Mechanism of Transport Enhancement of LHRH Through Porcine Epidermis by Terpenes and Iontophoresis: Permeability and Lipid Extraction Studies

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Purpose. The purpose of this study was to investigate the effect of 5% terpenes (i.e., limonene, carvone, thymol, and cineole)/ethanol (EtOH) and iontophoresis on the *in vitro* permeability of luteinizing hormone releasing hormone (LHRH) through the porcine epidermis and biophysical changes in the stratum corneum (SC) lipids by fourier transform infrared (FT-IR) spectroscopy.

Methods. The porcine epidermis was pretreated with enhancer for 2 h. The permeability measurement system included Franz diffusion cells, Ag/AgCl electrodes, and SCEPTOR® iontophoretic power source. FT-IR spectroscopy was performed to assess the possible contribution of lipid extraction to the transport enhancement of LHRH.

Results. Terpenes in combination with EtOH significantly ($p < 0.05$) increased the flux of LHRH in comparison with the control (epidermis which was not enhancer treated). Iontophoresis further enhanced ($p < 0.05$) the flux of LHRH through terpenes/EtOH treated epidermis in comparison with their passive permeability. Reversibility studies showed that the post-recovery passive flux of LHRH through 5% limonene in EtOH/iontophoresis treated epidermis was significantly ($p < 0.05$) decreased but did not significantly recover to the baseline flux (i.e., flux through control epidermis). The SC treated with terpenes/EtOH showed a decrease in peak heights and areas for both asymmetric and symmetric C-H stretching absorbances in comparison to untreated SC. A greater percent decrease in peak heights and areas was obtained by limonene/EtOH. However, treatment of the SC with terpenes/EtOH followed by iontophoresis did not further decrease the percentage of peak height and area over and above terpene/EtOH suggesting that iontophoresis alone does not cause SC lipid extraction.

Conclusions. Terpenes/EtOH increased LHRH permeability by enhancing the extraction of the SC lipids. Iontophoresis synergistically enhanced the permeability of LHRH through terpenes/EtOH treated epidermis. Thus, terpenes can be used as chemical enhancers in combination with iontophoresis to enhance the transdermal delivery of peptides such as LHRH.

KEY WORDS: luteinizing hormone releasing hormone; terpenes; fourier transform infrared spectroscopy; lipid extraction; porcine epidermis.

INTRODUCTION

The primary barrier to transdermal diffusion is the stratum corneum (SC), the thin outermost layer of the skin that is comprised of a regular array of protein rich cells embedded in a multilamellar lipid domain. The lipoidal domains are the integral components of the transport barrier, which must be breached if the drugs are to be administered at an appropriate rate (1). In quest for development of a transdermal delivery system for a peptide, iontophoresis in combination with an enhancer may permit the use of lower quantities of enhancer and current within the delivery system. Enhancer (Oleic acid/ethanol) in combination with iontophoresis synergistically enhanced the permeability coefficient of luteinizing hormone releasing hormone (LHRH) by increasing the lipid fluidity and through the loosening and swelling of cell layers of the porcine SC (2,3). Recently, the terpenes were reported to show an enhancement effect on percutaneous drug absorption (4).

Fourier transform infrared (FT-IR) spectroscopy provides information on the vibrational modes of its components and probes the structure on a molecular level. Recent reports suggest a correlation between enhanced permeation of a polar solute (mannitol) and extraction of lipids and proteins from human skin in the presence of 75% (v/v) aqueous alcohol solutions (5). Decreases in the absorbances and the areas of C-H asymmetric and symmetric stretching peaks have been linked to the extraction of SC lipids (6). Therefore the effect of iontophoresis in combination with enhancer on the biophysical properties of the SC needs to be adequately focused.

LHRH is used clinically for inducing ovulation in women with hypothalamic amenorrhea, inducing puberty and spermatogenesis in men with hypogonadotrophic hypogonadism, and in the treatment of prostatic carcinoma. It has also been used in endometriosis and polycystic ovary syndrome (7). In this study, we have investigated the effect of 5% terpenes (i.e., limonene, carvone, thymol, and cineole)/EtOH and iontophoresis on the *in vitro* permeability of LHRH through porcine epidermis and biophysical changes in the SC lipids by fourier transform infrared (FT-IR) spectroscopy.

MATERIALS AND METHODS

Materials

[^{3}H] LHRH (specific activity 51 Ci/mmol) was obtained from NEN Research Products, Wilmington, DE, USA. Ethanol was obtained from Curtison Matison Scientific, Houston, TX. Carvone, limonene, thymol, cineole, and NaCl were purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals used were of analytical grade. De-ionized water (resistivity $\geq$ 18 MΩ) was used to prepare all solutions. The purity of radiolabelled LHRH was evaluated, prior to use, by thin layer chromatography (TLC). The radiochemical purity of the peptide was 98%. Furthermore, electrochemical degradation of the peptide was examined by TLC during the course of the iontophoretic experiments. The electrochemical degradation of LHRH following 8 h iontophoresis was found to be minimal (described elsewhere in detail, ref. 2)

Preparation of Epidermis/SC

Porcine ears were obtained from the local slaughterhouse and after cleaning under cold running water, the outer region of the ear was cut. The whole skin was removed carefully from

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the underlying cartilage with help of a scalpel. The method of Kligman and Christophers (8), with slight modification was adopted to remove the epidermis. The epidermis was prepared by soaking the whole skin in water at 60°C for 45 sec. The skin was removed from the water, blotted dry and pinned with the dorsal side down. The intact epidermis was teased off from the dermis with forceps, washed with water and used in the *in vitro* transport studies.

The epidermis was incubated for 4 h in a 0.5% trypsin solution in phosphate buffered saline (pH 7.4) at 37°C. The tissue was then smoothed on a flat surface and the epidermis removed by rubbing with the moistened cotton tipped applicator. The transparent SC obtained was floated on water, blotted dry, stored in desiccator at room temperature.

In Vitro Studies

The epidermis was pretreated by immersing it in the enhancer solution for 2 h and then washing it with de-ionized water. Ethanol (EtOH), 5% thymol/EtOH, 5% cineole/EtOH, 5% carvone/EtOH, and 5% limonene/EtOH were used as penetration enhancers for pretreatment. Epidermis without pretreatment was used as a control. Franz diffusion cells modified for iontophoresis were used in all transport studies. The control/pretreated epidermis was sandwiched between the cells with the SC facing the donor compartment. The capacities of the donor and the receiver compartments were 2 ml and 5 ml, respectively. The surface area of the epidermis exposed to the solution was 0.785 cm^2. The donor compartment contained 1 ml of LHRH solution [0.2 μCi/ml of LHRH in 0.9% w/v sodium chloride (normal saline)], and the receiver compartment contained 5 ml of normal saline. The donor concentration of LHRH used was 3.92×10^{-3} n moles/ml. The cells were maintained at 37 ± 0.5°C by PMC Dataplate® stirring digital dry block heater (Crown Bioscientific Inc., NJ). The content of the receiver compartment was stirred with a magnetic bar at 100 rpm. At specific intervals, 0.5 ml samples were withdrawn from the receiver compartment and an equivalent amount of normal saline (0.5 ml) was added to maintain a constant volume. Ag/AgCl electrodes (99.99 + % Ag wire plated with AgCl) of 0.5 mm diameter and 4 cm length obtained from Keltronics Corporation, Oklahoma, were used. These electrodes are non-polarisable, reversible and therefore do not decompose water. The anode was placed in the donor and the cathode in the receiver for anodal iontophoresis. LHRH in normal saline is positively charged (9). A constant current of 0.2 mA/cm^2 was applied (SCEPTER^R, Keltronics Corporation, Oklahoma, USA). The results were expressed as the mean ± SD of four experiments.

The samples were assayed by liquid scintillation counting. Each sample was mixed with 10 ml of scintillation cocktail, (Econosafe®, biodegradable counting cocktail, Research Products International Corp., IL), and counted in a liquid scintillation counter (Packard, Tri Carb® 2100 TR, CT). The instrument was programmed to give counts for 10 min.

Reversibility Studies

Reversibility studies were carried out to address the issue of post-enhancer/iontophoresis recovery of LHRH flux. We designed a three step experiment. During step I, passive or iontophoretic *in vitro* transport was carried out for 8 h in the same way as detailed in the *in vitro* studies through either control or enhancer treated epidermis. In step II, the electrodes were removed. The receiver and donor solutions were withdrawn leaving the epidermis intact in the diffusion cells. The donor and receiver compartments with intact epidermal membrane were washed three times with fresh normal saline solution. Thereafter, the donor and receiver compartments were filled with normal saline solution and allowed to recover for the next 12 h. In step III, the receiver and donor compartments were replaced with fresh normal saline and LHRH solution (3.92×10^{-3} n moles/ml), respectively, and passive diffusion was carried out through control or enhancer/iontophoresis treated epidermis. This experimental strategy allowed each condition to serve as its own control. Introduction of a 12 h recovery period allowed the skin to recover and also helped eliminate the drug retention problem.

Following the experiments, passive flux (post-recovery) through the enhancer/iontophoresis treated epidermis were compared with the control (no enhancer/iontophoresis treated epidermis).

FT-IR Spectroscopy Studies

After enhancer and iontophoresis treatments of the SC in the same manner as in the *in vitro* transport study except using the peptide, the samples were vacuum dried (650 mm Hg) at 21 ± 1°C for three days to evaporate off the volatile terpenes and EtOH (10). The samples were then subjected to FT-IR spectroscopic study. FT-IR (Nicolet 210, Madison, WI, USA) was used to accomplish this study. Spectra were obtained in the frequency range 3000–28000 cm^{-1}. All spectra analyzed represent an average of 64 scans with a resolution of 1 cm^{-1}, Happ-Genzel apodized, zero-filling factor of none. Attention was focused on the decrease in the absorbance (i.e., measured in terms of peak height and peak area) of C-H stretching bands (near 2850 and 2920 cm^{-1}) sensitive to alkyl chains of the lipids. Omnic® FT-IR software (Nicolet instrument corporation, Madison, WI) was used to calculate the peak heights and areas. The lower limit for the peak heights and areas is 0.001. For each SC sample, peak heights and areas were measured before and after the enhancer and/or iontophoresis treatment. This experimental strategy allowed each sample to serve its own control.

Data Treatment

The receiver compartment concentration of LHRH was corrected for sample removal by using the equation given by Hayton and Chen (11). The cumulative amount of LHRH permeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as the steady-state flux. Statistical comparisons were made using ANOVA and Duncan's multiple range test with the help of an SAS program. The probability value of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

In Vitro Transport Studies

Figures 1 and 2 demonstrate *in vitro* passive and iontophoretic transport profiles, respectively, of LHRH through 5% ter-

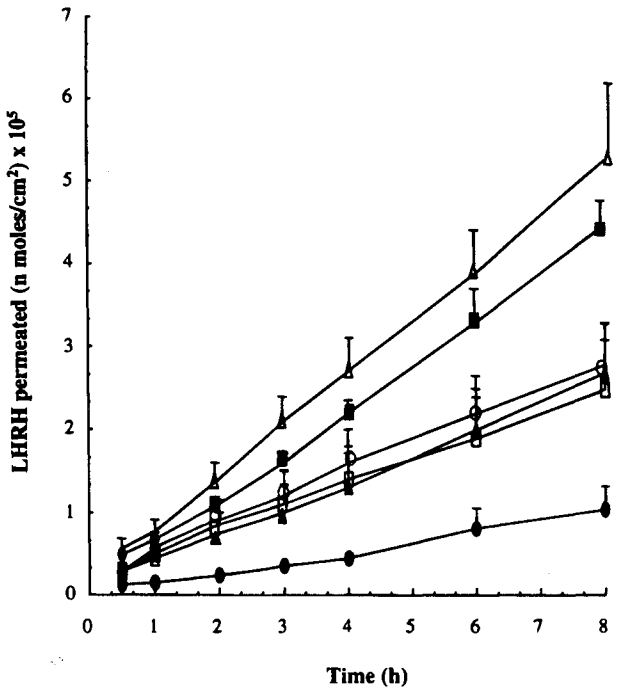

Fig. 1. The effect of 5% terpenes in combination with ethanol on the *in vitro* passive transport of LHRH through porcine epidermis. The donor concentration of LHRH used was 3.92×10^{-3} n moles/ml. Each data point is the mean ± SD of four determinations. Key: (●) control (no enhancer); (□) EtOH; (▲) 5% thymol/EtOH; (O) 5% cineole/EtOH; (■) 5% carvone/EtOH; (△) 5% limonene/EtOH.

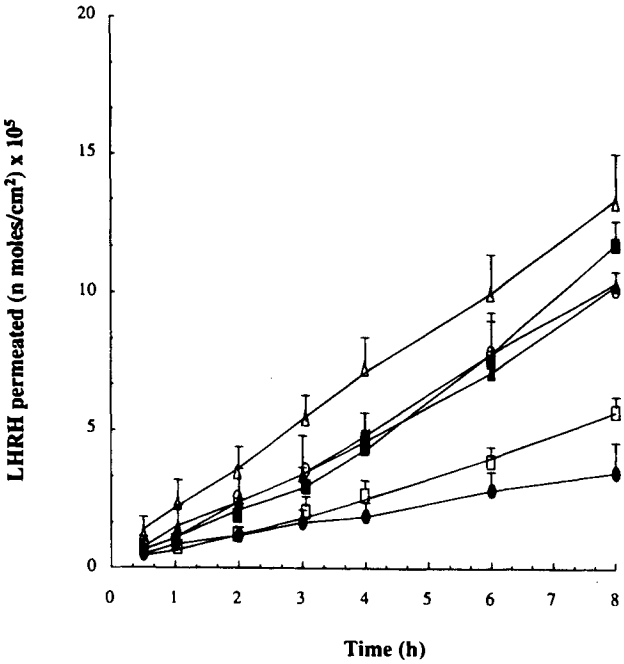

Fig. 2. The effect of 5% terpenes in combination with ethanol on the *in vitro* iontophoretic transport of LHRH through porcine epidermis. The donor concentration of LHRH used was 3.92×10^{-3} n moles/ml. Each data point is the mean ± SD of four determinations. Key: (●) control (no enhancer); (□) EtOH; (▲) 5% thymol/EtOH; (O) 5% cineole/EtOH; (■) 5% carvone/EtOH; (△) 5% limonene/EtOH.

penes (i.e., thymol, cineole, carvone, and limonene) in combination with EtOH pretreated epidermis. Pretreatment of epidermis either with 5% thymol, 5% cineole, 5% carvone, or 5% limonene in combination with EtOH increased the passive transport of LHRH. The flux and enhancement factors of LHRH are given in Table 1. All the terpenes (5%) in combination with EtOH significantly ($p < 0.05$) increased the flux of LHRH in comparison with control (no enhancer treated epidermis). The flux of LHRH by 5% terpenes in combination with EtOH pretreated epidermis in comparison to the passive flux through the control was 2.17, 2.10, 3.79, and 4.71, respectively (Table 1). Iontophoresis further increased the transport of LHRH through the 5% terpenes/EtOH pretreated epidermis (Figure 2). The iontophoretic flux of LHRH through 5% terpenes in combination with EtOH pretreated epidermis was significantly enhanced ($p < 0.05$) in comparison to the control epidermis. Also, a significant enhancement ($p < 0.05$) in the iontophoretic flux of LHRH was found through 5% terpenes in combination with EtOH pretreated epidermis in comparison with their passive flux (Table 1). Furthermore, iontophoretic flux of LHRH through 5% terpenes in combination with EtOH pretreated epidermis was significantly greater ($p < 0.05$) in comparison with iontophoretic flux of LHRH through EtOH pretreated epidermis signifying the enhancement effect of thymol, cineole, carvone, and limonene beyond EtOH. Enhancement in the iontophoretic flux of LHRH (E_2 value) through thymol, cineole, carvone, and limonene in combination with EtOH pretreated epidermis in comparison to iontophoretic flux through the control was 3.30, 3.40, 3.78, and 4.30, respectively (Table 1). Enhancement in the iontophoretic flux of LHRH (E_3 value) through thymol, cineole, carvone, and limonene in combination with EtOH pretreated epidermis in comparison to passive flux through the control was 9.80, 10.07, 11.21, and 12.75, respectively (Table 1).

Table 1. Flux and Enhancement Factor of LHRH Due to 5% Terpenes in Combination with Ethanol and Iontophoresis[a]

Enhancer	Flux (n moles/cm²/h) × 10⁶ (mean ± SD, n = 4)		Enhancement Factor		
	P	I	E_1	E_2	E_3
Control	1.40 ± 0.16	4.15 ± 1.26[f]	—	—	—
EtOH	2.94 ± 0.55[b]	7.94 ± 0.24[d,e]	2.1	1.9	5.7
5% thymol/EtOH	3.36 ± 0.57[b]	13.72 ± 1.26[d,e,f]	2.4	3.3	9.8
5% cineole/EtOH	3.03 ± 0.42[b]	14.10 ± 0.28[d,e,f]	2.2	3.4	10.1
5% carvone/EtOH	5.30 ± 0.50[b,c]	15.70 ± 2.98[d,e,f]	3.8	3.8	11.2
5% limonene/EtOH	6.60 ± 1.20[b,c]	17.85 ± 3.30[d,e,f]	4.7	4.3	12.8

[a] P = Passive; I = Iontophoresis; Control = no enhancer; EtOH = Ethanol. E_1 = [Flux with enhancer treated epidermis (passive)]/[Flux with no enhancer treated epidermis (passive)]; E_2 = [Iontophoretic flux with enhancer treated epidermis]/[Iontophoretic flux with no enhancer treated epidermis]; E_3 = [Iontophoretic flux with enhancer treated epidermis]/[Flux with no enhancer treated epidermis (passive)].
[b] Significantly ($p < 0.05$) different from control (passive no enhancer).
[c] Significantly ($p < 0.05$) different from ethanol (passive).
[d] Significantly ($p < 0.05$) different from iontophoresis alone (no enhancer).
[e] Significantly ($p < 0.05$) different from ethanol (iontophoresis).
[f] Significantly ($p < 0.05$) different from the corresponding passive permeability.

From above results, it can be inferred that iontophoresis is synergistic with enhancers, such as terpenes in combination with EtOH, to provide an additional driving force to maintain and control the target flux of LHRH. Researchers assessed a series of terpenes as percutaneous enhancers toward the model penetrants (12–14). The authors suggested that the terpenes increased the permeation of solutes by disrupting highly ordered structure of intercellular lipids, improving the partitioning of solutes in the SC and/or creating polar pathways through which ionic and polar drugs pass.

The ranking of the skin permeability of different species *in vitro* has been determined (15). The histological characteristics of pig and human skin have been reported to be comparable (16). Skin from the pig generally approximates the permeability of human skin (17). Although there is no commercial transdermal drug delivery device available for LHRH, such a device exists for the LHRH analog (nafarelin), which is approved in the form of a nasal spray application for the treatment of endometriosis. The dose recommended is 200 μg twice a day with a bioavailability of 3.6%, i.e., the amount reaching systemic circulation is 10–20 μg/day (18). Thus, a transdermal delivery formulation which could deliver ~ 1 μg/cm^2/day would need a 10–20 cm^2 patch size to be bioequivalent in terms of dose. In this study, we could achieve iontophoretic flux of 1.5×10^{-2} μg/cm^2/h through 5% limonene/EtOH treated epidermis at current density of 0.2 mA/cm^2 using a very low donor concentration (4 μg/mL) of LHRH. With the usual concentration (e.g., 5 mg/mL), it seems easy to attain the required therapeutic levels using a low current density and smaller patch size.

Reversibility Studies

Skin damage caused by electric current (19) or enhancer (20) has been studied by many researchers. In these studies, skin damage was assessed by comparing the passive permeability coefficient before and after iontophoresis (19). The above method has the advantage of self-comparison. However, skin has been shown to have a reservoir effect for many compounds (21). The drug/enhancer retained in the skin during iontophoresis and passive diffusion could conceivably diffuse into the receptor compartment. To overcome this problem, we designed a three-step experiment.

Table 2 shows the post-recovery passive flux of LHRH through enhancer(s)/iontophoresis treated epidermis. Ideally, if the epidermis has undergone a complete recovery in the 12 h after termination of enhancer(s)/iontophoresis application, then the post-recovery passive flux through enhancer(s)/iontophoresis treated epidermis should not be different than the baseline flux through the control (no enhancer/iontophoresis treated epidermis).

Table 2. Post-Recovery Passive Flux of LHRH Through Enhancer(s)/Iontophoresis Treated Porcine Epidermis[a]

Enhancer	Post-recovery passive flux (nmoles/cm^2/h) × 10^6 (Mean ± SD, n = 4)	
	P′	P″
Control	1.60 ± 0.23	1.95 ± 0.83
EtOH	1.74 ± 0.08	2.68 ± 0.12
5% limonene/EtOH	3.18 ± 0.89	6.31 ± 1.53

[a] P′ = Post-recovery passive flux through control or enhancer treated epidermis. P″ = Post-recovery passive flux through iontophoresis or enhancer and Iontophoresis treated epidermis.

Post-recovery passive flux through iontophoresis treated epidermis is not significantly different ($p > 0.05$) than the post-recovery passive flux through the control. However, post-recovery passive flux through EtOH/iontophoresis treated epidermis was significantly greater ($p < 0.05$) than the control. Also, the post-recovery passive flux was significantly higher ($p < 0.05$) through limonene/EtOH or limonene/EtOH in combination with iontophoresis treated epidermis in comparison to the post-recovery passive flux through the control. Thus, our results show that the post-recovery passive flux of LHRH through EtOH/iontophoresis or limonene/EtOH and iontophoresis treated epidermis was significantly lower ($p < 0.05$) than pre-recovery values (Table 1), but did not completely recover to the baseline post-recovery control flux.

Other investigators have also shown that the *in vitro* passive permeability is increased following application of an iontophoretic current (20,22). Laser scanning confocal microscopy (LSCM) and impedance spectroscopy were used to assess the recovery of enhanced calcein permeability through hairless mouse skin following 2 h of current passage at 0.5 mA/cm^2. The time required for hairless mouse skin to return to pre-iontophoresis levels was at least 18 h (22). Precise physiological changes that are induced in the skin by iontophoresis are not defined. One possible scenario is that the concentration of current flow through appendageal "shunts" or other skin imperfections results in significant local heating owing to the resulting high-current densities. This heating may disorder the adjacent intercellular lipids with a concomitant increase in overall skin permeability (23).

FT-IR Spectroscopic Studies

FT-IR studies were performed to further understand the possible contribution of lipid extraction to the transport enhancement of LHRH due to EtOH or terpenes/EtOH in combination with iontophoresis. The lipid extraction resulting from terpenes/EtOH in combination with iontophoresis was evaluated by comparing the peak heights and areas of the asymmetric and symmetric C-H stretching absorbances before and after penetration enhancer/iontophoresis treatment.

The IR spectra from 3000-2800 cm^{-1} of the SC treated with 5% terpenes/EtOH are shown in Figure 3. Tables 3 and 4 show the percent change in asymmetric and symmetric peak heights and areas, respectively, of the SC treated with 5% terpene/EtOH. SC treated with 5% terpenes/EtOH showed a decrease in peak heights and areas for both asymmetric and symmetric C-H stretching in comparison to untreated SC. A greater percent decrease in peak height was obtained by 5% limonene/EtOH, which was 61.61 and 66.00 for asymmetric and symmetric C-H stretching absorbances, respectively (Table 3). Also, SC treated with 5% limonene/EtOH showed a greater decrease in peak area for the asymmetric and symmetric C-H stretching absorbances of 60.13 and 63.04, respectively (Table 4). However, treatment of the SC with 5% terpenes/EtOH followed by iontophoresis did not further decrease the percent

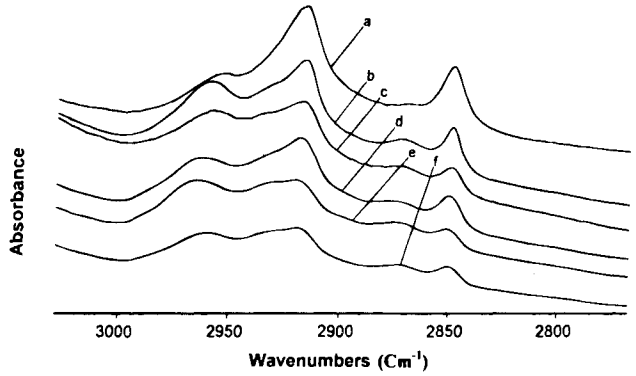

Fig. 3. FT-IR spectra from 3000-200 cm^{-1} of the porcine SC treated with 5% terpenes/EtOH. (a) Control; (b) EtOH; (c) 5% thymol/EtOH; (d) 5% cineole/EtOH; (e) 5% carvone/EtOH; (f) 5% limonene/EtOH.

of peak heights and areas over and above 5% terpene/EtOH depicting that iontophoresis alone does not cause SC lipid extraction (Tables 3 and 4).

Goates and Knutson (5) showed that at high concentration (75% v/v) EtOH leads to lipid and protein extraction. Bommannan *et al.* (6) found that EtOH did not produce lipid disordering, but caused appreciable lipid extraction *in vivo*, which may be responsible for rendering the SC more permeable in the presence of EtOH. Removal of protein and lipid components of the SC was suggested to be the primary source of alcohol-enhanced permeation of ionic solute through the skin (24). Osigo *et al.* (25) found a linear relationship between flux and removal of ceramides from hairless rat skin by limonene and cineole pretreatment, indicating that the removal of intercellular lipids would cause dramatic dilations between adherent cornified cells. This would facilitate the iontophoretic and passive transport of hydrophilic solutes due to the resultant increase in free volume. Our findings provide evidence that iontophoresis does

Table 3. Changes in Symmetric and Asymmetric C-H Stretching Absorbance Peak Heights After Treatment with 5% Terpenes in Combination with Ethanol[a]

	Peak height (mean ± SD, n = 3)					
	Asymmetric			Symmetric		
Enhancer	Control	Treatment	% decrease	Control	Treatment	% decrease
EtOH	0.30 ± 0.01	0.16 ± 0.01	48.7	0.18 ± 0.01	0.09 ± 0.00	50.3
EtOH (I)		0.16 ± 0.01	49.0		0.09 ± 0.00	51.4
5% thymol/EtOH	0.34 ± 0.01	0.16 ± 0.01	53.4	0.21 ± 0.01	0.10 ± 0.00	54.8
5% thymol/EtOH (I)		0.16 ± 0.00	53.4		0.10 ± 0.00	54.8
5% cineole/EtOH	0.35 ± 0.00	0.16 ± 0.00	54.1	0.23 ± 0.01	0.10 ± 0.01	55.8
5% cineole/EtOH (I)		0.16 ± 0.00	54.7		0.10 ± 0.01	56.8
5% carvone/EtOH	0.31 ± 0.00	0.13 ± 0.00	59.3	0.20 ± 0.00	0.08 ± 0.02	60.2
5% carvone/EtOH (I)		0.13 ± 0.00	58.5		0.08 ± 0.02	58.9
5% limonene/EtOH	0.31 ± 0.06	0.12 ± 0.03	61.6	0.20 ± 0.04	0.07 ± 0.01	66.0
5% limonene/EtOH (I)		0.12 ± 0.03	60.3		0.07 ± 0.01	66.0

[a] I = Iontophoresis; EtOH = Ethanol. % decrease = 100 − [(absorbance peak height due to treatment/absorbance peak height due to control) × 100].

Table 4. Changes in Symmetric and Asymmetric C-H Stretching Absorbance Peak Areas After Treatment with 5% Terpenes in Combination with Ethanol[a]

	Peak area (mean ± SD, n = 3)					
	Asymmetric			Symmetric		
Enhancer	Control	Treatment	% decrease	Control	Treatment	% decrease
EtOH	5.95 ± 0.06	2.99 ± 0.21	49.8	2.15 ± 0.06	0.98 ± 0.05	54.4
EtOH (I)		2.98 ± 0.21	50.0		0.96 ± 0.04	55.3
5% thymol/EtOH	7.39 ± 0.13	3.42 ± 0.25	53.8	2.59 ± 0.12	1.11 ± 0.02	57.2
5% thymol/EtOH (I)		3.42 ± 0.25	53.8		1.11 ± 0.02	57.2
5% cineole/EtOH	7.58 ± 0.08	3.34 ± 0.12	56.0	2.77 ± 0.07	1.20 ± 0.12	56.7
5% cineole/EtOH (I)		3.33 ± 0.11	56.1		1.15 ± 0.18	58.5
5% carvone/EtOH	6.57 ± 0.03	2.73 ± 0.59	58.5	2.33 ± 0.00	0.93 ± 0.02	60.1
5% carvone/EtOH (I)		2.79 ± 0.60	57.5		0.98 ± 0.11	58.0
5% limonene/EtOH	6.77 ± 1.38	2.70 ± 0.52	60.1	2.41 ± 0.51	0.90 ± 0.15	63.0
5% limonene/EtOH (I)		2.70 ± 0.60	60.1		0.90 ± 0.15	63.0

[a] I = Iontophoresis; EtOH = Ethanol. % decrease = 100 − [(absorbance peak area due to treatment/absorbance peak area due to control) × 100].

not cause lipid extraction (Tables 3 and 4). However, iontophoresis could utilize permeability defects of additional free volume created by lipid extraction due to chemical enhancers and enhance the permeability of ionic and hydrophilic solute such as LHRH.

CONCLUSIONS

The present studies identify the synergy between terpenes (i.e., limonene, carvone, cineole and thymol)/EtOH and iontophoresis as a technique to enhance and control the transdermal delivery of LHRH. Terpenes (5%) in combination with EtOH significantly ($p < 0.05$) increased the flux of LHRH in comparison with the control (no enhancer treated epidermis). Iontophoresis further increased the flux of LHRH through 5% terpenes/EtOH pretreated epidermis. Reversibility studies demonstrate that the recovery of enhanced LHRH flux through treated epidermis did not reach the baseline flux within a 12 h recovery period. Furthermore, our FT-IR results showed that the SC treated with 5% terpenes/EtOH decreased the percent peak heights and areas for both asymmetric and symmetric stretching absorbances in comparison to untreated SC. Thus, 5% terpenes/EtOH increased LHRH permeability by enhancing the SC lipids extraction.

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