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Transdermal Delivery of Valsartan: I. Effect of Various Terpenes

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The objective of the present study was to investigate the effect of various terpenes, including a diterpene, forskolin (FSK; a putative penetration enhancer), on skin permeation of valsartan. Permeation studies were carried out with Automated Transdermal Diffusion Cells Sampling System (SFDC 6, LOGAN Instruments Corp., NJ, USA) through rat skin and human cadaver skin (HCS) using ethanol: IPB (pH 7.4) (40:60) as vehicle. The efficacy of the study terpenes for permeation of valsartan across rat skin and human cadaver skin was found in the order of cineole > d-limonene > l-menthol > linalool > FSK and cineole > d-limonene > linalool > l-menthol > FSK, respectively. No apparent skin irritation (erythema, edema) was observed on treatment of skin with terpenes including FSK. FT-IR, DSC, and histopathological studies revealed that FSK enhanced the skin permeation of the active drug by disruption and extraction of lipid bilayers of SC in consonance with other terpenes.

Keywords valsartan; permeation; human cadaver skin; terpenes; FT-IR; DSC

INTRODUCTION

Valsartan is an angiotensin II receptor antagonist with actions of blocking the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptors. It is used in the management of hypertension and may also be used in patients with heart failure who are unable to tolerate ACE inhibitors (Jackson, 2006; Moffat, 2006).

Valsartan is rapidly absorbed following oral administration, with a low bioavailability of about 23%. It is not significantly metabolized and is excreted mainly via the bile as unchanged drug (86%) (Moffat, 2006). The steady state volume of distribution of valsartan after intravenous administration is small (17 L), indicating that valsartan does not distribute into tissues extensively. It is highly bound to serum proteins (95%), mainly serum albumin. It has low molecular weight (435.5) and melting point (116–117°C) with a favourable log partition coefficient (4.5) and mean biological half life (7.5 hours) (Jackson, 2006; Moffat, 2006). There are no reports of skin irritation attributed to valsartan. All the above characteristics make valsartan a good candidate for transdermal delivery.

Human skin is a remarkably strong barrier, thus causing difficulties for transdermal delivery of therapeutic agents. Only a few drugs have the characteristics required to permeate sufficiently across the stratum corneum (SC) to reach therapeutic blood concentration. In order to enhance transdermal absorption of the actives, different methodologies have been investigated and developed, including the use of drug derivatives, drug-saturated systems, and physical and chemical enhancers that facilitate the diffusion of drugs through the stratum corneum. The most popular approach of transdermal enhancement is the inclusion of chemical enhancer such as terpenes, azones, DMSO, and so forth (Hadgraft, 1999; Williams & Barry, 2003). Terpenes, which are derived from plant essential oils, have been previously used to enhance the skin permeation of both lipophilic drugs, such as indomethacin (Buyuktimkin, Buyuktimkin, & Rytting, 1993), ketoprofen (Wu et al., 2001), estradiol (Monti et al., 2002), haloperidol (Vaddi, Ho, & Chan, 2002), and hydrocortisone (Fuhrman et al., 1997) and hydrophilic drugs, including propranolol hydrochloride (Hori et al., 1991), 5-fluorouracil (Cornwell & Barry, 1993), and nicardipine hydrochloride (Krishnaiah, Satyanarayana, & Bhaskar, 2002). Recently Nokhodchi and coworkers (2007) studied the effect of terpenes (carvone, l-menthol, nerolidol, farnesol, limonenoxide) on permeation of diclofenac sodium and suggested that highest permeation rate obtained at 2.5% v/v concentration for all terpenes. Terpenes are less toxic with no irritancy property to skin. USFDA has approved terpenes as “GRAS” (generally regarded as safe).

The present work was carried out to investigate the feasibility of valsartan for transdermal drug delivery, to monitor the effect of terpenes, including forskolin (FSK; a putative penetration enhancer), on skin permeation of valsartan and to elucidate the mechanism of skin permeation enhancement by Fourier transform infrared spectroscopy (FT-IR), Differential scanning calorimetry (DSC), and histopathology studies.

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MATERIALS AND METHODS

Materials

Valsartan was received as gratis sample from Ranbaxy, India. FSK was generously gifted by Planta, Austria. Cineole, d-limonene, linalool, and l-menthol were purchased from Sigma-Aldrich, UK. Absolute ethanol was purchased from Merck, Germany. All other chemicals used were of reagent grade.

Solubility Studies

The equilibrium solubility of valsartan was determined in mixture of ethanol and isotonic phosphate buffer (IPB) (pH 7.4) systems with ethanol fraction ranging from 0 to 60% v/v at 37 ± 2°C with and without terpenes. In this determination, an excess amount of the drug was added to the ethanol: IPB (pH 7.4) systems present in different flasks and till saturated. Then, it was placed in mechanical shaker at 37 ± 2°C for 72 h (Nirmal International, Delhi, India). The samples were centrifuged at 4000 rpm for 10 min, and the supernatants were assayed by HPLC method reported by Tatar and Saglik (2002).

Preparation of Rat Skin

Preparation of Full Thickness Skin

The skin samples were harvested from the ventral surface of the male wistar rats. Hair on dorsal skin of animal was removed with depilatory, subcutaneous tissue was removed manually, and dermis side was wiped with isopropyl alcohol to remove residual adhering fat. The skin was washed with distilled water, wrapped in aluminum foil and stored in a freezer at −20°C.

Preparation of Dermatomed Skin

On the day of experiment, skin was brought to room temperature and then treated with 2 M sodium bromide solution in distilled water for 6 h. The hair and fat were removed manually. Epidermal sheet was cleaned by washing with distilled water, dried under vacuum, and examined for cuts or holes. It was stored in desiccator until further use (Jain, Thomas, & Panchagnula, 2001; Narishetty & Panchagnula, 2003).

Preparation of Human Cadaver Skin (HCS)

Preparation of Human Abdominal Skin

Human abdominal skin was obtained post-mortem from our University Teaching Hospital, sealed in evacuated polythene bags and stored at −20°C. The epidermal membranes were prepared by heat-separation technique (Yamane, Williams, & Barry, 1995). The whole skin was immersed in water at 60°C for 2 min, followed by careful removal of the epidermis. The samples were stored at −80°C until used. Before the permeation experiments, the membranes with SC side up were floated over 0.9% (w/v) sodium chloride solution containing 0.002% (w/v) sodium azide solution for three days to ensure essentially full hydration of the stratum corneum (Vaddi, Ho & Chan, 2002).

Preparation of Stratum Corneum (SC)

The human SC was prepared by following method reported by Vaddi, Ho, & Chan (2002). Briefly, epidermal membrane with SC side up was incubated in Petri dish over filter paper imbibed with 0.1% (w/v) trypsin in 0.5% (w/v) sodium bicarbonate solution at 37 ± 1°C for 3 h. The SC was removed, thoroughly washed, and dried in a vacuum desiccator. Finally, the SC was dipped in acetone solution for 20 s to remove sebaceous lipids and dried again.

Permeation Studies

Ex vivo permeation studies were carried out through rat skin and HCS using an Automated Transdermal Diffusion Cell Sampling System (SFDC 6, LOGAN Instruments, NJ, USA) (Figure 1) attached with a HPLC instrument. The system consisted of 3 side by side and 3 vertical diffusion cells with area of diffusion 0.636 cm^2. The water was warmed with the in built heater with a thermostat set at 37 ± 0.2°C. A pump circulated the warmed water throughout the system. A Teflon coated mini magnetic bead was kept in the receiver compartment for agitating the contained vehicle (ethanol: IPB (pH 7.4) (40:60) at 600 rpm. The receptor compartment was filled with vehicle, containing 0.003% w/v sodium azide as a preservative. Receptor fluid was sonicated to remove dissolved gases and equilibrated at 37 ± 0.2°C before placing in the receptor compartment. Hydrated skin samples were mounted into the diffusion cells with dermal side in contact with receptor phase, equilibrated at 37 ± 0.2°C for 8–10 h. Valsartan solutions of 2.5 mg/ml concentration in vehicle (4 ml) with or without enhancers (1–5% w/v) were applied in donor compartment. The donor and receiver compartment were covered with teflon plug. After application of drug solution, 500 µl samples were withdrawn from the receptor compartment at different time intervals and analyzed for drug content by HPLC method (Tatar & Saglik, 2002). Receiver volume was immediately replenished with the same amount of fresh vehicle. Permeation parameters like flux (j), enhancement ratio (ER), and lag time were calculated.

FT-IR Studies

Rat skin was prepared as mentioned earlier and SC was cut into small circular disc with approximate diameter of 1.5 cm. 0.9% (w/v) sodium chloride was prepared and 0.003% w/v sodium azide was added as antibacterial and antimycotic agent. Equal volume of 0.9% (w/v) sodium chloride solution was placed in different conical flask and SC of approximate diameter 1.5 cm was floated over for 3 days. After 3 days of hydration, these discs were thoroughly blotted over filter paper and FT-IR (Perkin Elmar, Germany) was recorded before enhancer treatment (control) in frequency range of 400 to 4000 cm^{-1},

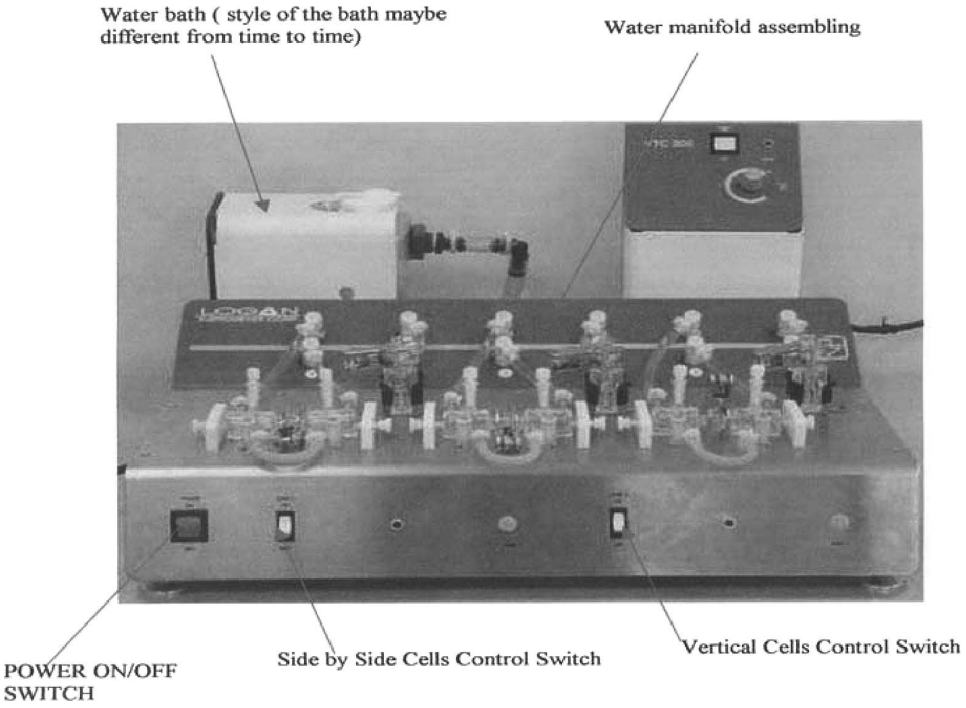

FIGURE 1. Automated transdermal diffusion cell sampling system (SFDC- 6, LOGAN Instruments, NJ, USA).

with resolution of 2 cm^{-1}. After taking FT-IR, the same discs were dipped into respective enhancer solution (1% w/v) in 4 ml ethanol: IPB pH 7.4 (40:60) systems for 24 h, (equivalent to the permeation studies) at 21 ± 1°C. Each SC disc after treatment was washed, blotted dry, and then air dried for 2 h. Samples were kept under vacuum in desiccators for 15 min to remove traces of solvent and enhancer completely. FT-IR spectra of all SC discs treated with enhancer were recorded again.

DSC Studies

Approximately 20 mg of freshly prepared rat SC was taken and hydrated over saturated potassium sulphate solution for 3 days. Percent hydration was calculated using the formula:

$$\% \text{ of hydration} = \frac{\text{Wt. of hydrated SC} - \text{Wt. of dry SC}}{\text{Wt. of dry SC}} \times 100 \quad (1)$$

Hydrated rat SC samples were dipped into respective enhancer solutions (1% w/v) in 4 ml ethanol: IPB pH 7.4 (40:60) systems for 24 h, at 21 ± 1°C. After the enhancer treatment, SC was removed and blotted to attain hydration of 20–25%, cut (5 mg) and sealed in aluminum hermatic pans, and equilibrated for 1 h before the DSC run. Then, the SC samples were scanned on a DSC6 Differential Scanning Calorimeter (Perkin Elmer, Germany). Scanning rate was at 5°C/min over the temperature range of 30 to 200°C (Krishnaiah, Satyanarayana, & Karthikeyan, 2002; Vaddi, Ho, & Chan, 2002).

Histopathological Studies

Abdominal skin of wistar rats was treated with optimized established enhancer, cineole (1% w/v) and new enhancer, FSK (1% w/v) in ethanol: IPB pH 7.4 (40:60). After 24 h, rat was sacrificed and the skin samples from treated and untreated (control) area were taken. Each specimen was stored in 10% formalin solution in phosphate buffer saline (pH 7.4). The specimen was cut into section vertically. Each section was dehydrated using ethanol, embedded in paraffin for fixing, and stained with hematoxylin and eosin. These samples were then observed under light microscope (Motic, Japan) and compared with control sample. In each skin sample, three different sites were scanned and evaluated for elucidation of mechanism of penetration enhancement (Fang et al., 2003).

Data Analysis

Flux is the rate of change of the cumulative amount of drug that passes per unit area and time through the skin. The equation derived from Fick's second law of diffusion can be used to calculate the steady-state flux (*J*).

$$J = \frac{V(dc/dt)}{A} (\mu g / cm^2 / hr) \quad (2)$$

Where V (ml) is the volume of the receiver compartment, dc/dt is the steady-state slope from the plot of the amount of drugs permeated through the skin versus time, and A is the effective diffusional area. Lag time (t_{lag}) is obtained by extrapolating the linear portion of the same graph with respect to the horizontal time (h) axis.

In order to express the extent of enhancement, enhancement ratio (ER) was calculated as follows:

$$ER = \frac{\text{Flux of valsartan with enhancer}}{\text{Flux of valsartan without enhancer}} \quad (3)$$

One-way analysis of variance (ANOVA) with Dunnett test was used for statistical comparison. The level of significance was taken as $p < 0.05$.

RESULTS AND DISCUSSION

In order to identify a suitable vehicle for ex vivo skin permeation studies, solubility of valsartan in different ratios of ethanol: IPB pH 7.4 mixture was determined. The above mixture in the ratio of 40:60 v/v was selected as the optimized vehicle (Table 1). The solubility of drug also was studied in selected vehicle containing terpenes. As there was no significant difference in solubility of the drug in the vehicle with and without terpenes, the results are not reported in the present manuscript. It is documented that solubility of valsartan increases with increase in the percentage of ethyl alcohol and pH of the solvent (Mbah, 2005). At 30% concentration of ethyl alcohol solubility was increased 4-fold. Whereas, solubility was increased to 2 times at pH 10.06. The result obtained here is well correlated with results reported by Mbah (2005). The mixture in the ratio of ethanol (40) and IPB (60) was selected as the optimum vehicle for skin permeation studies as it dissolved sufficiently high amount of drug.

The results of the ex vivo skin permeation studies are presented in Table 2 and Figures 2 and 3. The flux was obtained from slope of linear portion of graph between cumulative drug permeated and time. Good correlation was found between the amount of drug permeated across treated skin and time

TABLE 1
Solubility of Valsartan in Ethanol: IPB (pH 7.4)

S.NO.	VehicleEthanol: IPB (pH 7.4) in Different Ratios	Solubility (mg/ml) ± *SD*
1	0:100	1.61 ± 0.49
2	20:80	5.28 ± 2.31
3	40:60	47.08 ± 13.78
4	60:40	1093.5 ± 78.19

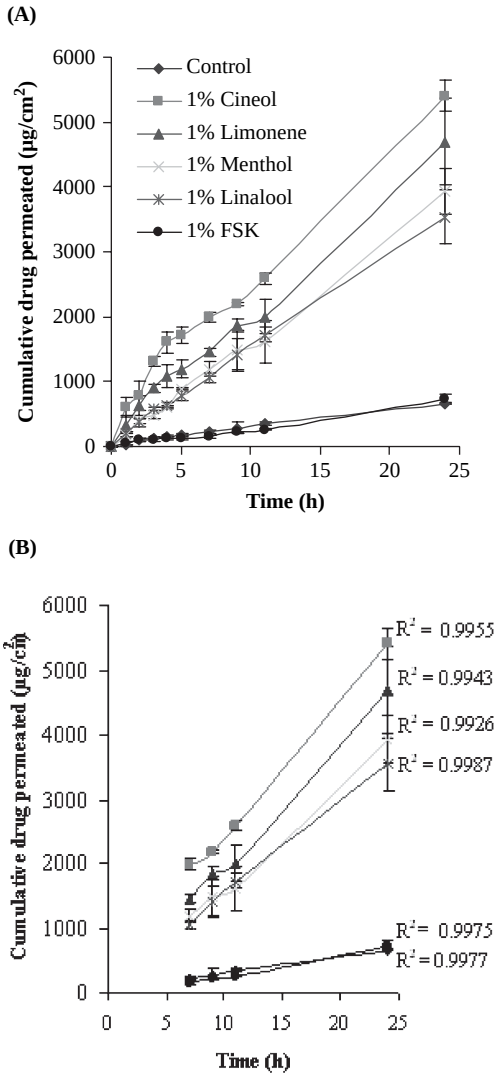

FIGURE 2. (A) Permeation profile of valsartan across rat skin in absence and presence of various enhancers (1% w/v) in vehicle; (B) Graph showing the data points selected for regression analysis along with correlation coefficients.

(Figures 2 and 3). The passive diffusion (without enhancer) of valsartan through wistar rat skin produced a flux of 27.1 µg/cm^2/h (Table 2), which was well short of the target flux required to achieve the therapeutically effective plasma concentration of valsartan (1.64 mg/L) (Jackson, 2006; Moffat, 2006).

All the enhancers, except FSK, used in the study provided a significant ($P < 0.05$) increase in the flux at concentration of 1% w/v across rat skin. The effectiveness of enhancers at 1% concentration was in the following order: cineole > d-limonene > l-menthol > linalool > FSK (Figure 2). The flux value dipped when the enhancer concentration was increased to 3% w/v except in case of linalool and FSK. At 5% w/v concentration, valsartan flux was generally lower than that at 1% w/v concentration for all the terpenes except in case of linalool and FSK where the difference was not

TABLE 2
Effect of Different Terpenes on Permeation of Valsartan Across Rat Skin and Human Cadaver Skin

		J(μg/cm2/h)* ± *SD*)		Kp × 10^3 (cm/h) ± *SD*		Tlag (h) ± *SD*		ER ± *SD*	
	% Enhancer	RS	HCS	RS	HCS	RS	HCS	RS	HCS
Control	—	+27.07 ± 2.80	17.76 ± 2.26	10.80 ± 0.001	7.11 ± 0.001	3.8 ± 0.68	7.3 ± 2.11	—	—
Cineole	1%	208.06 ± 32.81	*36.93 ± 7.91	83.22 ± 0.013	14.77 ± 0.003	0.5 ± 0.30	0.6 ± 0.17	7.70 ± 1.67	2.08 ± 0.22
	3%	*106.56 ± 19.32	—	42.62 ± 0.008	—	0.7 ± 0.38	—	3.90 ± 0.94	—
	5%	*75.76 ± 21.40	—	30.30 ± 0.009	—	2.2 ± 0.92	—	2.80 ± 0.92	—
D-limonene	1%	185.57 ± 18.98	*33.18 ± 5.45	74.23 ± 0.008	13.27 ± 0.002	1.8 ± 0.10	0.6 ± 0.27	6.90 ± 1.17	1.87 ± 0.10
	3%	*50.39 ± 9.20	—	20.16 ± 0.004	—	2.0 ± 0.50	—	1.90 ± 0.44	—
	5%	*71.42 ± 8.64	—	28.57 ± 0.003	—	1.2 ± 0.30	—	2.60 ± 0.49	—
Linalool	1%	146.01 ± 21.63	*29.73 ± 5.26	58.40 ± 0.009	11.89 ± 0.002	0.5 ± 0.30	1.0 ± 0.62	5.40 ± 1.13	1.67 ± 0.50
	3%	149.40 ± 15.55	—	59.76 ± 0.006	—	0.2 ± 0.10	—	5.50 ± 0.95	—
	5%	146.91 ± 16.96	—	58.76 ± 0.007	—	4.2 ± 0.61	—	5.40 ± 0.99	—
L-menthol	1%	161.03 ± 33.23	*28.23 ± 4.29	64.41 ± 0.013	11.29 ± 0.002	0.6 ± 0.12	2.1 ± 0.27	5.90 ± 1.55	1.59 ± 0.42
	3%	113.48 ± 13.41	—	45.39 ± 0.005	—	1.2 ± 0.52	—	4.20 ± 0.77	—
	5%	71.87 ± 25.01	—	28.75 ± 0.010	—	1.5 ± 0.50	—	2.60 ± 1.03	—
FSK	1%	28.67 ± 4.98	19.86 ± 6.01	11.47 ± 0.002	7.94 ± 0.002	3.0 ± 0.44	3.2 ± 0.36	1.10 ± 0.24	1.12 ± 0.48
	3%	*68.44 ± 22.54	—	27.38 ± 0.009	—	2.2 ± 0.99	—	2.50 ± 0.94	—
	5%	27.29 ± 11.00	—	10.92 ± 0.004	—	3.2 ± 0.27	—	1.00 ± 0.44	—

J = Flux; ER = enhancement ratio; Kp = permeability coefficient; t_{lag} = lag time; HCS = human cadaver skin; RS = Rat skin.
+Mean of three observations. *SD* values are given in parentheses.
*Significant at $p < 0.05$.

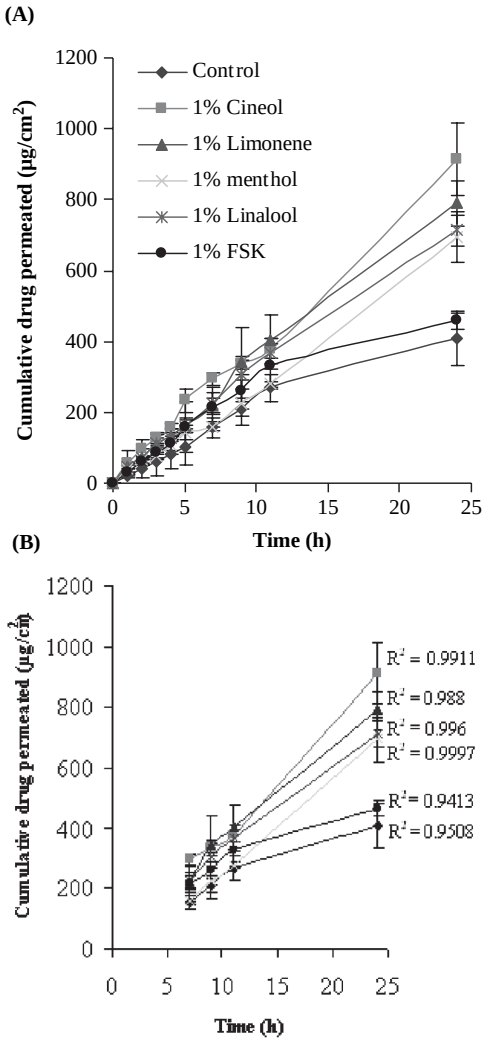

FIGURE 3. (A) Permeation profile of valsartan across human cadaver skin in absence and presence of various enhancers (1% w/v) in vehicle; (B) Graph showing the data points selected for regression analysis along with correlation coefficients.

significant ($p > 0.05$). This implies that 3% w/v is the optimized enhancer concentration in case of FSK which produced a 2.5-fold enhancement over control (without enhancer) (Table 2). Similar permeation profile for diclofenac sodium was reported by Nokhodchi et al. (2007) using different concentration of terpenes as enhancer through wistar rat skin. It was reported that diclofenac sodium flux increased as the concentration of farnesol and carvone was increased from 0 to 0.5% v/v and further at 1% v/v concentration, the flux decreased, and then increased again at 2.5% v/v concentration (Nokhodchi et al., 2007). In our study, the effects of enhancers were investigated on HCS at 1% w/v concentration only and these were found to increase in valsartan flux in the following order: cineole > d-limonene > linalool > l-menthol > FSK (Table 2, Figure 3). All the enhancer treatments including FSK resulted in significant reduction of lag time ($p < 0.05$) relative to control (Table 2).

Among the study terpenes, the boiling point (bp) of cineole (173°C) (Jain et al., 2001) is lesser than those of all the other terpenes. This is an indication of weak cohesive or self association of cineole molecule, which implies that oxygen of functional ether and carbonyl group is free for interaction. Therefore, the energy required for competitive hydrogen bonding in skin ceramide is relatively less for cineole, which can be correlated to higher flux, kp, ER, and lesser t_{lag} found with cineole (Jain, Thomas, & Panchagnula, 2001).

IR spectra of SC treated with terpenes exhibited a decrease in height and area of asymmetric and symmetric CH- stretching suggesting the extraction of lipids from SC by the above terpenes (Figure 4). The maximum extraction of lipids was observed with cineole as the decrease in peak height and area was the highest. Whereas, the extraction of lipids was apparently minimum in the case of d-limonene. The above observations conform to the ex vivo skin permeation results that indicated highest skin permeation rate (flux) values when cineole was added as an enhancer in the vehicle. There was clear difference in the IR spectra of control and FSK treated SC with prominent decrease in asymmetric and symmetric CH- stretching of peak height and area, leading us to conclude that FSK enhances permeation of valsartan by extracting SC lipids. The difference between the IR spectra of control and d-limonene treated SC was not very obvious, which is in contradiction of earlier reports that mention a significant percent decrease in the CH- peak height and area in the IR spectrum of d-limonene treated SC (Zhao & Singh, 1998). The peak shift to a higher wave number was not observed; it is clear that study terpenes did not fluidize the SC lipids (Jain, Thomas, & Panchagnula, 2001; Satyanarayana & Bhaskar, 2002).

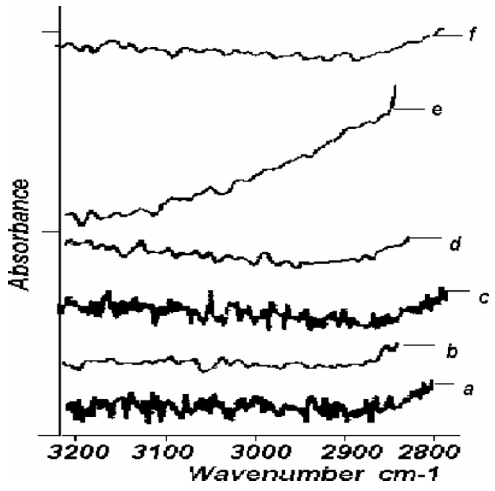

FIGURE 4. FT-IR spectra of rat SC. Change in lipid C-H stretching (2920 cm^{-1}) vibrations after 24 h treatment with (a) control, (b) cineole, (c) d-limonene, (d) linalool, (e) l-menthol, and (f) forskolin.

The rate limiting step or main barrier of transdermal drug delivery is lipophilic part of SC in which lipids (ceramide) are tightly packed as bilayers due to high degree of hydrogen bonding. The amide I group of ceramide is hydrogen bounded to the amide I group of another ceramide, forming a tight network of hydrogen bonding at the head of ceramide. This hydrogen bonding lends strength and stability to lipids bilayers and thus imparts barrier property to SC (Jain, Thomas, & Panchagnula, 2001). When skin is treated with terpenes ceramides may get loosened because of competitive hydrogen bonding leading to breaking of hydrogen bond network at the head of ceramides due to entry of terpenes into the lipid bilayers of SC. The tight hydrogen bonding between ceramides causes split in the peak at 1650 cm^{-1} (amide I) as shown in the control skin spectrum (Figure 5). Treatment with terpenes resulted in either two or single peak at 1650 cm^{-1}, which suggests breaking of hydrogen bonds by terpenes. There was no significant change in the pattern of amide I peak when d-limonene was used as an enhancer, suggesting that d-limonene is not able to break hydrogen bonds. This is further substantiated by the hypothesis that breaking or loosening of hydrogen bond network between ceramides head groups is caused by oxygen containing terpenes such as linalool, l-menthol, cineole and FSK (Jain, Thomas, & Panchagnula, 2001). Hence, d-limonene (terpene without –OH or C=O group) does not break hydrogen bond network between ceramides due to hydrogen bond accepting or donating group. The mechanism of skin permeation enhancement by the investigated terpenes was further elucidated by DSC studies.

DSC thermogram of untreated rat epidermis presented four endotherms (Figure 6). It was observed that both T_2 (82°C) and T_3 (105°C) endotherms completely disappeared or shifted to lower melting points in thermogram of SC treated with the vehicle and the terpenes (d-limonene, linalool, l-menthol, cineole, and FSK). This indicates that the ethanol and the terpenes present in the vehicle enhance skin permeation of drugs through extraction

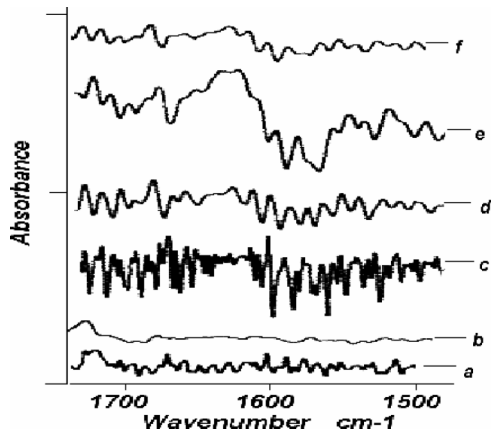

FIGURE 5. FT-IR spectra of rat SC. Change in amide I (1640 cm^{-1}) and amide II (1550 cm^{-1}) stretching (2920 cm^{-1}) vibrations after 24 h treatment with (a) control, (b) cineole, (c) d-limonene, (d) linalool, (e) l-menthol, and (f) forskolin.

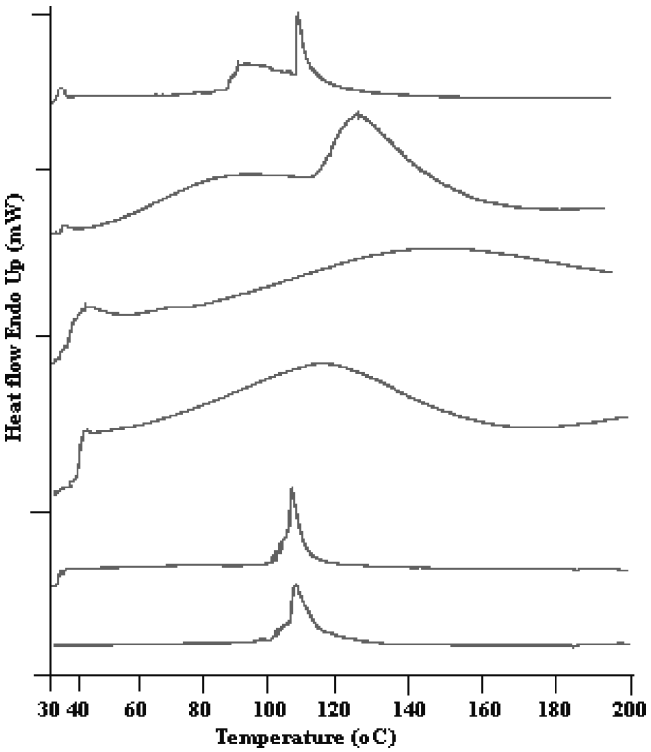

FIGURE 6. DSC thermogram of SC untreated and treated with 1% w/v terpene in vehicle for 24 h. Top to bottom: untreated SC, SC treated with cineole, d-limonene, linalool, l-menthol, and forskolin in vehicle.

of SC lipids (Vaddi, Ho, & Chan, 2002; Yamane, Williams, & Barry, 1995). Ethanol and terpenes (d-limonene, linalool, and FSK) decrease the protein endotherm T_4 to lower melting points, suggesting keratin denaturation and possible intracellular permeation mechanism in addition to the extraction of lipid bilayers by d-limonene, linalool, and FSK. Another observation was that T_4 increased up to 122°C in the case of cineole with a broadening of the peak (Figure 5). Shift to higher transition temperature (Tm) and peak broadening has been attributed to dehydration of SC (Vaddi, Ho, & Chans, 2002) as another mechanism of permeation enhancement in addition to extraction of lipid resulting in higher permeation of active as compared to other terpenes. Hence, it was no surprise that it produced highest flux values through rat skin as well as human cadaver skin.

The photomicrograph of untreated rat skin (control) shows well defined epidermal and dermal layers with layer of corneocytes and skin appendages (sweat glands, hair follicles) (Figure 7 A). When the skin was treated with terpenes (cineole), definite changes were observed in the skin morphology. The disruption and extraction of lipid bilayers was clearly evident as distinct voids and empty spaces visible in the epidermal region (Figure 7 B). The extraction of SC lipids might cause dehydration of SC with significant loss of moisture. It would have been interesting to have measured transepidermal water loss (TEWL) (Fang et al., 2003; Panchagnula et al., 2005; Pillai &

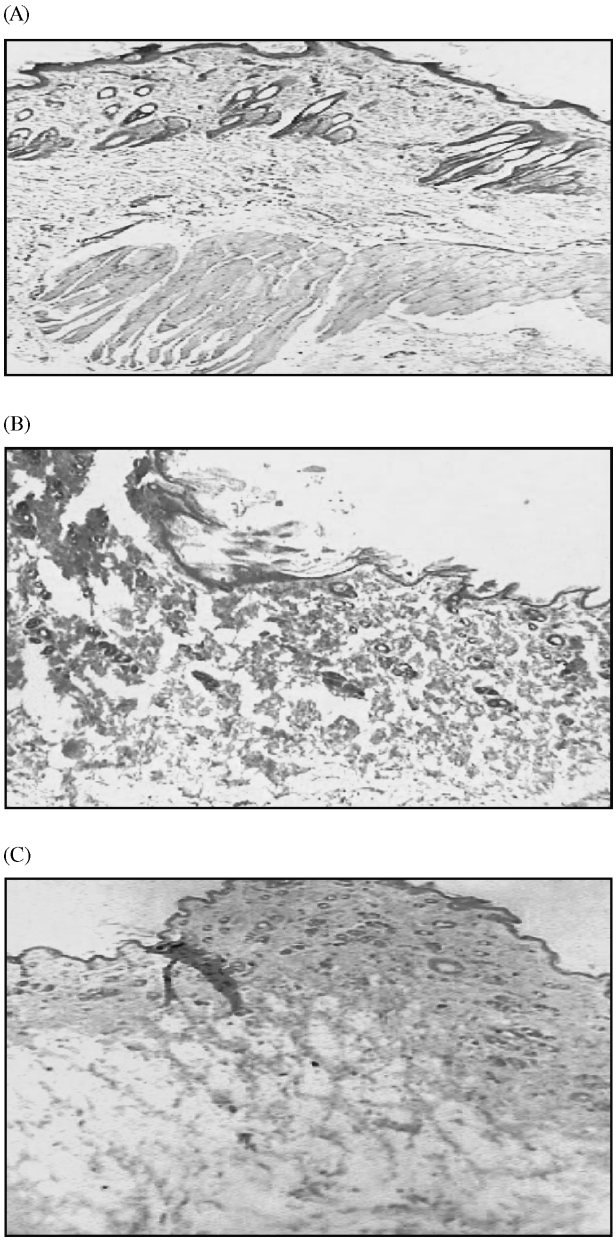

FIGURE 7. Light microscope photographs (× 100) of wistar rat skin after no treatment (A) and 24 h after treatment with 1% cineole (B), and 1% forskolin (C).

Panchagnula, 2003). This would have substantiated the above hypothesis of SC dehydration by cineole treatment. On treatment of rat skin with FSK aberration in skin morphology was very less prominent as compared to cineole treated SC (Figure 7C), which clearly indicates that cineole is far more effective penetration enhancer than FSK. These observations amply support the ex vivo skin permeation data which reflect the superiority of cineole over FSK as skin sorption promoter.

There were no apparent signs of skin irritation (erythema and edema) observed on visual examination of skin specimens treated with either cineole or FSK, indicating absence of any skin irritation as a consequence of terpene treatment. Moreover, terpenes have been proclaimed as Generally Regarded as Safe (GRAS) excipients. Still, further elucidation studies could have been performed to corroborate the above claim, for example, laser Doppler velocimetry (LVD), which is based on difference in measurement of blood flow to the application site before and after enhancer treatment (Panchagnula et al., 2005).

CONCLUSION

Cineole was found to be the most effective enhancer for diffusion of valsartan through rat skin and human cadaver skin and might be potentially used for transdermal delivery of valsartan. The efficacy of the studied terpenes for permeation of valsartan across rat skin and human cadaver skin was found in the order of cineole > d-limonene > l-menthol > linalool > FSK and cineole > d-limonene > linalool > l-menthol > FSK, respectively. Although FSK was found to be the least effective penetration enhancer for permeation of valsartan through rat and human cadaver skin in comparison to established enhancers (terpenes), it produced 2.5-fold enhancement in valsartan flux over control (passive diffusion without enhancer) when used in concentration of 3% w/v. No apparent skin irritation (erythema, edema) was observed on treatment of skin with study terpenes including FSK. FT-IR, DSC, and histopathological studies revealed that FSK enhanced the skin permeation of active medicament by disruption and extraction of lipid bilayers of SC like other terpenes. Further studies may be performed to substantiate the mechanism of action of FSK.

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