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COMBINATION TREATMENTS OF CHEMICAL ENHANCERS WITH LOW FREQUENCY ULTRASOUND FOR THE TRANSDERMAL DELIVERY OF HYDROCORTISONE

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ABSTRACT

The aim of this study was to investigate combination treatments of chemical enhancers and 20 kHz ultrasound across porcine skin in order to identify possible synergistic and/or additive effects. For this purpose, three different classes of permeation enhancers were selected. These were: terpenes, fatty acids and sodium lauryl sulphate (a surfactant). Terpenes were chosen as their low cutaneous irritancy makes them attractive for clinical use. Fatty acids were chosen due to their general potency, widespread historical use and established status as dermal enhancers. Sodium lauryl sulphate was chosen as it has already been proven to act synergistically with low frequency ultrasound. Throughout, hydrocortisone was used as a model drug screen the selected ultrasound application with various chemical enhancer pre-treatments. The 300s concurrently-applied beam, at a 10% duty cycle was used as an application protocol for the study. Synergism with menthone and sodium lauryl sulphate occurred. More interesting was the fact that ultrasound exposure following 1% SLS treatment caused a highly significant synergistic 8.8-fold increase in hydrocortisone delivery. Treatment with 0.25% SLS and ultrasound caused a significant additive effect. A simultaneous administration of all three treatments could be more effective and probably simpler to apply to hydrocortisone compared with passive transdermal delivery.

Keywords: Sonophoresis, chemical enhancers, low frequency ultrasound, transdermal, skin permeation, hydrocortisone.

INTRODUCTION

Terpenes are naturally occurring volatile oils that have been tested fairly extensively as percutaneous chemical enhancers (Vaddi *et al*., 2002; Williams and Barry, 2004). Although these compounds can exhibit good transdermal enhancement ability, they usually cause only low cutaneous irritancy. Hence, terpenes have been given the designation of "generally recognized as safe" (GRAS) by the Food and Drug Administration (FDA) (Godwin and Michniak, 1999; Asbill and Michniak, 2000).

Application of differential scanning calorimetry, X-ray diffraction and infra-red techniques have shown that the terpenes act at least in part by modifying the intercellular lipids of the stratum corneum and disrupting their highly ordered structure (El-Kattan *et al*., 2001). Differential scanning calorimetry research on hydrated stratum corneum samples showed that applied 1, 8-cineole did in fact act in this way (Cornwell and Barry, 1993). Narishetty and Panchagnula (2004) chose 1, 8-cineole, menthol, α-terpineol and (+)-carvone and studied the effects of each of these oxygen-containing terpenes on zidovudine permeation across rat skin *in vitro*. All of these terpenes significantly increased the transdermal flux of the hydrophilic drug. Molecular modelling showed that terpene molecules probably hydrogen bond with intercellular lipid head groups of the stratum corneum. This disrupts the pre-existing interlamellar hydrogen bonding network and increases the distance between two opposite lamellae. This effectively creates new polar pathways or channels through the stratum corneum barrier. Terpenes seem to be more effective as enhancers when they are formulated in propylene glycol (Vaddi *et al*., 2002).

Over the years, many terpene studies have been performed in order to rationalise the selection of the best terpene for any specific percutaneously applied drug. Okabe and co-authors (Okabe *et al*., 1989) studied ten different cyclic monoterpenes. They reported that terpenes with lipophilic indices greater than zero were most effective at enhancing indomethacin delivery *in vitro*. In general, it appears that lipophilic terpenes such as the hydrocarbons tend to be effective for promoting the absorption of lipophilic drugs. In contrast, more hydrophilic oxygen-containing terpenes such as alcohols and ketones are effective for promoting the absorption of hydrophilic drugs (Williams and Barry, 2004; Hori *et al*., 1991; Williams and Barry, 1991a; Williams and Barry, 1991b; Fang *et al*., 2007).

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Up to our knowledge there seems to be one literature report by Mutalik *et al*. (2009) dealing with the combined effect of terpenes and low frequency ultrasound on transdermal drug absorption using full thickness mouse skin which is generally more permeable than human skin due to a high density of hair follicles as well as other structural and biochemical differences (Walters and Roberts, 1993). The GRAS status of terpenes means that they probably would be good candidates for combined use with sonophoresis if the treatments acted synergistically. In order to explore this idea, four monocyclic terpenes were selected for use in the sonophoresis studies. These were (+)-carvone, 1,8-cineole, menthone and α-terpineol. It is interesting to note that three of these are chemically-distinct structural isomers -1,8-cineole (an ether), α-terpineol (an alcohol), and menthone (a ketone). These three terpene molecules have a molecular weight of 154 Da. (+)-carvone is also a ketone but with a molecular weight of 150 Da. All four of these hydrophilic terpenes have a free hydroxyl or oxygen group and so they should be able to perturb stratum corneum lipid packing through the formation of hydrogen bonds (Vaddi *et al*., 2002; Fang *et al*., 2007). All four terpenes have a similar density of ~0.9 g/ml.

Fatty acids are one the most extensive studied group of transdermal permeation enhancers (Wang *et al*., 2004). It is known that these enhancers act by disrupting intercellular lipid packing in the stratum corneum, allowing any applied drug to more readily permeate through the layer. The exact mechanisms are not yet fully understood. It is possible that certain fatty acids can extract a fraction of the endogenous stratum corneum membrane components, causing phase separation to occur. This will lower the proportion of crystalline lipids and create more permeable fatty acid-rich domains (Rowat *et al*., 2006). Chemically, fatty acids consist of an aliphatic hydrocarbon chain and a terminal carboxyl group. They differ from each other in various parameters such as hydrocarbon chain length as well as the number, position and configuration of double bonds. Other differences include the extent of hydrocarbon chain branching or the presence of additional functional groups. The enhancer potency of a fatty acid is known to be related to these molecular properties.

For saturated fatty acids, a hydrophobic chain length of about 12 carbon atoms was found to be optimal, generally possessing an optimal balance between partition coefficient, solubility parameter and affinity to skin (Aungst *et al*., 1986; Aungst, 1989; Elyan *et al*., 1996). For unsaturated fatty acids, chain lengths of about 18 carbon atoms appear to be the best (Williams and Barry, 2004). The presence of double bonds will affect the efficiency of fatty acids as chemical enhancers. It is well established that unsaturated fatty acids are usually more potent than saturated ones in permeating the skin and facilitating enhanced drug delivery (Aungst *et al*., 1986; Bhatia and Singh, 1998). It is important to note that the fatty acids are usually formulated in a propylene glycol vehicle. This allows the so-called "solvent drag mechanism" to take place (Wang *et al*., 2004; Aungst *et al*., 1990) causing better skin penetration of both the fatty acid and the vehicle. In a study by Cotte *et al*. (2004), it was reported that myristic acid was able to penetrate deeper into epidermal layers of the skin when propylene glycol was used as the vehicle. Deeper penetration of the fatty acid leads to improved enhancer activity. Importantly, propylene glycol is widely used in topical formulations (Ho *et al*., 1998) because of its low toxicity to the skin (Fang *et al*., 2003).

The aim in the present study was to explore the combination treatment of each of three fatty acids with low frequency ultrasound. The fatty acid molecules chosen were; oleic acid, linoleic acid and stearic acid.

It can be seen that all three molecules have the same chain length of 18 carbons but differ in the number of double bonds. Linoleic, oleic and stearic acids have two, one and zero double bonds, respectively.

Sodium lauryl sulphate is also of particular interest in relation to sonophoresis as it has already been shown to act synergistically with low frequency ultrasound in permeabilising the skin barrier. Mitragotri *et al*. (2000) studied the combining effect of 20 kHz ultrasound with SLS in an *in vitro* full thickness porcine skin model. It was determined that treatment with SLS alone as well as ultrasound alone both increased the skin permeability of mannitol. Application of SLS alone for 90 minutes produced an approximate 3-fold increase in mannitol permeation, while application of ultrasound alone for 90 min produced an approximate 8-fold enhancement. In the present study, SLS and 20 kHz ultrasound effects were investigated with *in vitro* transport studies, using hydrocortisone as a model drug.

MATERIALS AND METHODS

Materials

menthone, 1,8-cineole, α-terpineol, (+)-carvone, linoleic acid, oleic acid, stearic acid, sodium lauryl sulphate (SLS), hydrocortisone and propylene glycol were purchased from Sigma-Aldrich (Poole, UK). Phosphate buffer saline tablets (pH 7.4), potassium dihydrogen orthophosphate and disodium hydrogen orthophosphate were purchased from Sigma-Aldrich (Poole, UK). [1,2,6,7-3H]-hydrocortisone (74 Ci/mmol) was purchased from Amersham Biosciences (Buckinghamshire, UK). Scintillation fluid (Optiphase HiSafe 3) and scintillation vials were purchased from Fisher Scientific (Loughborough, UK) and Packard Instrument Co. (Meriden, CT), respectively. Double distilled, de-ionised water was employed throughout.

Transport studies

Preparation of donor and receiver solutions

A degassed PBS solution (pH=7.4) was prepared by taking five tablets of PBS tablets and dissolving those in 1 L of double-distilled water to form a solution with a; phosphate concentration of 0.01 M, potassium chloride concentration of 0.0027 M and sodium chloride concentration of 0.137 M. The pH was 7.4. This was used as the receiver fluid in all the studies described in this chapter. A 10 mg mass of hydrocortisone powder was weighed out and dissolved in 100 ml of PBS to form a 0.01% (w/v) solution. A suitable small volume of tritiated hydrocortisone was added and mixed in to form a solution exhibiting an activity of 1 μCi/ml. This was used as the donor solution for the studies.

Preparation of full-thickness skin samples

Porcine ears (Landrace species) were obtained immediately after slaughter from a local abattoir but before steam sterilisation of the tissue. The skins were cleaned under cold running water. The external surface of each ear was sectioned horizontally by scalpel to yield whole skin samples of area ~8 cm^2. The skin sections were visually checked for integrity and then stored in a frozen state (-20°C) for a maximum period of 3 months. Immediately prior to the permeation studies, the porcine skin sections were thawed at room temperature and cut into smaller samples of surface area ~1 cm^2. These skin samples were mounted on the Franz cells.

Preparation of chemical enhancer solutions

For menthone, 1,8-cineole, α-terpineol and (+)-carvone, each enhancer was dissolved in propylene glycol to form a 2% (3/v) solution. Most transdermal studies that involve terpenes incorporate these enhancers in a propylene glycol vehicle in the 1% to 5% w/v concentration range (Nokhodchi *et al*., 2007). A 2% w/v solution in propylene glycol was also used for linoelic acid and oleic acid. For stearic acid, 1.69 g of flakes were weighed and dissolved in ethanol to make a 2% (w/v) stearic acid solution. Ethanol was used as a solvent as this enhancer did not dissolve in propylene glycol. For sodium lauryl sulphate (SLS), 0.25 g and 1 g of the surfactant were each dissolved in separate volumes of PBS to make two solutions of 0.25% (w/v) and 1% (w/v) of aqueous SLS. The 1% w/v value is the FDA-approved concentration that is widely deployed in many cosmetic products. All of these prepared chemical enhancer solutions were each used as pre-treatments in the transport studies.

Exposure to chemical enhancers and ultrasound

The full-thickness skin samples were mounted in Franz cells with associated water jacket system (37ºC) while the receptor solution consisted of pre-degassed PBS (pH= 7.4). The skins were initially left to hydrate in the diffusion cells for 1 hour. During this period, the cells were occasionally inverted so as to allow the escape of any air bubbles that had accumulated on the skin underside.

After the 1 hour hydration period, a 20μl aliquot of the selected chemical enhancer solution was deposited on the pig skin surface and the donor cell was sealed with Parafilm M®. After 2 hours, any available test enhancer solution on the skin surface was removed by pipette. Each porcine skin was left exposed to the air for 30 minutes and at that point the surface appeared dry. Subsequently, 0.5 ml of hydrocortisone solution was deposited on to each skin surface. In the case of ultrasound treatment experiments, the transducer tip of the ultrasound generator was immersed in the donor solution. The application of pulsed ultrasound (10% duty cycle) at a SATA intensity of 3.7 W/cm^2 was then applied for 300s as it was used previously by Sarheed and Frum (2012). Apart from during periods of sonication, the donor compartments were always covered with a strip of Parafilm M® in order to prevent evaporation.

All permeation studies were allowed to proceed for a total period of 28 h. At selected time points, a 100 μl aliquot of receiver solution was withdrawn and replaced by a blank PBS volume. From analysis by liquid scintillation counting and Excel software calculations, it was possible to obtain cumulative permeation data. Each individual study consisted of at least 6 replicate runs. Where needed, a two-way ANOVA was used to test for synergistic effects. These calculations were performed on an IBM-compatible computer using the software package, Prism version 2 (GraphPad Software, San Siego, CA, USA).

RESULTS

Hydrocortisone permeation data

Figures 1 to 9 inclusive show the hydrocortisone cumulative permeation graphs for each of the tested enhancers. Each graph includes a plot representing treatment with; ultrasound alone, chemical enhancer alone, ultrasound and chemical enhancer as well as control conditions. For hydrocortisone permeation, it was determined that under passive conditions, the average permeability coefficient was 3.59×10^{-4} cm/h. We could not find any data relating to hydrocortisone penetration through full-thickness pig skin, but our value is the same order of magnitude as the permeability coefficient value of 1.7×10^{-4} cm/h reported by Fuhrman *et al.* (1997) for hydrocortisone absorption into full-thickness human skin. In our studies, sonication caused the steady state flux to increase significantly by 2.4 times to an average permeability coefficient of 8.78×10^{-4} cm/h. All the calculated steady state flux values and permeability coefficients and enhancement ratios are shown in table 1.

Table 1. Steady state data for the chemical enhancer –ultrasound combination studies.

Treatment (replicates)	J_{ss} (μg cm^{-2} h^{-1}) Mean ± s.e.m	$k_p \times 10^{-4}$ (cm h^{-1}) Mean ± s.e.m	E. R. [#]
Control (n=6)	0.03 ± 0.01	3.6 ± 0.75	1
US only (*n*=7)	0.08 ± 0.01	8.8 ± 1.34	2.4
1,8-cineole (*n*=7)	0.25 ± 0.04	24.5 ± 4.73	6.8
1,8-cineole + US (*n*=7)	0.15 ± 0.01	14.6 ± 1.94	4.1
Menthone (*n*=7)	0.12 ± 0.01	11.7 ± 1.31	3.2
Menthone + US (*n*=6)	0.27 ± 0.04	26.7 ± 4.24	7.4
(+)-carvone (*n*=7)	0.13 ± 0.01	13.2 ± 1.19	3.7
(+)-carvone + US (*n*=7)	0.11 ± 0.01	11.4 ± 1.02	3.2
α-Terpineol (*n*=7)	0.21 ± 0.01	20.7 ± 1.37	5.8
α-Terpineol + US (*n*=7)	0.06 ± 0.01	6.4 ± 1.30	1.8
Linoleic acid (*n*=7)	0.32 ± 0.06	31.7 ± 6.26	8.8
Linoleic acid + US (*n*=7)	0.29 ± 0.08	28.6 ± 8.43	7.9
Oleic acid (*n*=7)	0.55 ± 0.08	55.2 ± 8.75	15.3
Oleic acid + US (*n*=7)	0.51 ± 0.05	51.2 ± 5.81	14.2
Stearic acid + (*n*=7)	0.05 ± 0.01	5.09 ± 0.72	1.4
Stearic acid + US (*n*=7)	0.05 ± 0.01	4.57 ± 1.32	1.3
0.25% SLS (*n*=7)	0.19 ± 0.02	19.3 ± 2.75	5.4
0.25% SLS + US (*n*=7)	0.26 ± 0.02	25.9 ± 2.45	7.2
1% SLS (*n*=7)	0.15 ± 0.01	14.7 ± 1.83	4.1
1% SLS + US (*n*=7)	0.32 ± 0.07	31.6 ± 4.07	8.8

[#]Where E.R. represents the enhancement ratio relative to passive hydrocortisone flux in the absence of ultrasound or chemical treatment.

Terpenes and ultrasound

Figure 1 shows the hydrocortisone permeation plots under the influence of 20 kHz ultrasound and 1,8-cineole - a cyclic ether terepene. When 1,8-cineole was applied to porcine skin in the absence of ultrasound, a subsequent mean drug flux of 0.24 μg/cm^2/h was measured. This is equivalent to a permeability coefficient of 24.5 × 10^{-4} cm/h. This represented a significant enhancement ($p \leq 0.05$) of about 6.8-fold relative to passive hydrocortisone flux. Figure 1 also shows the effect of combining ultrasonication with 1,8-cineole pre-treatment. Surprisingly, the combination treatment produced a lower mean flux of 0.14 μg/cm^2/h. This is equivalent to a permeability coefficient of 14.63 × 10^{-4} cm/h. It represents a 4-fold increase in flux over control levels.

The data for menthone is shown in figure 2, it can be seen that menthone only application produced an average steady state hydrocortisone flux of 0.11 μg/cm^2/h. This means the average permeability coefficient was 11.78 × 10^{-4} cm/h. This is about 3.2-fold higher than the passive hydrocortisone delivery. Menthone combined with ultrasound caused a further significant increase in the permeation of hydrocortisone so that the mean steady state value was 0.26 μg/cm^2/h. This is approximately 7.4 times the mean control value. From table 1, it can be seen that ER [Menthone + Ultrasound] (7.4) > ER [Menthone] (3.2) + ER[Ultrasound] (2.4), strongly suggesting that a synergistic effect is occurring. However, this simple relationship just relates to the mean enhancement values. In order to be sure that synergism is developing and not just an additive effect, it is necessary to take into account the effect of the variability (error bars) of each measurement. This was done by performing a 2-way ANOVA. It was shown that there was indeed a significant interaction ($p = 0.038$) taking place between the ultrasound and the menthone. So synergism was occurring.

Figure 3 depicts the effect of ultrasound and (+)-carvone pre-treatment on hydrocortisone permeation. Application of (+)-carvone alone caused a significant increase ($p \leq 0.05$) in mean hydrocortisone flux (0.13 μg/cm^2/h), representing an enhancement of 3.7-fold over average passive flux levels. Treatment with (+)-carvone followed by low frequency ultrasound caused a slight but statistically insignificant ($p>0.05$) decrease in hydrocortisone permeation compared to the effect of chemical enhancer alone. In this case, the mean flux was 0.11 μg/cm^2/h and this can be converted to a permeability coefficient of 11.23 × 10^{-4} cm/h. This represents a 3.2-fold increase over mean passive flux.

Figure 4 shows the influence of α-terpineol treatment on transdermal hydrocortisone penetration. Treatment of the skin samples with α-terpineol alone resulted in an average

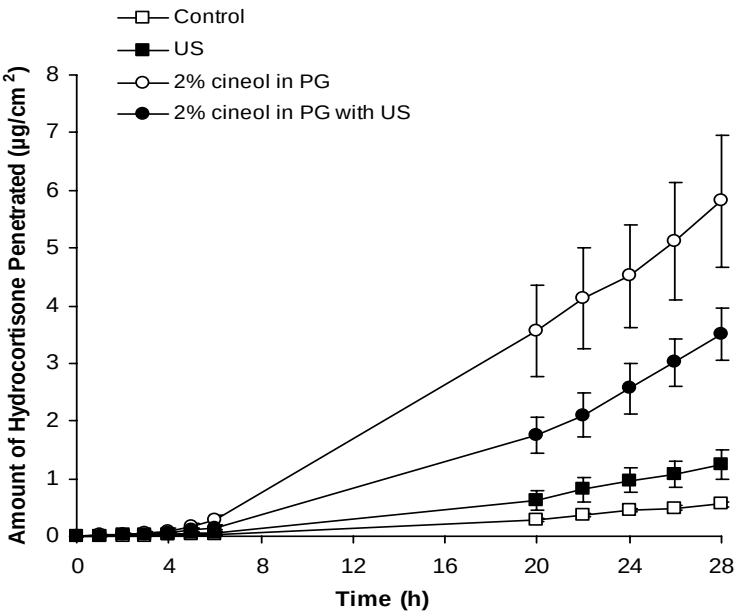

Fig. 1. The influence of 1,8-cineole and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

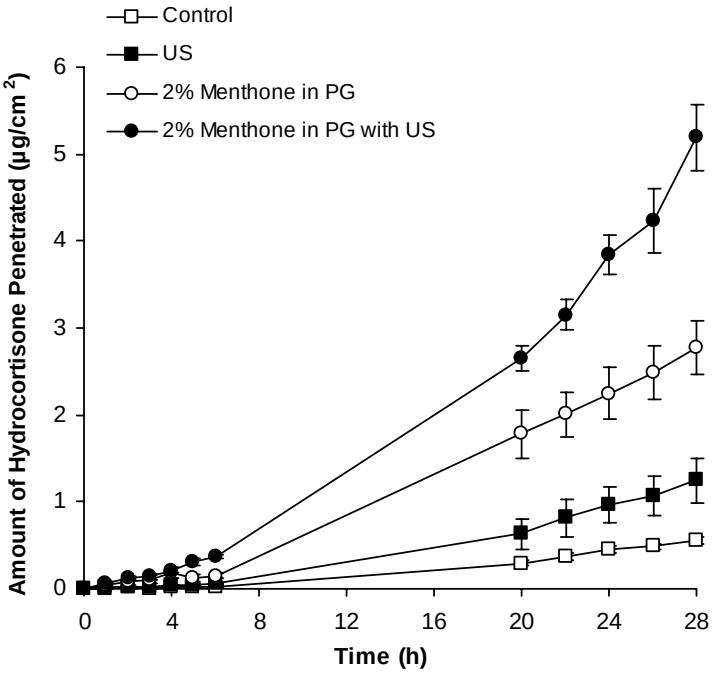

Fig. 2. The influence of menthone and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

hydrocortisone flux of 0.21 µg/cm^2/h, which is a mean permeability coefficient of 20.7 × 10^{-4} cm/h. This represents a statistically significant ($p \leq 0.05$) 5.8-fold increase in drug flux in comparison to control levels. Interestingly, application of ultrasound to α-terpineol-treated skin samples produced less drug permeation. In those experiments, hydrocortisone permeation was decreased to a significant extent ($p \leq 0.05$) to a mean value of 0.06 µg/cm^2/h. This is close to the flux value produced by ultrasound alone treatments.

Fatty acids and ultrasound

Figure 5 presents the effects of linoleic acid pre-treatment and /or ultrasound exposure on hydrocortisone permeation. Deposition of linoleic acid on the skins increased subsequent mean drug flux to 0.32µg/cm^2/h

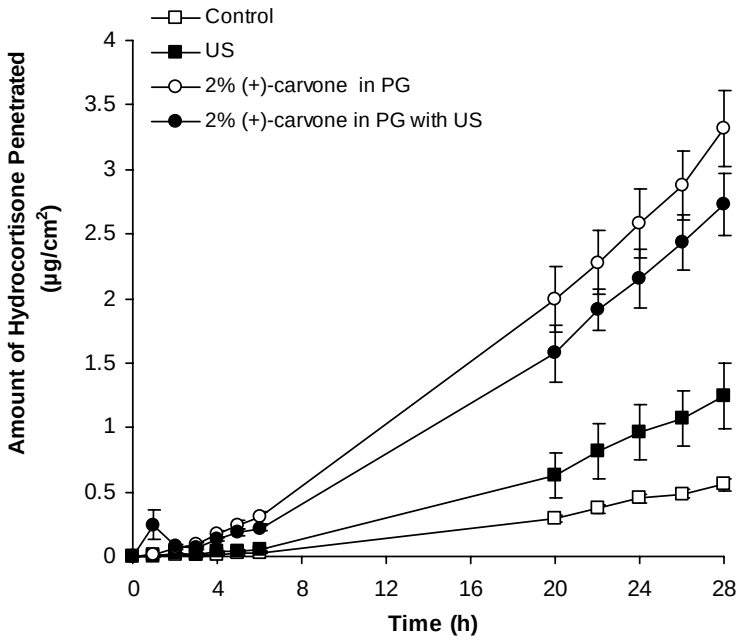

Fig. 3. The influence of (+)-carvone and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

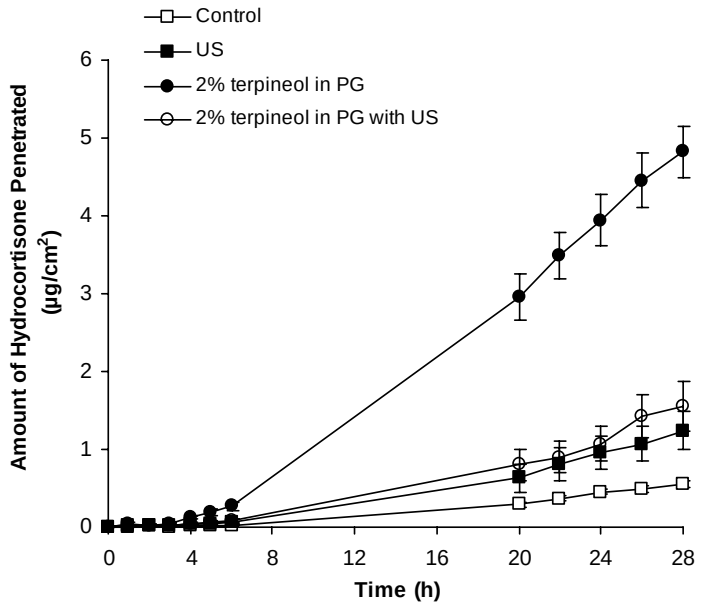

Fig. 4. The influence of α-terpineol and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

(permeability coefficient of 31.72×10^{-4} cm/h). This represents an enhancement ratio of 8.8 over passive hydrocortisone flux. It can be seen that sonication following linoleic acid treatment did not really change hydrocortisone permeation. Average steady state flux was insignificantly reduced to 0.29 µg/cm^2/h, meaning the mean permeability coefficient was 28.67×10^{-4} cm/h.

The cumulative permeation data for oleic acid is shown in figure 6. It should be noted that the plot representing oleic acid only treatment is mostly obscured by the plot representing oleic acid with sonication. Nevertheless, from table 1, it can be seen that the calculated steady state hydrocortisone flux was 55.2 ± 8.75 µg/cm^2/h. This penetration rate is about 15 times higher than that obtained in the absence of enhancer. Interestingly, later

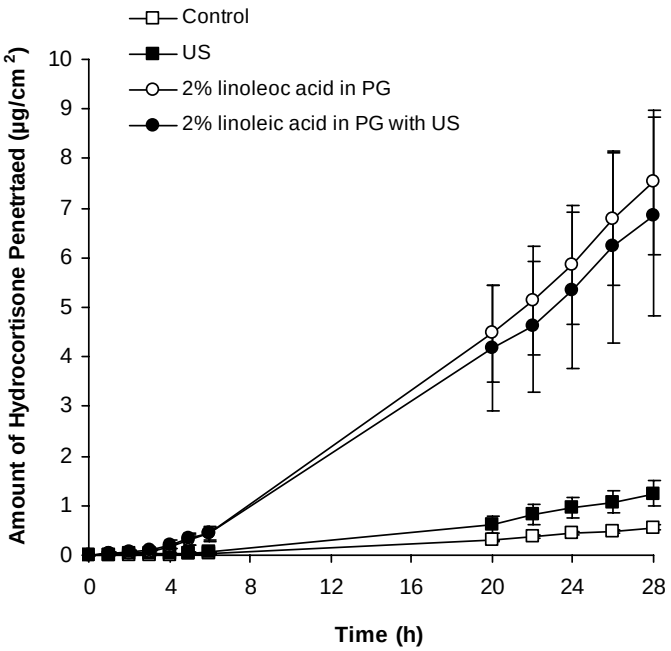

Fig. 5. The influence of linoleic acid and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

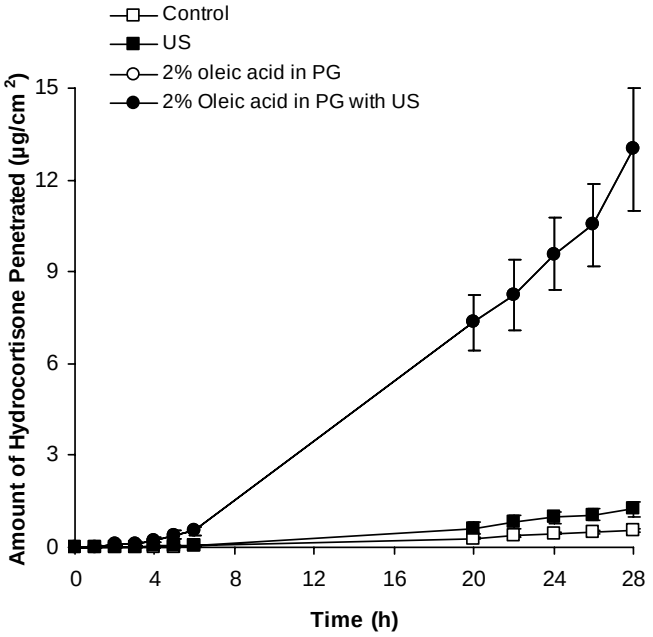

Fig. 6. The influence of oleic acid and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

application of 20 kHz ultrasound did not significantly affect drug absorption in comparison to oleic acid only treatment.

Figure 7 presents the plots for the stearic acid data. It is apparent that both the chemical enhancer only and chemical enhancer with sonication protocols produced relatively mild drug transport enhancement of 1.4-fold and 1.3-fold, respectively.

Sodium lauryl sulphate (SLS) and ultrasound

Figure 8 depicts the hydrocortisone permeation data when 0.25% SLS was employed as the chemical enhancer. It can be seen that skin treatment with SLS only

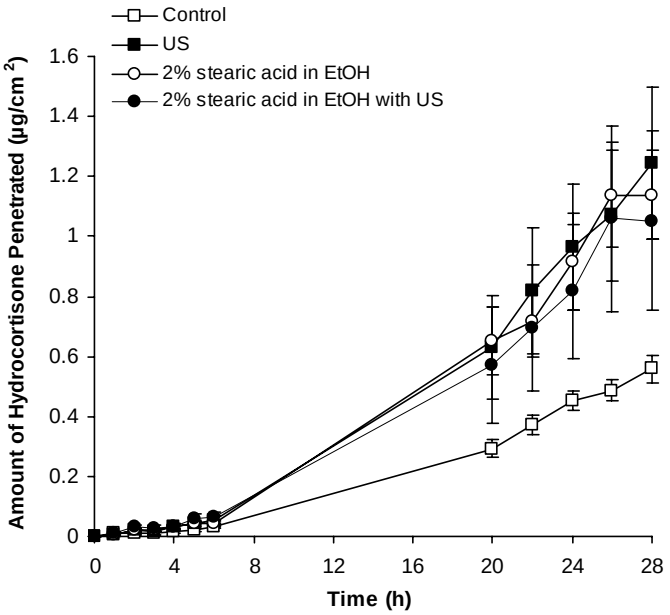

Fig. 7. The influence of stearic acid and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

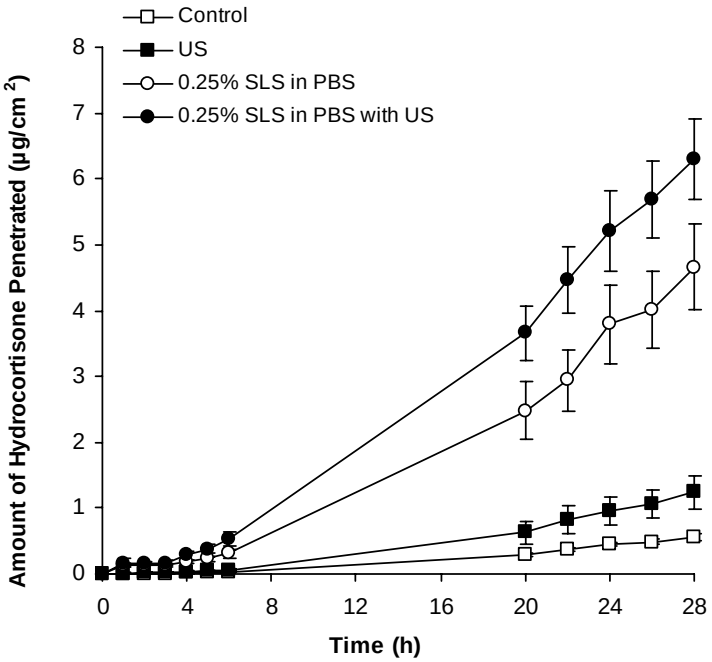

Fig. 8. The influence of 0.25% SLS and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

significantly enhanced steady state hydrocortisone flux by over 5-fold relative to control levels. In fact, a mean flux of 0.19 µg/cm^2/h was measured. When SLS-treated skin was exposed to 20 kHz ultrasound, drug flux was significantly enhanced even further to 0.26 ± 0.02 µg/cm^2/h. By examining table 1, it can be shown that ER [0.25% SLS+ Ultrasound] (7.2) < ER [0.25% SLS] (5.4) + ER [Ultrasound] (2.4). This means that a synergistic effect could not be developing.

Figure 9 presents the hydrocortisone permeation plots when a more concentrated 1% SLS solution was employed as the pre-treatment. Application of the surfactant solution significantly promoted drug flux over

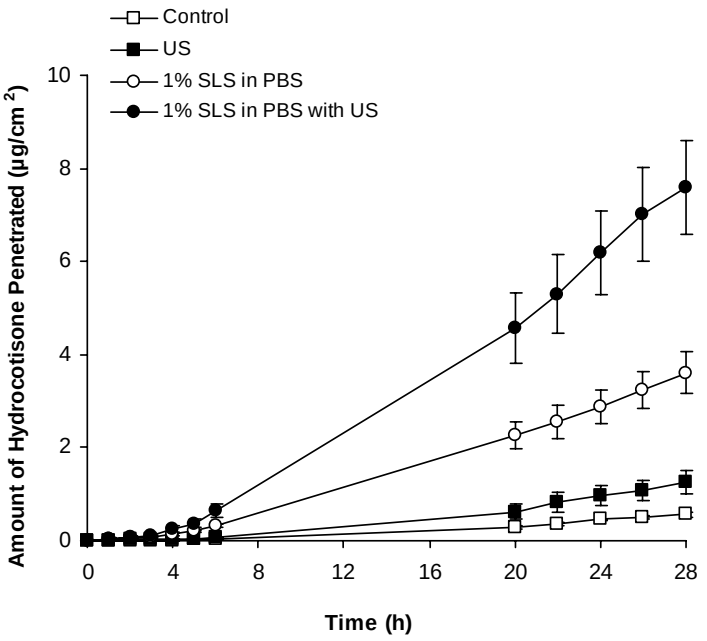

Fig. 9. The influence of 1% SLS and ultrasound on hydrocortisone permeation through porcine skin. Error bars represent s.e.m values.

control levels to achieve a steady state value of 0.15 ± 0.01 µg/cm^2/h. Later exposure of the skin sections to ultrasound resulted in a further significant increase of drug flux to 0.32 ± 0.07 µg/cm^2/h.

In terms of the mean enhancement ratios, it can be shown that: ER [1% SLS+ Ultrasound] (8.8) > ER [1% SLS] (4.1) + ER [Ultrasound] (2.4). This means a synergistic interaction between the surfactant treatment and ultrasound may have developed. A Two-way ANOVA indicated that there was indeed a significant interaction (p = 0.026) taking place between the ultrasound and the 1% SLS, providing evidence of synergism.

DISCUSSION

With regards to the terpenes, our results indicated that application of these chemicals in the absence of ultrasound produced significant enhancement of hydrocortisone delivery. The enhancement ratios produced in decreasing order of potency were: 1,8-cineole (6.8) > α-terpineol (5.8) > (+)-carvone (3.7) > menthone (3.2). This rank order is somewhat similar but not identical to that reported by others who measured hydrocortisone flux through hairless mouse skin *in vitro* (El-Kattan *et al*., 2000). That group reported an order of: menthone > 1,8-cineole > α-terpineol > (+)-carvone. The very different nature of the tested skins may explain the different ranking for menthone. Ultrasonication of skins following terpene application did not actually improve hydrocortisone permeation except where the terpene was menthone. Here, we identified synergism causing a 7.4-fold increase in drug flux over control levels.

For the tested fatty acids, our enhancement ratios for hydrocortisone permeation occurred in the following order: oleic acid (15.3) > linoleic acid (8.8) > stearic acid (1.4). This type of pattern in which oleic acid seems to be the optimal enhancer has been reported many times before in the literature, with perhaps the research of Kim et al. (2008) being the most recent example. The very low potency of stearic acid might be due to the fact that an ethanolic vehicle was used to dissolve this enhancer and it is known that ethanol can under certain circumstances reduce the permeation of some drugs (Fang *et al*., 2003). Interestingly, ultrasonication of each fatty acid-treated skin samples did not cause any further increase in hydrocortisone delivery.

With respect to SLS, our findings indicated that the 0.25% concentration was marginally more permeabilising to the skin than the 1% concentration. It is unclear why this should be the case. More interesting was the fact that ultrasound exposure following 1% SLS treatment caused a highly significant synergistic 8.8-fold increase in hydrocortisone delivery. Treatment with 0.25% SLS and ultrasound caused a significant additive effect.

CONCLUSION

To summarize, the main finding of this work is that using the fatty acids or the terpenes (excepting menthone) in

combination with ultrasonication does not produce a synergistic or even additive flux enhancement effect. In contrast, use of SLS followed by ultrasound does yield additive or synergistic activities, depending upon the SLS concentration applied. A possible explanation for these differences is due to the lipid-protein partitioning (LPP) concept (Williams and Barry, 1991a; Williams and Barry, 1991b; Barry, 2006). This theory classifies chemical enhancers into three categories depending upon how they work. Terpenes and fatty acids fall into the first class of enhancers. These chemicals modify the structured intercellular lipid domains of the stratum corneum, making the stratum corneum more permeable. In contrast, ionic surfactants such as SLS fall into the second class of enhancers that act at stratum corneum desmosomes and protein structures (Barry, 2006). Considerable evidence suggests that low frequency ultrasound causes stratum corneum disordering of lipids that may be relatively similar to the changes provoked by terpenes and fatty acids. It may be that a combination of lipid domain and protein domain changes is required for synergism to take place.

Finally, it should be mentioned that in our studies, skin samples were treated with chemical enhancers while the drug solution and ultrasound were applied later. This methodology has the advantage that it avoids complex three way interactions occurring between the drug, the enhancer and the ultrasound. However, a simultaneous administration of all three treatments could be more effective and probably simpler to apply in a clinical setting.

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DECLARATION OF INTERESTS

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