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Effects of Phosphatidylcholine on the Topical Bioavailability of Corticosteroids Assessed by the Human Skin Blanching Assay

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Abstract—A non-occluded multiple application skin blanching assay has been used to determine the effect of applied phosphatidylcholine (PC) on the bioavailability of corticosteroids. One forearm of each of ten volunteers was treated twice daily for one week with PC presented as a liposomal suspension in Sørensen's (pH 5·0) phosphate buffer (2·5 mg PC/0·5 mL) while the other arm was treated with 0·5 mL of phosphate buffer. For the following two weeks this treatment regimen was continued but in addition, each of four corticosteroid formulations (containing (i) hydrocortisone 0·1%, (ii) clobetasone butyrate 0·05%, (iii) betamethasone 0·1% and (iv) clobetasol propionate, 0·05%) was applied to sites on both arms. 5 ± 1 mg of each cream was applied twice daily to the sites on day 1, then once daily for a further four days; after two days of no application, the protocol was repeated. Estimation of pallor was usually made four times daily. At the end of the second week of corticosteroid application the blanching response to all four formulations on the PC treated arms was significantly higher than on the buffer treated arm. Tachyphylaxis to the applied corticosteroids was markedly less apparent on the lipid-treated arms. It is proposed that the applied phospholipid either supplements the lipid content of the skin or provides a thin film in intimate epidermal contact. Such a film may promote hydration of the stratum corneum and also provide an environment into which corticosteroids initially partition before a subsequent, more controlled, release to the underlying tissue.

Lipids are an integral part of the skin, present in both the intercellular matrix and cell membranes. However, little is known of their contribution to the percutaneous absorption of various drugs. It has been assumed traditionally that polar and non-polar substances traverse the stratum corneum via the intracellular route, but recent studies have indicated that intercellular lipids are a more important determinant of percutaneous absorption, particularly for lipophilic substances, than either the stratum corneum thickness or the total number of its cell layers (Elias et al 1981). Furthermore, it has been reported that the total lipid content appears to be a more important factor in the transdermal passage of drugs than the types of lipid species that are found (Elias 1981). The application of liposomes incorporating sodium pyroglutamate to the skin (Oleniacz 1976) as well as solar filter/moisturizer combinations (Vanlerberghe & Handjani 1980) has been suggested to have certain cosmetic advantages. Of greater scientific interest is the improved disposition of corticosteroids within the skin layers of rabbits which was claimed following the topical application of preparations containing triamcinolone acetonide incorporated into liposomes (Mezei & Gulasekharam 1980, 1982). However, the direct effect of phospholipid application to intact skin upon the absorption of subsequently applied drugs has not been investigated previously. The purpose of this study was to examine the effect of pre-treating skin with phosphatidyl choline (PC), presented as a liposomal suspension, on the bioavailability of four formulations of corticosteroids with different inherent potencies. The human skin blanching assay using a non-occluded multiple dosage regimen (see Haigh & Kanfer 1984) was used for this purpose.

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Materials and Methods

Liposome preparations

Quantities (1 mL) of a 50 mg mL^{-1} PC (Grade I, Lipid Products, S. Nutfield, Surrey, UK) in chloroform–methanol (6:1) solution were evaporated to dryness under nitrogen in round-bottomed flasks. Flasks were rotated during evaporation to facilitate an even deposition of lipid as a film. Flasks were stored under vacuum at 4°C overnight to ensure the complete removal of solvent. Liposomal suspensions were formed following the addition of 10 mL of Sørensen's phosphate buffer (pH 5·0, I = 0·015 M) to each flask at 40°C with subsequent vigorous shaking at 15 min intervals for 1 h.

The final concentration of PC in the liposomal suspensions was 5 mg mL^{-1}. Fresh batches of liposomes were prepared weekly during the study and stored at 4°C throughout.

Corticosteroid formulations

Commercially available packs of the four corticosteroid preparations, hydrocortisone 0·1% cream (Dioderm, Dermal Laboratories Ltd., Gosmore, Hertfordshire, UK), clobetasone butyrate 0·05% cream (Eumovate, Glaxo Laboratories, Greenford, Middlesex, UK), betamethasone 0·1% (as valerate) cream, (Betnovate, Glaxo Laboratories) and clobetasol propionate 0·05% cream (Dermovate, Glaxo Laboratories) were stored at room temperature (20°C) and the first gram of product squeezed from each tube was discarded.

Volunteers

Ten Caucasian volunteers were selected without reference to

their sex (3 male, 7 female; age range 21–31 years), each having previously demonstrated a consistent response to a standard preparation (Betnovate Cream). None had received corticosteroids topically or systemically for at least 3 months before this study.

Blanching assay

Liposomal suspension 0·5 mL, was applied to the flexor surface of the 'test' forearm twice (between 07–0800 h, 13–1400 h) daily for 7 days before any steroid application. Sørensen's phosphate buffer was similarly applied to the 'control' arm. The solutions were spread evenly, using a latex rubber finger cot (Macarthy Surgical Ltd, Dagenham, UK), for as long as was necessary to disperse any surface liquid present. This twice daily treatment of control and test arms with either buffer or lipid suspension, respectively, was maintained throughout the following two weeks of study with application not less than 1 h previous to that of any steroid (see Fig. 1).

A quantity (5 ± 1 mg) of each steroid formulation was applied to 7×7 mm sites on the flexor surface of each forearm by extruding a standard length of each product from a 2 mL disposable syringe. The syringes were filled immediately before use, to minimize any possible interaction between the preparation and the plastic matrix of the syringe barrel. Two sites were allocated randomly to each preparation on both arms.

Once delivered to the site, the creams were spread for 1 min using a glass rod. Holes cut in a 1 mm thick transparent PTFE sheet template were used to locate accurately the application sites. The template boundaries of the sites were marked with indelible ink to ensure the precise location of subsequent applications.

The preparations were applied twice on day 1 (between 09–1000 h and also 15–1600 h) to provide a loading dose and then once daily (between 09–1000 h) on days 2–5. No corticosteroids were applied on days 6 and 7 but from day 8 the application procedure was repeated to the same sites (Fig. 1).

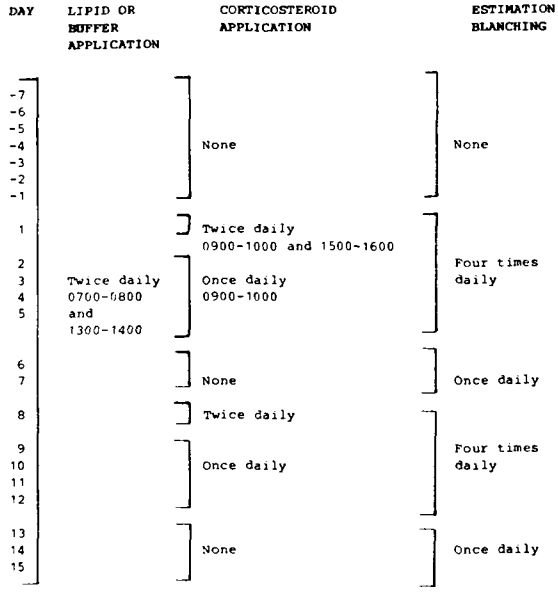

FIG. 1. Summary of the protocol employed.

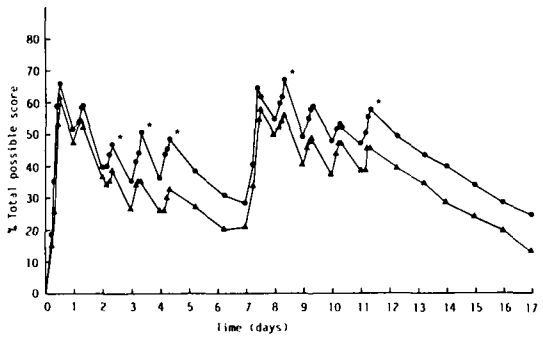

FIG. 2. % TPS values for clobetasol propionate cream applied to buffer treated (▲) and PC-treated (●) arms with time (* $P<0{\cdot}05$).

Estimation of pallor was made either four times daily (from days 1 to 5 and 8 to 12) or once daily (from days 6 and 7 and 13 to 15). Blanching was estimated using a 0–4 scale with half-point ratings for intermediate readings based on that of Barry & Woodford (1974). No guards over the sites were used but the volunteers were requested to take care not to smear the creams for up to 2 h after application. Volunteers were asked to avoid elevated temperatures and excessive arm contact with water. A 2-tailed *t*-test was used to determine whether significant differences existed between daily peak responses on the control and test arms.

Results

The blanching scores obtained for each preparation on both test (PC-treated) and control (buffer-treated) arms were summed for all volunteers at each time, expressed as a percentage of the total possible score (% TPS) and plotted as a function of time (Barry & Woodford 1974).

The blanching profile for clobetasol propionate cream (Fig. 2) applied to the buffer treated arms exhibited a maximum response on day 1, with daily peak response values falling sharply over the following two days. This trend continued over days 4 and 5 but the reduction in peak response was less marked. The peak response achieved on the PC-treated arms on day 1 was marginally higher than that for the control arms. The daily peak response values on the test arms also decreased on successive days from days 1–3, although the net reduction was less than on control arms. However, unlike the profile obtained for the control arms, on days 3–5 no marked change in peak response was observed

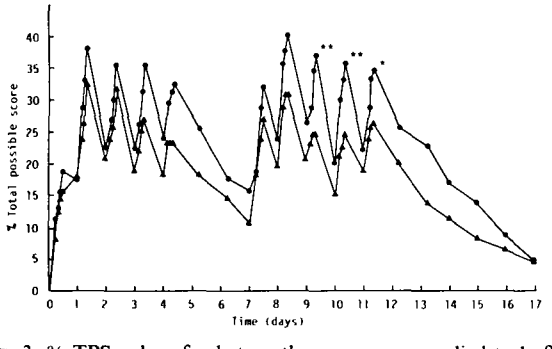

FIG. 3. % TPS values for betamethasone cream applied to buffer-treated (▲) and PC-treated (●) arms with time (* $P<0{\cdot}05$, ** $P<0{\cdot}01$).

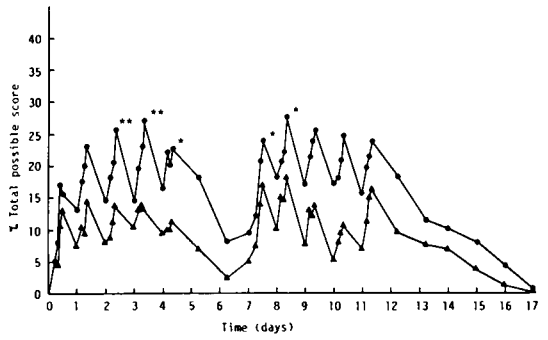

FIG. 4. % TPS values for clobetasone butyrate cream applied to buffer-treated (▲) and PC-treated (●) arms with time (* $P<0.05$, ** $P<0.01$).

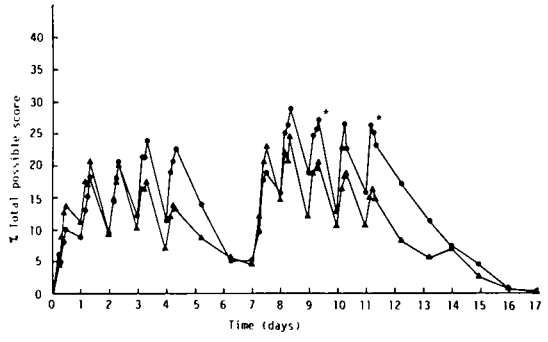

FIG. 5. % TPS values for hydrocortisone cream applied to buffer-treated (▲) and PC-treated (●) arms with time (* $P<0.05$).

and consequently the two profiles diverge. Week 2 shows the PC profile remaining consistently higher than the buffer profile with both series of responses following a similar pattern. A small reduction in peak response values from day 8 to day 10 is apparent, with all values obtained after day 9 higher than those obtained on the corresponding days in week 1 (i.e. days 3–7).

The blanching profiles for betamethasone cream (Fig. 3) were similar to those obtained for the clobetasol propionate, however, for betamethasone the blanching response was maximal on day 2 as opposed to day 1 and the test and control profiles diverged from day 3 to day 5. In contrast to the results obtained for clobetasol propionate, the peak response values obtained on day 8 were much lower than those on day 9, repeating the pattern seen in week 1.

The largest differences between PC-treated and buffer-treated arms were revealed by the blanching profiles for clobetasone butyrate cream in Fig. 4. Scores for the PC treated arms were up to 100% greater than corresponding values on the control arms. The successive diminution of peak response values observed for both clobetasol propionate and betamethasone was not evident for clobetasone butyrate with the possible exception of the control arms during week 2. Indeed, responses increased over the first four days on the PC-treated arms and peak values appeared to remain constant on the control arms.

The blanching profiles for hydrocortisone cream (Fig. 5) show much smaller differences between test and control arms than seen with the other preparations. On day 1 the buffer arms achieved a higher score than the PC arms, the magnitude of this difference reducing with time until the profiles intersect at day 3. On days 4 and 5 the PC-treated arms scored higher, the difference between profiles being greatest on day 5 of week 1 (in common with all preparations). During week 2, daily peak values on the test arms were highest on day 9 and remained relatively constant over the next three days. Peak values became progressively lower on the control arms from day 9 to day 12, and all scores achieved in week 2 were higher than the corresponding daily peak values in week 1. All volunteers developed dry and flaky skin patches at the sites treated with this formulation of hydrocortisone and this apparent change in membrane integrity became increasingly evident from day 8 onwards. Such flaking of skin was, however, noticeably less severe on the PC-treated arms.

For a direct assessment of the effect of treatment with exogenous lipid on blanching activity the results for the four corticosteroid formulations were summed for all volunteers at each reading time and expressed as % TPS. Differences between the daily peak responses obtained from PC treated (T) and control (C) arms are shown in Table 1.

Discussion

Percentage TPS values for each preparation were summed for all times (Table 2) to enable comparison of the overall potency and bioavailability of each corticosteroid on both the control and test arms. The rank order for overall blanching intensity was found to be clobetasol propionate > betamethasone > clobetasone butyrate > hydrocortisone for the PC treated arms, with the positions of clobetasone butyrate and hydrocortisone reversed for the control arms. The rank order obtained for the PC-treated arms was consistent with their respective potency ratings in both the British National Formulary and the United Kingdom Monthly Index of Medical Specialities. A recent comparison of the blanching activities of clobetasol propionate and clobetasone butyrate and betamethasone, using a single application, non-occluded assay also confirms the order found in the present study (Meyer et al 1988). It seems likely

Table 1. Peak blanching responses (% TPS) induced by multiple steroid applications; sum of all preparations.

DAY:	1	2	3	4	5	8	9	10	11	12
						(% TPS)				
T	27·5	34·5	32·2	34·2	31·4	34·2	41·3	37·2	33·6	34·7
C	26·1	29·8	26·1	23·1	20·0	30·8	31·7	26·7	25·2	25·5
T-C	1·4	4·7	6·1	11·1**	11·4*	3·4	9·6**	10·5**	8·4**	9·2**

* $P<0.05$, ** $P<0.01$ using a 2-tailed t-test.
T = PC-treated arms, C = Buffer-treated control arms, T-C = Different between treatments.

Table 2. Summed % TPS for corticosteroid formulations on control and test arms.

Corticosteroid Cream	PC arms (% TPS)	Buffer arms (% TPS)
Clobetasol propionate	2269	1855
Betamathasone	1254	988
Clobetasone butyrate	843	476
Hydrocortisone	773	643

that the positions of clobetasone butyrate and hydrocortisone were reversed on the buffer treated arms as a result of the apparent disruption to the skin integrity induced by some component of the particular formulation of hydrocortisone used in this study. This phenomenon may have facilitated the penetration of hydrocortisone and thereby increased vasoconstriction. The observation that dry and flaky areas were noticeably fewer and less severe on arms treated with the lipid would suggest that PC confers some protection against assault by potentially damaging substances applied topically.

Tachyphylaxis was first demonstrated for topically applied corticosteroids by Du Vivier & Stoughton (1975). On the buffer-treated control arms a tachyphylactic response was demonstrated for each corticosteroid preparation. The onset of this phenomenon was rapid for clobetasol propionate, with a large reduction in the peak blanching response occurring between days 1 and 5 (see Fig. 2). The blanching profile for betamethasone (Fig. 3) shows tachyphylaxis occurring from days 2–5 and for clobetasone butyrate (Fig. 4) between days 4 and 5. The magnitude of the reduction in peak response apparently diminishes with the potency of the steroid preparation. These finding are in agreement with those reported by Du Vivier & Stoughton (1975), that the more potent the steroid, the greater the degree of vasoconstriction produced initially but the faster the onset of tachyphylaxis. The tachyphylactic response observed for hydrocortisone (Fig. 5) was, however, greater than would have been anticipated for a corticosteroid of that potency and this may again be related to the alteration in epidermal character which was observed with prolonged use. The blanching profiles obtained for clobetasol propionate and betamethasone (Figs 2, 3) on the buffer-treated arms are similar to those obtained for a series of amcinonide creams by Woodford et al (1983) investigating possible dosage regimens for topical steroids. Those workers also reported that with a loading dose on day 1, the response peaked and then diminished as a result of tachyphylaxis. A recovery of the blanching response after a rest period of a few days was clearly demonstrated in this and previous studies (Du Vivier & Stoughton 1975; Woodford et al 1983), with daily peak values during week 2 being consistently higher than the corresponding days in week 1. The residual pallor from week 1 would also contribute to the blanching scores obtained during week 2, and this was most apparent for clobetasol propionate cream where residual blanching scores of over 20% TPS were recorded. It would appear that a repeated treatment of skin with PC, presented as a liposomal suspension, causes an alteration of the normal blanching profile. The nature of this effect is revealed by the peak blanching response values obtained by combining the results for each corticosteroid preparation (see Table 1). During the course of week 1 from days 1–5 the blanching profiles diverge and significant differences are found on days 4 ($P<0.01$) and 5 ($P<0.05$). The increasing difference with time appears to be owing to a reduction in the tachyphylactic response on the PC treated arms. Between days 9–12 the daily peak response values remain much higher on the test arms, with this difference being highly significant ($P<0.01$). This effect appears to varying degrees with all the individual preparations, although it was most notable for clobetasone butyrate when the blanching intensity was nearly 100% higher on the PC-treated arms.

The reason for the differences in blanching behaviour between phospholipid- and buffer-treated arms is open to speculation. It is possible that the PC treatment leads to a small increase in the total lipid content of the stratum corneum, particularly within the intercellular regions. Alternatively, the applied lipid may form a thin film after evaporation of the aqueous phase which remains in intimate contact with the skin. By increasing the amount of lipid present, either as a film or within the skin itself, the steroids could partition more favourably into those regions. The steroid depot thus formed would lead perhaps to a more controlled delivery of the steroids to the lower skin layers where blanching occurs. Tachyphylaxis may be reduced as local concentrations, following the loading and subsequent doses, fail to reach the high levels achieved in the buffer treated arms.

If a film of PC is formed in intimate contact within the skin it is possible that water loss from the epidermis will be retarded, leading to a greater hydration of the stratum corneum. This would resist any potential insult from applied vehicle components and be likely to promote the absorption of corticosteroids. The natural lipids of the stratum corneum contain predominantly saturated hydrocarbon chains which have phase transition temperatures above body temperature and the resistance of the stratum corneum to water permeation has been attributed to lipids being in the crystalline or gel state (Downing et al 1986). The present study employed egg PC which contains a mixture of phosphatidylcholines and has a phase transition temperature within the range -7 to $-15^{\circ}C$, well below skin temperature. The occlusive capacity of PC is likely therefore to be less than for lipids having phase transitions greater than about $30^{\circ}C$.

Mezei & Gulasekharam (1980) claimed an improved disposition of triamcinolone acetonide within the skin layers of rabbits following the topical application of a formulation with the steroid incorporated into dipalmitoyl phosphatidylcholine (DPPC) liposomes containing cholesterol. They also suggested a mechanism of action involving the passage of intact liposomes through the lipid-rich outer skin layers to the aqueous dermis where they became localized. The hypothesis was received critically (Ganesan et al 1984) because of the unlikely concept of relatively large lipid vesicles traversing the densely packed outer skin layers to arrive intact, and then localizing within the dermis. A recently published study compared the skin permeation rates of hydrophobic and hydrophilic compounds when presented topically in DPPC liposomes (Ho et al 1986). The relatively lipophilic steroids hydrocortisone and progesterone were found to have permeation coefficients similar to those of the free drug in solution. However the skin transport of glucose

(which is entrapped in the aqueous regions of the liposome), was markedly slowed when compared with the free species and its release from the liposome was shown to be the rate-determining step. These observations indicated that liposomes were not absorbed as intact entities and also suggested the absence of fusion of liposomes with the stratum corneum. Further evidence that liposomal lipids do not penetrate the skin appears in a study of the preservative activity of the antimicrobial butylparaben, when radiolabelled DPPC was found to remain on the skin surface of guinea-pigs following topical application (Komatsu et al 1986). In addition, others have also confirmed that liposomes do not enter the surface layer of rabbit skin (Saket 1986). Interestingly, the methodology employed by Mezei & Gulasekharam (1980) involves washing the skin surface with ethanol, which, as discussed by Kellaway (1985), is likely to destroy liposome integrity, yield high drug activity at the skin surface, and thereby in part explain the reported drug loading of epidermis and dermis.

When a liposomal suspension is applied to the skin, loss of water through evaporation will lead to a structural rearrangement of the liposomal lipid resulting in the formation of a liquid crystalline matrix. When the corticosteroid formulation is subsequently applied, the steroid may intercalate in the lipid bilayers. High concentrations of hydrocortisone have been achieved in an ointment vehicle consisting of PC/water liquid crystals (Wahlgren et al 1984). The preparation was stable and the diffusion coefficient for hydrocortisone within the liquid crystalline phase was found to be four times higher than the corresponding value for skin. The mechanism of topical delivery of lipophilic steroids appears to involve the direct transfer of the steroids between the lipid bilayer and the lipid phase of the stratum corneum, as liposomal release rates of these steroids into the aqueous phases are slow (Ganesan et al 1984). Penetration of the steroids into the lipid rich regions of the stratum corneum may also be affected by lipid exchange occurring between the relatively fluid PC and native skin lipids.

Conclusions

The present investigation indicates that lipid application itself may be a factor in determining the bioavailability of concomitantly administered corticosteroid formulations applied topically. The repeated skin treatment with a PC solution may increase the total lipid content of the skin and thereby alter the bioavailability of steroids. Alternatively, the application of PC could create a relatively fluid lipid film in intimate association with the skin surface, into which the steroids partition and enter the lipid regions of the stratum corneum by diffusion or lipid exchange. A steroid depot thus formed may be therapeutically significant, especially if a more controlled rate of delivery of the steroid to the lower skin layers results, as indicated by the significant reduction in the tachyphylactic response observed in this study. Such a lipid film, by possessing a degree of occlusion, is likely to promote the hydration of the stratum corneum and thereby facilitate corticosteroid absorption.

This study was approved by the Ethical Committee of Brighton Area Health Authority.

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