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The influence of lecithin and urea on the in vitro permeation of hydrocortisone acetate through skin from hairless mouse[1]

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Abstract

Many workers have attempted to modify the permeability characteristics of skin. In the present work, the in vitro permeation of a model lipophilic drug (hydrocortisone acetate) was studied through hairless mouse skin. The drug was applied in poloxamer 407 gels containing known concentrations of lecithin or urea as penetration enhancers. The results of permeation studies showed that the flux and the retention of the drug were dependent on the concentration of the penetration enhancers. Lecithin (8.0% w/v) caused a retention of seven times more than that of urea (12.0% w/v). The mechanism of penetration enhancement was investigated by differential scanning calorimetry (DSC) and FTIR spectroscopy of the stratum corneum (SC) from hairless mouse treated with solution of lecithin and urea. The lecithin was found to have a significant influence on the lipid matrix of the SC, suggesting a disruption of the intercellular lipid lamellar structure. © 1997 Elsevier Science B.V.

Keywords: Hairless mouse skin; Penetration enhancers; Lecithin; Urea; DSC; FTIR

1. Introduction

It has been assumed that the transport of the substances across the stratum corneum (SC) occurs via the intra- and intercellular route, but studies have indicated that intercellular lipids are a more important determinant of percutaneous absorption for most permeants (Abraham et al.,

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1995). The transport of drug molecules across the SC can be increased by penetration enhancers. An enhancer or enhancer/vehicle combination will be effective if it affects the SC protein: lipid phases within the 'horny layer'. Many substances have been used successfully as penetration enhancers. However, their relative skin toxicity can lead to them being unsuitable for use in dermatological preparation (Michniak et al., 1993). Lecithin and urea associated with poloxamer gels seem to be attractive as penetration enhancers, due to their non toxic characteristics, biocompatibility with the skin and different solubility characteristics (Morimoto et al., 1990).

The effect of penetration enhancers on the cutaneous absorption profile of drugs has been quantified by permeation parameters, such as flux of the drug across the skin and drug skin retention (Baker and Hadgraft, 1995). Physical techniques such as DSC have been used to characterize the thermal properties of the SC (Potts et al., 1991). Four transition temperatures were detected for hydrated human SC (Van Duzee, 1975), which may be regarded as lipoprotein domains. Changes in these transition temperatures may be used as a indicator of an interaction between added substrances and the SC. In addition, Fourier transform infrared (FTIR) provides information at the molecular level about the interaction of substances with the SC (Naik et al., 1995). These studies have provided considerable insight into the mechanism of action of penetration enhancers on the SC.

The aim of this work was to characterize the penetration enhancement of hydrocortisone acetate produced by lecithin and urea present in poloxamer 407 gels as a model vehicle, as well as to elucidate their influence on the lipid-protein domains of the SC from hairless mouse.

2. Materials and methods

2.1. Materials

The following reagents were used as received: hydrocortisone acetate (HCA), dexamethasone acetate, soy bean lecithin (90% L-α-phosphatidyl choline) and trypsin Type III (Sigma, St. Louis, MO), poloxamer 407 (BASF). All others chemicals were BDH reagent grade. The solvents use in the HPLC analysis were HPLC grade.

2.2. Preparation of gel formulations

The poloxamer gels used in this study were prepared by mixing poloxamer 407 (25.0% w/v) in distilled water with penetration enhancers in the concentration range of 2, 4, 8 and 12% w/v of urea and 1, 2, 4 and 8% w/v of lecithin (L-α-phosphatidyl choline) at 5°C. Liquid paraffin (5.0% w/v) was added in order to disperse the lecithin. HCA (1.0% w/v) was previously dispersed in propylene glycol (5.0% w/v). Upon warming to room temperature, clear and opaque viscous gels were formed when either urea or lecithin was added, respectively.

2.3. In vitro percutaneous absorption experiment

The full-thickness skin was excised from the abdominal surface of 4–6 week old hairless mice HRS/J strain (Jackson Laboratories, Bar Harbor, ME). Adhering fat and other visceral tissues were removed and the skin was mounted in a modified Franz diffusion cell. The available diffusion area of the diffusion cell was 2.54 cm^2. The receptor phase was isotonic phosphate buffer (pH 7.2) with 0.01% w/v of thiomersal. To maintain sink conditions in this system, 0.5% w/v of polyoxyethylene 20 cetyl ether was added to improve the aqueous solubility of the HCA in the receptor phase. Then, 1.5 g of the formulations (infinite dose) was placed on the membrane. Samples from the receptor phase were withdrawn at predetermined times over a 24-h period, and the amount of HCA present was analysed by HPLC. After 24 h, the skin was removed, cleaned with cotton soaked in methanol, homogeneized in methanol, filtered, and the amount of HCA was analysed by HPLC. The skin penetration parameters for the model drug contained in the formulations, i.e. lag time (L) and flux across the membrane (J) were calculated. Permeation profiles were constructed by plotting log total amount of HCA transported across the hairless mouse skin (g/cm^2) against the

time (h). The X intercept of the extrapolated linear region on the profile gave the value of L in hours. J was calculated from the slope of the graph and expressed as log g/cm^2/h.

2.4. HPLC analysis

Analysis of all samples was performed by a CG (Instrumentos Cientifícos) HPLC System, model 480-C, UV detector at 254 nm, C18 reversed-phase column 125 × 4 mm (5 m), C18 pre-column 4 × 4mm (5 m), Hewlett Packard 3390A integrator, and 0.02 AUFS. The mobile phase used was methanol:water (60:40) at 1 ml/min and the extraction was carried out using chloroform. Dexamethasone acetate (200 ng/ml) was used as internal standard. The retention time for HCA and internal standard were 3.92 and 6.10 min, respectively. The method was linear to the concentration of 50–800 ng HCA/ml (Bentley, 1994).

2.5. Preparation of hairless mice SC samples for DSC and FTIR studies

SC samples were prepared by floating abdominal full-thickness skin for 14 h on a solution of trypsin Type III (0.1% w/v) and sodium bicarbonate (0.5% w/v) at room temperature, following a rinsing of the SC sheets with distilled water. SC samples were pressed between paper tissue, spread on filter paper and dried by storage in a desiccator over silica-gel, for a maximum of 2 weeks prior to use. Delipidized SC samples were obtained by exhaustive extraction of dried SC sheets with a mixture of chloroform:methanol (2:1, v/v) for 24 h followed by soaking in acetone for 4 h and then hexane for 24 h, followed by treatment with ethanol:diethylether (8:92, v/v) for 24 h. The lipid extraction was carried out at room temperature with constant stirring. At the end of the procedure the delipidized SC sheets were dried at room temperature and stored as described above. Dried SC samples of known weight were incubated for 12 h with aqueous solutions of urea (12.0%, w/v) or solution of lecithin (8.0%, w/v) in liquid paraffin. A control sample using liquid paraffin was carried out. At the end of the incubation period with urea or lecithin the samples were rinsed with distilled water or ethanol, respectively, dried for several hours over silica-gel and reweighed. All samples were then hydrated to 30–60% w/w by storage for 3 days in a chamber at constant relative humidity (97% at 25°C) containing saturated potassium sulphate solution. The hydration level was defined as: [(weight of the hydrated sample − weight of the dry sample)/ weight of the dry sample] × 100. Untreated SC samples served as controls.

2.6. DSC measurements

The DSC studies were performed using a Seiko 2400 (Seiko Instruments, Tokyo, Japan) linked to a Seiko 2100 Data Analysis station. The apparatus was calibrated with 2–3 mg of indium (99.99% pure) and 20–25 mg of SC samples were equilibrated to 30–60% w/w hydration and hermetically sealed in steel pans to avoid evaporation of water. The samples were scanned over the temperature range of 0–120°C at a heating rate of 5°C/min.

2.7. FTIR measurement

FTIR studies of the SC were performed using an ATI Mattson Genesis Series FTIR spectrometer. Approximately, 1 cm in diameter SC samples at 30–60% w/w hydration were sealed between two IR-zinc sulfide transparent windows using a Perkin Elmer solution cell as a support. The ensemble was mounted in the path of the IR beam. All spectras were record at ambient temperature in the frequency range of 4000–400 cm^{-1} (1 cm^{-1} resolution, representing the average of 100 scans).

3. Results and discussion

A linear relationship was obtained when the log total amount of HCA in the receptor phase was plotted against time, indicating that the hairless mouse skin is permeable to the model drug and that the percutaneous transport seems to obey first order kinetic (Fig. 1). The concentration of urea and lecithin influenced the in vitro percu-

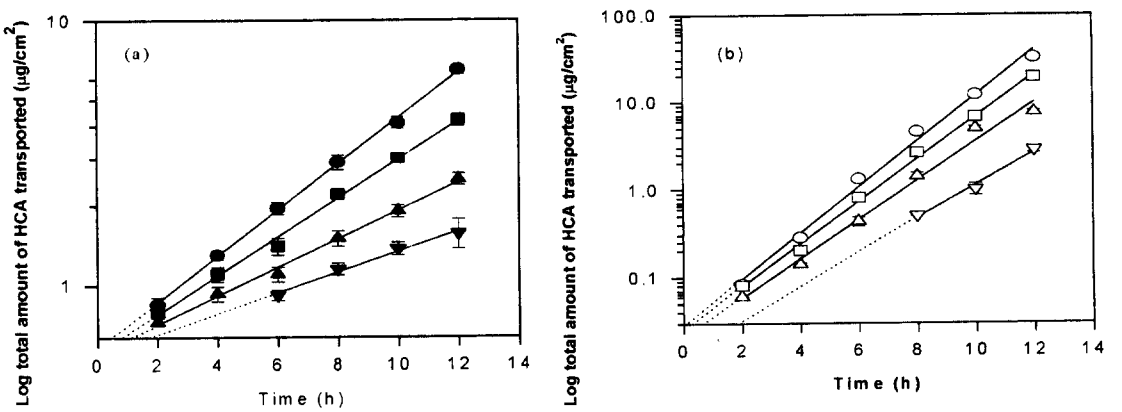

Fig. 1. Amount of HCA transported through hairless mouse skin as a function of the time, from poloxamer 407 gels containing different concentration of penetration enhancers: (a) urea: (●) 0%, (■) 2%, (▲) 4%, (▼) 8% w/v; (b) lecithin: (○) 0%, (□) 1%, (△) 2%, (▽) 4% w/v. Vertical bars indicate mean ± S.D. ($n = 4$).

taneous absorption of HCA from the poloxamer gel. Table 1 shows the parameters characterizing transport after treatment of SC with different concentration of urea or lecithin. There was no transfer of drug from formulations containing 12.0% w/v urea and 8.0% w/v lecithin until 12 h. The lag time (L) and the flux (J) were influenced by the amounts of urea and lecithin in the poloxamer gel. As the L increased the J decreased with the increasing concentration of these additives. In other words, the percutaneous absorption of HCA occured more slowly.

The skin retention of HCA and the total amount of drug absorbed percutaneously, after 24 h as a function of concentration of enhancers urea and lecithin are shown in Fig. 2. The results indicate that as concentration of urea or lecithin increased there was an increase in the amount of HCA retained, accompanied by a decrease of its percutaneous absorption.

Others workers (Shah et al., 1992), have suggested that the maximum cutaneous efficacy and minimal exposure to systemic absorption are among the attributes of dermatological preparations. On this premise, the formulations containing urea at 12.0% w/v or lecithin at 8.0% w/v were selected, because they provided the greatest drug skin retention and minimum flux of the drug across the hairless mouse skin (Fig. 2). The different HCA lag times (L) and flux (J) in the absence of lecithin and urea may be due to the presence of liquid paraffin, which could well influence the drug absorption. Lecithin provided a skin retention in the order of seven times more than that of urea. In our previous research, the lecithin increased the skin retention of dexamethasone acetate in the order of three times more than that of urea (Bentley et al., 1995).

These observation can be accounted for in terms of the mechanism of action of these penetration enhancers. Urea would increase the SC hydration and cause an exfoliative effect (Kim et al., 1993). Thereby decreasing the barrier effect of the SC and so improving the absorption of drugs into the skin. Lecithin would either supplement the lipid content of the skin or provide a film on epidermal contact, which would increase the partition of drugs into the skin. The natural lipids of the SC are predominantly saturated hydrocarbon chains which have phase transition temperatures above body temperature and the resistance of the SC to water permeation has been attributed to these lipids being in the crystalline or gel state (Jacobs et al., 1988). For the model lipophilic drug studied (HCA), the effect of the lecithin on the skin seems to enhance its skin retention, probably due to the drug's polarity. The main route of skin penetration of lipophilic drugs is by the apolar route. The mechanism of topical delivery of lipophilic corticosteroids has been reported to

Table 1
In vitro percutaneous absorption data of HCA from poloxamer gels containing penetration enhancers[a]

Penetration enhancer	Concentration (%)	L (h)	J (Log g/cm^{2}h^{-1})
Urea	0	0.55(±0.03)	2.98(±0.17)
	2	0.90(±0.05)	2.54(±0.19)
	4	1.20(±0.02)	2.23(±0.11)
	8	1.75(±0.15)	1.67(±0.07)
	12%[b]	—	—
Lecithin	0	0.10(±0.007)	11.45(±0.23)
	1	0.25(±0.010)	9.65(±0.22)
	2	0.75(±0.033)	7.98(±0.98)
	4	1.90(±0.12)	6.78(±0.92)
	8[b]	—	—

Abbreviations are as follows: L, lag time; J, flux.
[a]Results are expressed as mean ± S.D. ($n = 4$).
[b]In this concentration of urea or lecithin no drug was delivered until 12 h.

involve the direct transfer of these drugs between the lipid bilayer and the lipid phase of the SC, as the release rates of these drugs into the aqueous phase are less significant (Ganesan et al., 1984). The reason for the differences in permeation and retention of HCA between lecithin and urea may be due to the increase in the lipid content of the SC caused by the phospholipid, particularly within the intercellular regions. In this way the corticosteroid could partition more favourably into those regions, which formed a depot to HCA. In addition, the lecithin could have some influence on the structure and organization of the lipid matrix (Bonina et al., 1995).

In order to characterize the possible changes caused by lecithin and urea on the SC from hairless mouse and, thus elucidate their penetration enhancement mechanism, DSC and FTIR studies were carried out. Transition in the DSC thermal profile of the SC reflect the 'melting' of the structural domains of the lipids. These changes in the FTIR reflect changes in the vibrational modes and, thus, provide information at the molecular level. A combination of these methodologies will provide complemetary information about the structure of the SC (Potts et al., 1991).

Fig. 3 shows the DSC thermograms of untreated and treated (lecithin or urea) hydrated SC from hairless mouse skin. Three major transitions in the DSC profiles of untreated SC were observed around 30, 73, 87 and 104°C. The first three transitions were not present in the delipidized SC samples, suggesting that they derive from melting process of the SC lipids, in agreement with other workers (Cornwell and Barry, 1993). The fourth transition was heat irreversible and present after the delipidization of the SC, indicating denaturation of protein. The transition at about 87°C is considered to represent the lipids associated with proteins (Barry, 1991). Table 2 presents the values of transition and enthalpies for the interaction of the enhancers with SC obtained from the DSC thermograms (Fig. 3). The transition at about 73°C was clearly identified in each thermogram, and was considered to be the most useful parameter to compare the thermal profile of the SC samples. The treatment with urea did not change significantly the transition and enthalpy of the SC. The presence of lecithin produced a more pronounced effect on the lipid transition of the SC, with a transition temperature shift of about 8°C, as well as a considerable decrease in the enthalpy. It may be argued that such an effect could be attributed to the liquid paraffin used to solubilize the lecithin. However, the treatment with liquid paraffin alone only caused a small decrease of the enthalpy, indicating a possible extraction of the lipids of the SC. The lipid transitions involve decreased packing order

relative to the initial state. Furthermore, a decrease in the transition reflects a thermal transition starting from a less ordered, more heterogeneous state (Golden et al., 1987). In others words, the thermal profile changes seen following treatment with lecithin suggest that the incorporation of these substance into SC results in a decreased lipid order.

The FTIR spectra of hairless mouse SC from 2800 to 3000 cm^{-1} at room temperature and 30–60% w/w hydration are shown in Fig. 4. Of particular interest in this study are the peaks near 2800 and 2920 cm^{-1} due to symmetric and asymmetric carbon-hydrogen (C–H) stretchings, respectively. The major contribution to the C–H stretching peaks of the SC is the absorbance of

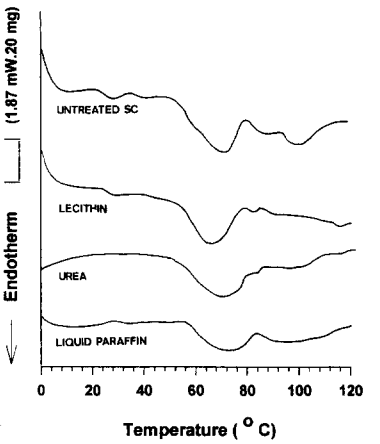

Fig. 3. DSC spectra of hairless mouse SC hydrated to 30–60% w/w: untreated SC; treated SC with lecithin (8% w/v); urea (12% w/v) and liquid paraffin, respectively.

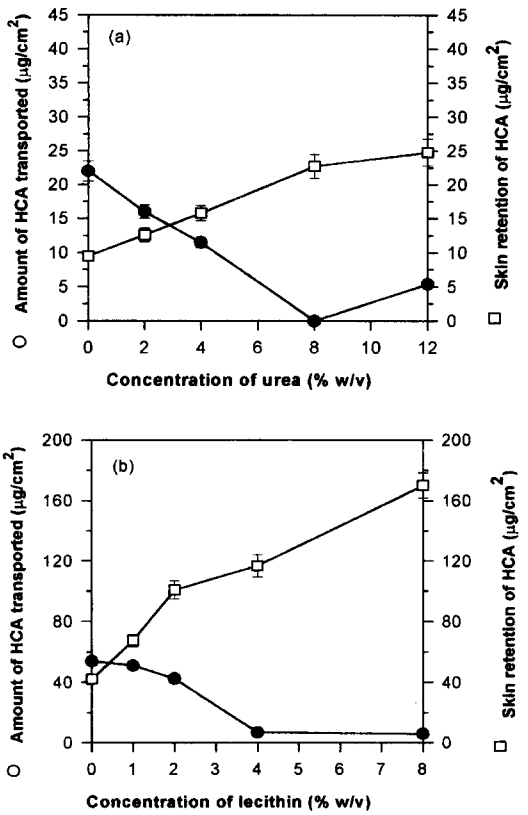

Fig. 2. Skin retention and amount of HCA transported after 24 h as a function of concentration of penetration enhancers: (a) urea; (b) lecithin. Vertical bars indicate as mean ± S.D. ($n = 4$).

the hydrocarbon chains of lipids. Hence, the delipidization treatment dramatically reduced both asymmetric and symmetric C–H stretching absorbances. Fig. 5 illustrates the values of shift of C–H stretchings of the SC samples. It can be seen that the treatment with aqueous solution of urea (12.0% w/v) induced a small shift of both C–H stretchings. The lecithin showed a higher level of interaction with the lipids of the SC, due to a pronounced blue shift of both C–H stretchings to a higher wavenumber, that is, the lipid–chain disorder was increased, reducing the efficiency of the packing and causing an increase of the energy necessary to vibrate the C–H bonds. The influence of liquid paraffin can not be considered relevant, due to the very small shift of the C–H stretching. DSC results showed that the liquid paraffin only brought about an extraction of the intercellular lipids and a minor influence on the fluidity of the lipid matrix (Table 2).

The DSC and FTIR results favour the hypothesis that the lecithin has a more pronounced interaction with the intercellular lipids than urea. This behaviour may be a consequence of the different mechanisms of action of these enhancers on the route of penetration into the skin. The possible effect of hydration of the urea on the SC was less effective promoting the penetration of the lipophilic model drug (HCA) into the hairless

Table 2
Influence of penetration enhancers on the thermal properties of hairless mouse SC[a]

Treatment of the SC	Transition temperature (°C)	Transition temperature shift (°C)[b]	Transition enthalpy (mJ/mg)
Untreated	73.3 ± 0.5	—	14.1 ± 1.5
Lecithin	65.3 ± 0.9	−8.0	8.7 ± 0.3
Urea	72.1 ± 1.2	−1.3	14.9 ± 0.9
Liquid paraffin	72.7 ± 0.7	−0.6	10.7 ± 0.6

[a]All data are summarized as mean S.D. ($n = 4$).
[b]Obtained by the difference between the average of transition temperature of the treated SC and untreated SC.

mouse skin. The action of the lecithin on the lipids of the SC was proved by the DSC and FTIR results, and accompanied by a greater retention of HCA in the skin. The lecithin may have caused a disturbance of the organized structure of the lipid matrix of the SC, producing an increase of the permeability of the skin, thus faciliting the penetration of the drug. The retention effect may be explained by the fact that HCA is a very lipophilic drug and the lecithin provides a depot for the HCA in the skin layers. In vivo studies of blanching assay of corticosteroids have shown that the pretreatment with lecithin can improve the bioavailability of these drugs in the skin (Jacobs et al., 1988). Phospholipid-drug complexes have been reported to prolong the activity of the drugs in the skin due to the increase of the lipid content of the SC layers and the decomplexation process (Bombardelli and Spelta, 1991). Bonina et al. (1995) attributed the effect of the lecithin gels containing methyl nicotilate to the supramolecular aggregation structure of the phospholipids, probably causing strong interactions with the SC lipids. Other substances that are constituents of the SC, such as fatty acids, can induce a pertubation of SC lipid lamellae and improve the penetration of drugs into the skin (Kitagawa et al., 1995). In the present work, the penetration studies showed that the formulation containing lecithin (8.0% w/v) provided a great retention of the HCA after 24 h, about 170 mg/cm^2, and a low flux of the HCA throught hairless mouse skin (Fig. 2). This behaviour can be considered suitable for local drug delivery therapy, as for example steroidal anti-inflamatories. It may be concluded that lecithin can be used to improve the performance of the dermatological formulations for lipophilic drug delivery into the skin, when the local effects are desirable.

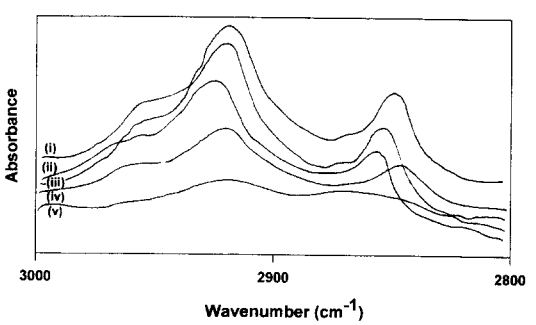

Fig. 4. FTIR spectra of hairless mouse SC hydrated to 30–60% w/w in the C–H stretching region between 2800 and 3000 cm^{-1}: (i) untreated SC; (ii) SC treated with lecithin (8% w/v); (iii) SC treated with urea (12% w/v); (iv) SC treated with liquid paraffin and (v) delipidized SC, respectively.

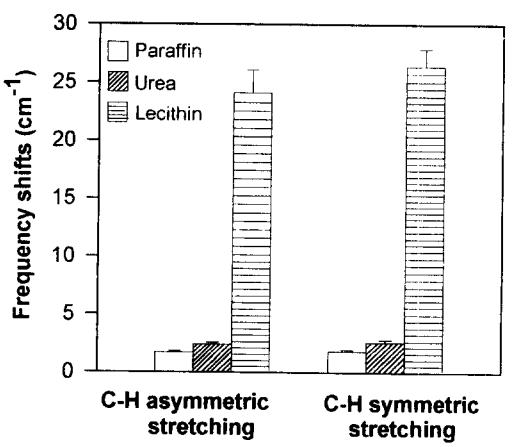

Fig. 5. Shifts of the C–H asymmetric and symmetric stretching caused by the differents treatments. Vertical bars indicate as mean ± S.D. ($n = 4$).

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