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Bile acids as enhancers of steroid penetration through excised hairless mouse skin

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Summary

Excised hairless mouse skin (EHMS) is used to evaluate the potential of sodium choleate (NaCOL), an ox bile extract containing the sodium salts of taurocholic, glycocolic, desoxycholic and cholic acids, and of the free choleic acids (HCOL) to enhance the transcutaneous penetration of progesterone (PGT) and prednisolone (PDN). EHMS is pretreated with aqueous dispersions of the enhancers, then the steroids are allowed to permeate through the pretreated EHMS from normal saline under occluded conditions. NaCOL is ineffective whereas HCOL produces structural modifications of the stratum corneum, resulting in increased skin permeability of both steroids. The chloroform-soluble components of HCOL interact strongly with stratum corneum lipids, as demonstrated by differential scanning calorimetry, thus facilitating PGT penetration. The chloroform-insoluble components of HCOL interact with more polar structures of stratum corneum, thereby promoting PDN transport. The data also suggest the existence of a parallel drug co-transport mechanism by the more lipophilic HCOL components.

Introduction

Excised hairless mouse skin (EHMS) has been used extensively for studies of transdermal drug penetration enhancement. A prospective clinical application of the penetration-enhancing agents studied is generally not the sole scope of these studies. In fact, the research in this field has often been aimed at clarifying the correlations among the enhancer-induced structural modifications of the biological membrane, the penetration enhancement effect, and the physicochemical properties of the penetrant, in view of rationalizing the choice of the proper enhancing agent for a given drug.

Some authors have warned that using EHMS in place of human skin might lead to substantial overestimations of drug permeability (Bond and Barry, 1988). Nevertheless, EHMS may still be a useful model for studies of the mechanism of enhancement of solute transport across the horny

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layer, not requiring similar permeabilities of human and model skin. Indeed, the type of human skin is typically not well controlled, leading to variation in age, diet, disease state, etc., and in turn, to excessive variability of permeability coefficients.

In the present study, EHMS has been used to evaluate the potential of sodium choleate (NaCOL), a cheap and readily available ox bile extract containing the sodium salts of taurocholic, glycocolic, desoxycholic and cholic acids, and of the free choleic acids (HCOL), obtained by acidification of NaCOL, to interact with skin components and structures and to enhance the penetration through skin of steroids having very different polarity, such as progesterone (PGT) and prednisolone (PDN).

The ability of the putative penetration enhancers to affect the degree of order of the lipid bilayers in the horny layer has been assessed by differential scanning calorimetry (DSC) and correlated with the penetration enhancing effect, in order to gain information on the mode of action of the agents.

Bile acids are known to be effective promoters of drug transport across epithelial membranes. Yet, little effort has so far been made to test their action on skin. The present work was intended to provide preliminary indications for more in-depth investigations with specific components of NaCOL.

Materials and Methods

Materials

The following commercially available materials were used as received: progesterone (PGT) and prednisolone (PDN) (Merck, Darmstadt, Germany), sodium choleate (NaCOL) and trypsin from porcine pancreas (type II: Crude) (Sigma Chemical Co., St. Louis, MO, U.S.A.).

The strongly acidic ion exchanger Amberlite IR-118H (Sigma) was thoroughly rinsed with deionized water prior to use.

The excised hairless mouse skin (EHMS) specimens were dorsal sections taken from 6–10 week old animals, killed by dislocation of the spinal cord at the neck. They were used immediately after isolation.

Preparation of choleic acids (HCOL) and their chloroform-soluble (HCOL(I)) and chloroform-insoluble (HCOL(II)) fractions

A portion of NaCOL was converted into the acidic form by gradually adding an 11% aqueous NaCOL solution (pH 6.27) with the ion exchanger Amberlite IR-118H, until a constant pH of 1.67 was reached. The initially clear solution turned into a turbid dispersion, which showed no tendency towards sedimentation. The ion exchanger was removed by filtering through glass wool, the dispersion was evaporated to dryness under reduced pressure with the aid of anhydrous ethanol, and the residue was vacuum dried at 50°C and pulverized in a mortar (HCOL).

A portion of HCOL was repeatedly extracted at ambient temperature with 500-ml volumes of chloroform until no appreciable residue was found in the evaporated extract. The chloroform-soluble (HCOL(I)) and chloroform-insoluble (HCOL(II)) materials were vacuum dried at 50°C and pulverized in a mortar. They were in the proportion of 1 : 2 w/w.

Preparation of isolated stratum corneum and dermis

Isolated stratum corneum sheets were obtained by treating EHMS with trypsin according to a previously described technique (Campigli et al., 1988).

The HCOL(I) fraction was used to remove the epidermis from EHMS by the following procedure.

Water was absorbed into HCOL(I) by levigation, until a spreadable, though viscous, paste resulted. The EHMS specimen was placed, dermis side down, on a teflon plate, and the HCOL(I) paste was spread on the epidermis. Next, the system was occluded with aluminum foil and kept for 24 h at 30°C, after which the paste, and the epidermis sticking to it, could easily be removed from the dermis by gently rubbing with a spatula. Microscope observation showed that separation had neatly occurred at the basal layer level. The

dermis was immediately used for the permeation experiments.

Permeation experiments

The permeation cells for semisolid vehicles described in a previous report (Campigli et al., 1988) were used for these experiments. Prior to the permeation experiment, the epidermis side of each EHMS specimen was maintained in contact with the agent to be tested for 24 h at 30°C. This was carried out by assembling the empty cell with the epidermis side of EHMS facing the receiving compartment, and then introducing 300 μl of the pretreatment agent into this compartment. Following pretreatment, the cell was disassembled, the skin flushed with normal saline containing 0.035% formaldehyde as a preservative (NSF), the donor compartment filled with a paste-like suspension of the steroid under study in NSF, the cell reassembled with the epidermis side of EHMS (or the stripped side of the dermis, in some cases) in intimate contact with the steroid suspension, and the receiving compartment filled with 5 ml of NSF. In a few cases to be specified no EHMS pretreatment was performed and a paste-like suspension of the drug in the vehicle under study was used as the donor phase. Four cells were generally run concurrently at 30°C. The drug flux measurements started after at least 6 h from the beginning of permeation. At time t_1 from the start of permeation the receiving solution was completely renewed, at time t_2 the amount of drug, Q, having penetrated into the receiving phase during the interval t_2–t_1 was determined, and the flux, J, at time $t = t_1 + (t_2 - t_1)/2$ was computed according to the equation: $J = Q/(A(t_2 - t_1))$, where A is the cross-sectional area of permeation. In no case was the interval t_2–t_1 so long as to allow the permeant concentration in the receiving phase to exceed 10% of solubility.

In order to determine the drug content in the receiving phase, each sample (3 ml) was shaken with 8 g of chloroform. After centrifugation (4000 rpm, 20 min) the aqueous phase was removed by suction and the organic phase (7 g) was transferred to a new centrifuge tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in 200 μl of methanol and the solution analyzed by HPLC, using a Partisil ODS-3 column (C_{18}- 5 μm, 25 cm × 4.6 mm, Whatman, Clifton, NJ, U.S.A.) and UV detection at 248 nm. For PGT, the mobile phase was a methanol-water (7:3) mixture, while a methanol-acetonitrile-water (3:3:4) mixture was used for PDN. The flow rate was 1.0 ml/min. The standard aqueous solutions used for calibration were processed in the same way as the samples. Testosterone and cortisone acetate were used as internal standards for PGT and PDN, respectively.

Determination of stratum corneum-NSF partition coefficients

Small sheets of isolated stratum corneum taken from three animals were pooled, then divided into two portions of which one was immersed in a 30% HCOL dispersion in NSF (HCOL30), the other in NSF, for 24 h at 30°C. Subsequently, each portion was processed as follows. The stratum corneum pieces were withdrawn, thoroughly rinsed with water, blotted dry with filter paper, and vacuum dried at ambient temperature. A weighed amount (around 20 mg) was immersed in 4 ml of an 8 μg/ml PGT solution in NSF, the suspension was deaerated by suction in order to ensure thorough wetting of the stratum corneum, and kept at 30°C for 60 h. Thereafter, it was passed through a teflon filter (pore size, 5 μm) and the clear solution was analyzed for PGT as described above. Partition coefficients of 1022 and 292 (expressed as w/w ratios) were determined for the stratum corneum treated with HCOL30 and NSF, respectively.

DSC measurements

A Mettler TA 3000 system, consisting of a TC 10 TA Processor, a DSC 20 measuring cell and printer/plotter, was used for these studies.

Isolated stratum corneum pieces taken from the same animal were treated with the agents to be studied for 24 h at 30°C. Then, after thoroughly rinsing with water, blotting dry with filter paper, and vacuum drying (around 10 Torr) at ambient temperature for 24 h, the samples were weighed (accuracy: $\pm 10^{-5}$ g) in sealed aluminum pans and scanned over a temperature range of

-10 to $+80$°C, at a heating rate of 10 K/min (the measuring cell worked in a freezer at -45°C). Type 8 was chosen as the integral baseline. This is a curve joining the measured points at the start and end of integration, allowing determination of the pure energy of transition in cases where a change in specific heat is associated with the transition.

Results

The pretreatment technique was adopted in this study in order to demonstrate and to assess modifications of skin, relevant to drug flux, caused by the agents. The permeation experiments following the pretreatment were always carried out with a drug suspension in NSF, as the donor. Under these conditions, the effects of the agents could be gauged, in the same manner as that described in previous papers by the present authors (Di Colo et al., 1989; Nannipieri et al., 1990), by means of the $R(J)$ value, defined as the ratio of drug flux through skin pretreated with the agent to that taken from the same animal, pretreated with a reference. Where required, the statistical significance of the difference between agent and reference was evaluated by Student's t-test for paired data.

Permeation studies employing PGT

In Fig. 1 the time profiles for PGT flux across full-thickness skin pretreated with NSF and isolated dermis taken from the same animal are displayed for the purpose of comparison. The flux for the full-thickness skin shows a trend toward gradual increase during the first 3 days of permeation, then increases sharply on day 4, after which the flux values closely match those relative to the isolated dermis.

According to these data, the resistance of the epidermis to PGT flux became negligibly small compared to that of the dermis after little more than 4 days of contact with NSF (1 day of pretreatment plus 3 days of permeation). Deterioration of the barrier function of EHMS is known to occur after prolonged exposure to aqueous media, due to microbial growth (Van der Merwe and

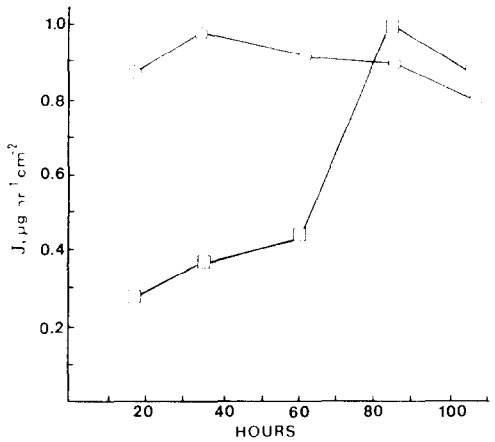

Fig. 1. Comparison between flux (J) vs time profiles for PGT through full-thickness EHMS pretreated with NSF (□) and isolated dermis (○) taken from the same animal. Each profile is the mean of two EHMS specimens taken from different dorsal areas. Data representative of three experiments.

Ackermann, 1987; Van der Merwe et al., 1988). Very recently it was shown that such a low formaldehyde concentration in the receiving phase as that used in the present permeation experiments is not sufficient to provide full protection for EHMS against microbial damage (Sloan et al., 1991). Nonetheless, the data in Fig. 1 show that for the first 3 days of permeation the epidermis, although becoming damaged, still presented a significant resistance to PGT flux. In particular, the flux values in the interval 30–70 h were similar and could be mediated. A virtually constant flux during such an interval was also observed with EHMS pretreated with all of the agents under study. Therefore, meaningful $R(J)$ values could be calculated from average flux values in the interval 30–70 h.

Consideration of the data in Fig. 1 suggests that with PGT as the permeant and NSF as the reference, if the permeability of the dermis to the drug is not enhanced by the agent under study, then $R(J)$ values substantially greater than 2 are unlikely to be attained, however effective the agent may be in reducing the diffusional resistance of the epidermis. This is due to the strongly lipophilic nature of PGT whereby the diffusional resistances of the epidermis and dermis are of the same order of magnitude. Similar considerations

have been made about the penetration of PGT through full-thickness human skin (Barry and Bennet, 1987).

Data on the effect of pretreating EHMS with the agents under study are listed in Table 1. Comparison between a 30% NaCOL solution in NSF (NaCOL30) and NSF resulted in no significant difference, which indicates that the choleate solution as such was unable to produce any stronger modification of the PGT permeability through EHMS than that caused by NSF itself. This consideration is confirmed by the strict similarity of the flux vs time plots for EHMS pretreated with NaCOL30 or NSF, illustrated in Figs 2 and 1, respectively. On the other hand, a 30% dispersion of the free bile acids in NSF (HCOL30) led to an $R(J)$ value in accord with a significant penetration enhancement with respect to NSF.

The permeability of the inner aqueous tissues of EHMS was substantially unaffected by HCOL30, as shown by the virtual correspondence, seen in Fig. 2, between flux values relative to full-thickness skin pretreated with HCOL30 and to isolated dermis.

The lipid-soluble components of HCOL were removed from HCOL by extraction with chloroform, and the residue, HCOL(II), was used to investigate the relevance of such components to

TABLE 1

Effect of EHMS pretreatment with different agents on the R(J) value for PGT

Reference	Jr (S.D.) [a] ($\mu g\ h^{-1}\ cm^{-2}$)	Agent/ Reference	$R(J)$ (S.D.) [a]
NSF	0.47 (0.12)	HCOL30/ NSF	2.08 (0.88)
		HCOL(II)30/ NSF	1.24 (0.23)
		NaCOL30/ NSF	0.93 (0.28)
HCOL(II)30	0.69 (0.13)	HCOL30/ HCOL(II)30	1.62 (0.18)

[a] Mean and standard deviation (S.D.) for four runs. The value for each run was an average of those in the 30–70 h interval. $R(J)$: ratio of drug flux through skin pretreated with the agent to that, taken from the same animal, pretreated with a reference (Jr).

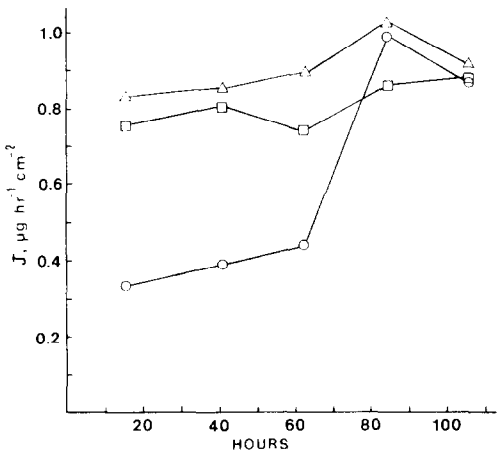

Fig. 2. Flux (J) vs time profiles for PGT through EHMS pretreated with HCOL30 (△) or NaCOL30 (○), or through the isolated dermis (□). All profiles were obtained with EHMS specimens taken from the same animal. Data representative of three experiments.

the overall penetration enhancing effect of HCOL. No statistically significant difference resulted between the effects of a 30% HCOL(II) solution in NSF (HCOL(II)30) and NSF. On the other hand, direct comparison between the effects of HCOL30 and HCOL(II)30 indicated a significantly greater effectiveness of the former ($P < 0.01$). Hence, the lipid-soluble components of HCOL can safely be considered of great relevance to the enhancing effect of this material on PGT penetration through EHMS.

Permeation studies employing PDN

Fig. 3 compares the time profiles of PDN flux through full-thickness skin and isolated dermis taken from the same animal. The flux values for the dermis exceed those for the full-thickness skin by two orders of magnitude. This demonstrates that the epidermis is virtually the only barrier to PDN permeation through EHMS. Hence, the in vitro flux of PDN is expected to be much more sensitive to changes in epidermis permeability induced by the agents under study than was the PGT flux. Fig. 3 also shows that the PDN flux through full-thickness skin remained virtually constant for the first 4 days, after which it tended to increase. This finding is in accord with the

progressive microbial spoilage of the skin, previously noted with PGT as the penetrant. Accordingly, the effects of the agents were evaluated based on the $R(J)$ values calculated from average J values in the 30–70 h interval, as previously performed when dealing with PGT.

The results are reported in Table 2. It should be stressed that the effects of a given agent on epidermis permeability to PDN and PGT cannot be compared through the corresponding $R(J)$ values, since the PGT flux through EHMS is strongly influenced by the dermis layer. As demonstrated by the data in Table 2, the penetration accelerating effects of both HCOL30 and HCOL(II)30 were remarkable. In fact, no statistically significant difference was observed between the two agents.

These results indicate little if any relevance of the lipid-soluble components of HCOL to the effect of pretreatment with this agent on PDN penetration through EHMS. Since the reverse was found with PGT as the penetrant, there appears to be more than one mode of action of HCOL on skin. Discussion of this point will be resumed later.

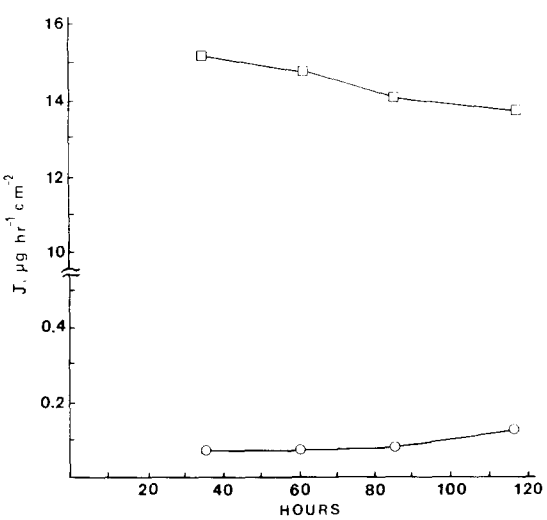

Fig. 3. Comparison between flux (J) vs time profiles for PDN through full-thickness EHMS pretreated with NSF (○) and isolated dermis taken from the same animal (□). Each profile is the mean of two EHMS specimens taken from different dorsal areas. Data representative of three experiments.

TABLE 2

Effect of EHMS pretreatment with different agents on the R(J) value for PDN

Reference	Jr (S.D.), [a] ($\mu g\ h^{-1}\ cm^{-2}$)	Agent/ Reference	$R(J)$ (S.D.) [a]
NSF	0.10 (0.02)	HCOL30/ NSF	20.3 (6.7)
		HCOL(II)30/ NSF	15.1 (5.4)
HCOL(II)30	1.33 (0.33)	HCOL30/ HCOL(II)30	1.15 (0.49)
HCOL(HN)30	0.42 (0.08)	HCOL30/ HCOL(HN)30	3.23 (0.95)
		HCOL15/ HCOL(HN)30	1.43 (0.33)

[a] Mean and standard deviation (S.D.) for four runs. The value for each run was an average of those in the 30–70 h interval. $R(J)$: ratio of drug flux through skin pretreated with the agent to that, taken from the same animal, pretreated with a reference (Jr).

The HCOL30 dispersion had a pH of 1.5, i.e., well below the physiological value for skin. However, the permeation enhancing effect of semi-neutralized HCOL30 (HCOL(HN)30), which was a dispersion of 15% HCOL and 15% NaCOL in NSF with a pH of 3.5, was substantially weaker than that pertaining to HCOL30, as appears from the $R(J)$ value for HCOL30/HCOL(HN)30 in Table 2. Such a difference between the effects of HCOL30 and HCOL(HN)30 is not believed to be due solely to the difference in their pH values, since we ascertained that the flux of either PGT or PDN through EHMS pretreated with isotonic HCl, pH 1.5, was not significantly different from the respective values determined with NSF as the pretreatment solution. The penetration enhancing effect of HCOL(HN)30 was even weaker than that of a dispersion of 15% HCOL in NSF (HCOL15, see Table 2), as evaluated based on the paired comparisons method ($P < 0.05$). Indeed, the presence of NaCOL may decrease the effectiveness of HCOL through the formation of mixed micelles.

The effect of HCOL concentration in the pretreatment dispersion on PDN flux was investigated by determining the $R(J)$ value for a given

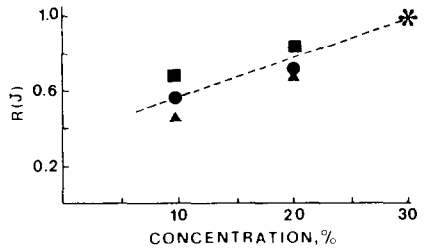

Fig. 4. Effect of HCOL concentration in the pretreatment vehicle on PDN flux through EHMS. The $R(J)$ value for the different concentrations was calculated with HCOL30 as the reference. Each series of data points was obtained with the same animal.

concentration relative to the reference concentration of 30%. The results are shown in Fig. 4. As can be seen, a reduction of the HCOL concentration from 30 to 10% corresponds to a decrease in flux by around 50%.

Permeation experiments where PDN suspensions in HCOL30 or HCOL(II)30 were applied to non-pretreated EHMS were carried out to investigate possible drug cotransport phenomena by some components of HCOL. The flux data obtained are represented in Fig. 5. Comparison between the J values for the two vehicles corresponding to each value of time by the paired comparisons method indicated a significantly higher flux for HCOL30 ($P < 0.01$) since the initial period. This result can be reconciled with

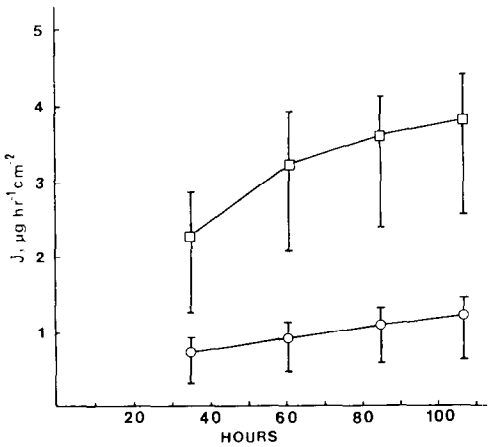

Fig. 5. Flux (J) vs time profiles for PDN permeation through EHMS from HCOL30 (□) or HCOL(II)30 (○). Each data point represents the mean of four values. Vertical bars represent the range.

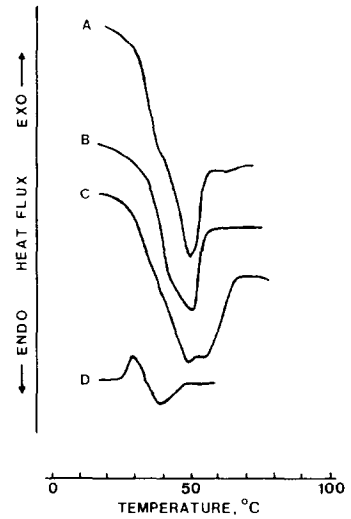

Fig. 6. DSC traces for isolated stratum corneum taken from a single animal, pretreated with HCOL(II)30 (trace A, $\Delta H =$ 14.0 J/g (49.8°C)), or NaCOL (trace B, $\Delta H = 15.8$ J/g (47.9°C)), or NSF (trace C, $\Delta H = 18.0$ J/g (48.5°C)), or HCOL30 (trace D, $\Delta H = 2.3$ J/g (40.8°C)). The thermograms are normalized by the weight of the sample. Data representative of three animals.

those of the pretreatment experiments, which demonstrated a non-significant difference between the effects of HCOL30 and HCOL(II)30, by hypothesizing that drug cotransport across the skin by some lipid-soluble components of HCOL represents a mechanism of permeation enhancement parallel to structural modification of EHMS.

DSC studies

It has been reported that thermal transitions of the stratum corneum lipids associated with increased mobility occur in the temperature range 27–67°C (Knutson et al., 1985). In Fig. 6 thermograms are compared for stratum corneum treated with the agents under study for the same time and at the same temperature as for the pretreatment of EHMS employed for the permeation studies. The transitions shown by the thermograms in Fig. 6 can safely be ascribed to a disordering of stratum corneum lipids. It clearly appears from such thermograms that the treatment with HCOL30 was the only one causing a dramatic decrease in both the enthalpy and the peak temperature of the transition, while NaCOL30

and HCOL(II)30 produced slight, barely significant changes. In order to ascertain whether the effect of HCOL30 was due to interactions of the more lipophilic bile acids with the lipid bilayers within the stratum corneum or to extraction of lipids from the stratum corneum, the effect of stratum corneum treatment with HCOL30 on the stratum corneum-NSF partition coefficient for PGT was determined. The choice of PGT for this test was prompted by the lipid affinity of this steroid. A value of 1022 was found for the stratum corneum treated with HCOL30 vs that of 292 for the stratum corneum treated with NSF. This finding and the DSC data are in support of a strong interaction between the more lipophilic bile acids and stratum corneum lipids within the stratum corneum.

Discussion

The pretreatment of EHMS with aqueous Na-COL resulted in no significant effect on skin permeability to either PGT or PDN. Nor did this agent produce any structural modification of the horny layer lipids that could be detected by DSC. Yet, bile salts such as sodium cholate and a combination of taurocholate and glycocholate have recently been found to enhance the percutaneous penetration of indomethacin and the hypocalcemic peptide elcatonin, respectively (Chiang et al., 1991; Ogiso et al., 1991). In order to resolve such an apparent contradiction, it should be considered that in the cases cited the bile salts were kept in contact with the skin throughout the permeation experiment, so they might favour drug penetration by some cotransport mechanism.

On the other hand, HCOL gave rise to a structural modification of EHMS resulting in increased permeability to both steroids. The free carboxylic function of the acids appears from these results to be of fundamental importance to the interaction of these acids with EHMS. The DSC studies have suggested that the lipid-soluble components of HCOL interact strongly with the stratum corneum lipids, thus perturbing the order of the bilayers. Such an effect is expected to facilitate the transcutaneous penetration through the lipid domain of the stratum corneum. Conversely, the chloroform-insoluble components of HCOL should exert a minor effect on such a route of penetration, as indicated by their failure to substantially alter the thermal transitions of the stratum corneum lipids. This reasoning is supported by the permeation data for PGT, which is a highly lipophilic molecule, supposed to traverse the lipid pathway. The permeation of PGT was indeed enhanced by HCOL30, whereas little if any enhancement was found with HCOL(II)30. The chloroform-insoluble components of HCOL were probably able to interact with more polar structures of the stratum corneum, in order to facilitate the penetration of PDN. This hypothesis is based on the following considerations. The PDN flux was enhanced to a non-significantly different degree by HCOL30 and HCOL(II)30. We determined a value of 37 for the PDN octanol-water partition coefficient. This value is of the same order of magnitude as the octanol-water partition coefficients for the hydrocortisone derivatives that were found to partition preferably in the protein domain of the horny layer (Raykar et al., 1988), therefore, it is reasonable to expect PDN to penetrate mainly by the polar route.

As already pointed out, the data in Fig. 5 suggest that the lipid-soluble components of HCOL, in addition to altering the structure of the lipid bilayers, can also accelerate steroid permeation by a cotransport mechanism. Such a phenomenon could be detected by permeation experiments only with PDN, since the dermis control of PGT penetration hindered the measurement of acceleration effects with this steroid.

In conclusion, the multiple effects of HCOL on drug penetration through EHMS demonstrated in this paper warrant future work aimed at identifying the individual components of the mixture responsible for such effects.

Acknowledgement

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References

Barry, B.W. and Bennet, S.L., Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin. *J. Pharm. Pharmacol.*, 39 (1987) 535–546.

Bond, J.R. and Barry, B.W., Hairless mouse skin is limited as a model for assessing the effects of penetration enhancers in human skin. *J. Invest. Dermatol.*, 90 (1988) 486–489.

Campigli, V., Di Colo, G., Nannipieri, E. and Serafini, M.F., Silicon dioxide as support for amorphous and metastable crystalline forms of indomethacin. Correlation between drug thermodynamic activity and percutaneous absorption in vitro. *Il Farmaco, Ed. Pr.*, 43 (1988) 57–70.

Chiang, C.H., Lai, J.S. and Yang, K.H., The effects of pH and chemical enhancers on the percutaneous absorption of indomethacin. *Drug Dev. Ind. Pharm.*, 17 (1991) 91–111.

Di Colo, G., Giannessi, C., Nannipieri, E., Serafini, M.F. and Vitale, D., Influence of drug-surfactant and skin-surfactant interactions on percutaneous absorption of two model compounds from ointment bases in vitro. *Int. J. Pharm.*, 50 (1989) 27–34.

Knutson, K., Potts, R.O., Guzek, D.B., Golden, G.M., McKie, J.E., Lambert, W.J. and Higuchi, W.I., Macro- and molecular physical-chemical considerations in understanding drug transport in the stratum corneum. *J. Controlled Release*, 2 (1985) 67–87.

Nannipieri, E., Carelli, V., Di Colo, G., Giorgi, I. and Serafini, M.F., Vehicle influence on the permeation of a highly lipophilic molecule. An in vitro technique to evaluate skin-vehicle interactions. *Int. J. Cosm. Sci.*, 12 (1990) 21–31.

Ogiso, T., Iwaki, M., Yoneda, I., Horinouchi, M. and Yamashita, K., percutaneous absorption of eleatonin and hypocalcemic effect in rat. *Chem. Pharm. Bull.*, 39 (1991) 449–453.

Raykar, P.V., Fung, M.C. and Anderson, B.D., The role of protein and lipid domains in the uptake of solutes by human stratum corneum. *Pharm. Res.*, 5 (1988) 140–150.

Sloan, K.B., Beall, H.D., Weimar, W.R. and Villanueva, R., The effect of receptor phase composition on the permeability of hairless mouse skin in diffusion cell experiments. *Int. J. Pharm.*, 73 (1991) 97–104.

Van der Merwe, E. and Ackermann, C., Physical changes in hydrated skin. *Int. J. Cosm. Sci.*, 9 (1987) 237–247.

Van der Merwe, E., Ackermann, C. and Van Wyk, C.J., Factors affecting the permeability of water through nude mouse skin in vitro. *Int. J. Pharm.*, 46 (1988) 169–171.