be delivered systemically from transdermal gel formulation containing carvone at rate of 0.9 mg/cm^2/24 h in rats. In order to achieve the minimum effective concentration of azidothymidine in humans (0.27 μg/ml), a delivery rate of 1 mg/cm^2/h from transdermal patch of 25 cm^2 in size was needed. Therefore, these studies demonstrated the inability of carvacrol to achieve the required delivery rate of azidothymidine in humans (22). Another investigation, however, demonstrated *in vitro* flux ranging from 1.03 to 1.79 mg/cm^2/h across rat skin by using hydro alcoholic solutions of various terpenes as permeation enhancers. The magnitude of permeation rates documented in this study show a great promise of terpenes in achieving the required systemic delivery rate of azidothymidine in humans (19).

In vitro permeation studies of nicorandil across rat abdominal skin from transdermal formulations containing carvone or limonene revealed, respectively, 1.6-fold and 1.7-fold greater permeation than that required for achieving their systemically effective concentration in rats. However, the observed enhancement (1.6-fold) of permeation of nicorandil across rat skin did not seem capable of providing effective plasma concentration in humans due to the higher resistance of human SC, which is three times less permeable than rat skin (40,89). Nevertheless, using carvone as enhancer and adhesive-coated EVA 2825 membrane, the average steady state of 20.5 ng/ml in humans was maintained through 24 h (89). The lag time was observed to reduce to 2.8 h and a steady state plasma concentration of nicorandil was maintained at 21.3 ng/ml through 24 h when limonene was employed as enhancer and adhesive-coated EVA 2825 as rate controlling membrane (40).

The suitability of adhesive-coated EVA 2825 membrane using limonene (90) or menthol (91) as enhancers for nicardipine hydrochloride was tested by employing excised rat skin. Limonene or menthol enhanced the *in vitro* permeation of nicardipine hydrochloride, respectively, by 2.08-fold and 1.8-fold. The systemic delivery of nicardipine hydrochloride in humans was maintained by limonene or menthol, respectively, at 32.1 or 21.2 ng/ml. Limonene maintained the plasma steady state concentration over 20 h, while menthol maintained it over 26 h. Although, these patches were not evaluated for their effectiveness from pharmacodynamic view point, their ability to maintain constant drug level in plasma of human volunteers suggested sustained systemic drug delivery (90,91).

TERPENES AND THEIR DISPOSITION

Despite the ubiquitous occurrence and importance of terpenes in food, little is known about their metabolic fate. After ingestion, terpenes are well absorbed and metabolized via hydroxylation or epoxidation by microsomal monooxidases. The primary metabolites are further transformed into more polar compounds and are excreted via urine. Most of the data available pertains to rat (92–95) or rabbits (96,97) which were fed with terpene rich diets. However, it is not certain that these dispositional characteristics can be extrapolated to humans, particular in view of the relatively large doses administered to animals. Terpene concentration might affect the type of metabolite formed (98,99).

Studies on metabolism of monoterpenes, carvone and pulgeon in humans using MICA (Metabolism of Ingestion Correlated Amounts) approach have been carried out.

Metabolites formed were identified as α,4-dimethyl-5-oxo-3-cyclohexene-1-acetic acid (dihydrocarvonic acid), α-methylene-4-methyl-5-oxo-3-cyclohexene-1-acetic acid (carvonic acid), and 5-(1,2-dihydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one (uroterpenolone) on the basis of mass spectral analysis in combination with synthesis and NMR experiments of the acidic fraction. Minor metabolites were identified as reduction products of carvone, namely, the alcohols carveol and dihydrocarveol. The previously identified major *in vivo* metabolite in rabbits, 10-hydroxycarvone (97), could not be detected, indicating either concentration dependent effect or interspecies differences. No differences in metabolism between *S*-(+)- and *R*-(−)-carvone were detected (100).

There have been several studies of (*R*)-(+)- and (*S*)-(−)-pulegone metabolism in rats (99–103), *ex vivo* experiments with rat liver microsomes (104–107) and human cytochrome *P*450 enzymes (108). Engel identified 2-(2-hydroxy-1-methylethyl)-5-methylcyclohexanone (8-hydroxymenthone), 3-hydroxy-3-methyl-6-(1-methylethyl)cyclohexanone (1-hydroxymenthone), 3-methyl-6-(1- ethylethyl)cyclohexanol (menthol), and E-2-(2-hydroxy- 1-methylethylidene)-5-methylcyclohexanone (10-hydroxypulegone) as the four major metabolites of pulegone. 10-hydroxypulegone was identified as a minor metabolite of (*S*)-(−)-pulegone. However, it was one of the major metabolites of (*R*)-(+)-pulegone. In addition, 3-methyl-6-(1-methylethyl)-2-cyclohexenone (piperitone,) and R,R,4-trimethyl-1- cyclohexene-1-methanol (3-*p*-menthen-8-ol) were identified as minor metabolites (109). Menthofuran was identified early as a metabolite of pulegone formed after ingestion of large amounts of pennyroyal oil (110), however it was later found that menthofuran was most probably an artifactual product formed during workup from known (10-hydroxypulegone) and/or unknown precursors. The reaction of 10-hydroxypulegone forming menthofuran is considerably faster in aqueous solution at room temperature at any pH, leading to complete transformation of 10-hydroxypulegone to menthofuran, usually within hours. Therefore, this reaction was considered as a major source of menthofuran. As a consequence, the amount of menthofuran detected in metabolism experiments was strongly dependent on the workup method. The precursor, 10-acetoxypulegone, was also unstable and it slowly isomerized to form 9-acetoxypulegone (111). Engel was not able to clarify that *p*-cresol was a major metabolite of menthofuran and, therefore, whether it was responsible for the toxic effects associated with pulegone ingestion. The difference in toxicity between (*S*)-(−)- and (*R*)-(+)-pulegone could be explained by the strongly diminished ability for enzymatic reduction of the double bond in (R)-(+)-pulegone, which might lead to further oxidative metabolism of 10-hydroxypulegone and the formation of currently undetected metabolites. These metabolites could possibly account for the observed hepatotoxic and pneumotoxic activity observed in humans (109).

CONCLUSION

Terpenes, the naturally occurring volatile oils, appear to be clinically acceptable penetration enhancers as indicated by high percutaneous enhancement ability, reversible effect on the lipids of SC, minimal percutaneous irritancy at low concentration (1–5%) and good evidence of freedom from toxicity. Moreover, a variety of terpenes have been shown to increase percutaneous absorption of both hydrophilic and lipophilic drugs when judiciously selected and combined with solvents. Hence, use of terpenes can be expected to yield satisfactory permeation of drugs across skin from transdermal formulations. However, proper selection of terpenes based on the functional groups, log *p* values, metabolic disposition would be important. However, it seems more rationale and essential to actually test the terpene containing formulations in humans in order to arrive at a reliable conclusion regarding systemic efficacy of the transdermal patches.

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