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Surfactant effects in percutaneous absorption II. Effects on protein and lipid structure of the stratum corneum

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Summary

The effects of several known penetration enhancers on the lipid structure of human stratum corneum have been investigated by differential scanning calorimetry. Effects on the birefringence were also examined. A correlation was found to exist between the ability of a compound to increase the birefringence of the stratum corneum and its reported tendency to induce local irritation when applied to the skin.

Introduction

Advances in instrumentation have made thermal analysis of the stratum corneum (SC) possible. Wilkes et al. (1973) used differential thermal analysis in conjunction with X-ray diffraction to study the lipid structure of SC. Analysis of rat SC showed a peak occurring between 60 and 70°C which was attributed to a transition of intercellular lipids and a second peak (approx. 110°C) attributed to denaturation of proteins.

Van Duzee (1975) studied thermotropic transitions of human SC by differential scanning calorimetry (DSC) and found peaks occurring at approx. 40, 70, 80 and 110°C. Considerable inter-subject variations (up to 7°C) were also noted. The authors postulated that the first two peaks were due to lipid transitions and the last two to denaturation of proteins. More recently, Potts et al. (1986) reported only three reproducible transitions occurring in human SC (hydrated to 50%). These were found at 65–70, 75–80 and 95–100°C. When lipids were removed by washing with a chloroform/methanol mixture the SC gave one peak at approx. 100°C. DSC of the extracted lipids also gave one peak, at approx. 65°C. It is thought that the peak occurring at 70°C is probably due to transitions of intercellular lipids and the 100°C peak is due to proteins. The intermediate peak was postulated to be caused by a protein/lipid complex in the corneocyte membrane. Further evidence for this was provided by Benga and Holmes (1984) who showed that association

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of lipids with proteins can increase lipid transition temperatures. Potts also reported a small transition at approx. 35°C which they believed to be due to surface sebaceous lipids. This is supported by the results of Bore et al. (1980) who found an endothermic peak at approximately the same temperature in extracted human sebum.

Golden et al. (1987) reported changes in the C-H symmetric and asymmetric stretching bands between 65 and 80°C in stratum corneum. This is also consistent with the hypothesis that structural changes in the lipid structure occur over this temperature. The flux of tritiated water through porcine SC was measured over a range of temperatures and from an Arrhenius plot of the log of the permeability coefficient versus the reciprocal of temperature, the activation energy of penetration of water vapour through SC could be deduced. It was found that above 70°C activation energy decreased from 70 to 23 kJ/mol. The activation energy of water penetration through lipid stripped porcine SC is 26 kJ/mol indicating that the SC loses almost all its barrier properties to water when the lamellar structure of the lipids is broken down.

Barry and Goodman (1986) have shown that the thermogram of SC can be drastically altered by treatment with penetration enhancers which appear to act on the lipid structure of the SC. The effects of penetration enhancers on SC proteins have also been studied. Putterman et al. (1977) studied swelling and showed that ionic surfactants cause expansion of the SC while nonionic surfactants and lipid solvents have no effect. Amongst alkyl anionic surfactants the greatest expansion was produced by an alkyl chain length of 12. Surfactant concentrations of 1/30 CMC were used.

Blank and Shappirio (1955) showed that treatment of human callus with soaps removes water soluble material and reduces the ability of the skin to absorb water. Middleton (1969) proposed that anionic surfactants alter the barrier function of the SC allowing removal of water-soluble agents that normally act as plasticisers. It was postulated that the ability of a surfactant to remove skin lipids would correlate with its ability to reduce hydration and cause irritation and roughness. Imokawa et al. (1975) obtained a better correlation between skin roughness and the effect of surfactants on the optical activity of bovine serum albumin solutions. Imokawa (1979), however, later reported that both SLS and nonirritating sodium lauryl phosphate alter the optical activity of BSA solutions indicating that the ability to denature a protein is not, in itself, enough to produce irritation.

Scheuplein and Ross (1970) reported that treating isolated sheets of SC with a 5% solution of sodium laurate caused a reversible decrease in its birefringence. They suggested that this indicated an uncoiling of keratin from an α-helix to a β-sheet. Oertel (1977) studied protein conformation of human SC by IR spectroscopy and found that although exposing SC to 11.7 M DMSO caused substantial α-helix to β-sheet conversion of keratin, exposure to 1 M DMSO for up to 24 h generated only trace amounts of β-sheet. No evidence of sheet formation was found in SC treated with 5% SLS and extracting skin lipids with nonpolar solvents also failed to affect the protein structure.

Previous in vitro (Part I) and in vivo (Gershbein, 1979; Hwuang and Danti, 1983) work has indicated that although ionic and nonionic surfactants can both increase the permeability of the SC the mechanisms of penetration and enhancement may be different. We have now investigated the modes of action of topically applied compounds with both the protein and lipid structures of the SC.

Materials and Methods

Cetyl trimethyl ammonium bromide (CTAB) and bovine trypsin (type III-S) were purchased from Sigma Chemical Co. (MO). Sodium lauryl sulphate (SLS) and Brij 36T (B36T) were obtained from BDH Chemicals, U.K. Azone® was donated by Whitby Research and the Pluronic surfactant, PF-127, by Atochem. Decyl methyl sulphoxide (DMS) was obtained from Cyclo Products (CA). Excised human abdominal skin was obtained at autopsy from Caucasian donors aged

between 47 and 83 years and stored at −20°C until used.

Subcutaneous fat was removed from full thickness skin and the epidermis isolated by heat separation (Christophers and Kligman, 1963). Viable epithelial cells were removed from the SC by soaking the epidermis in 0.0001% trypsin for 4 h at 37°C and then gently brushing to remove adhering epithelial cells. Samples were cut into 4 mg pieces and treated in surfactant solutions for 1 h before being desiccated over $CaCl_2$. The SC samples were rehydrated over a saturated solution of $BaCl_2$ (relative humidity 97%) for 24 h. Hydrated SC was then loaded into hermetically sealed pans and analysed by DSC using a Perkin Elmer DSC 7 linked to a data handling system. Samples were heated from 10 to 110°C at a heating rate of 10°C/min.

Birefringence of SC was assessed using the apparatus shown in Fig. 1. Samples were cut into pieces approx. 1 cm × 2 cm, spread over microscope slides and desiccated. The transmittance of each sample was measured using a Kontron Uvicon 860 scanning spectrophotometer. The birefringence was also measured over a wide range and the optimum wavelength for measurements determined. This was the wavelength giving the greatest birefringence to absorbance ratio, and was found to be 655–665 nm. All subsequent measurements were performed over this range. The birefringence of desiccated SC was measured before and after surfactant treatment (described above). The effect of Azone® was investigated by soaking the SC in 5% Azone® in ethanol for 1 h

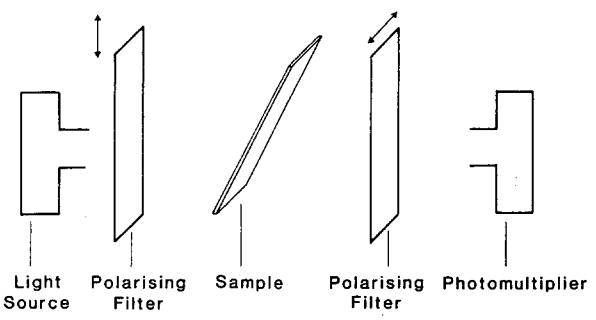

Fig. 1. Apparatus used to determine the birefringent effect of isolated sheets of human stratum corneum.

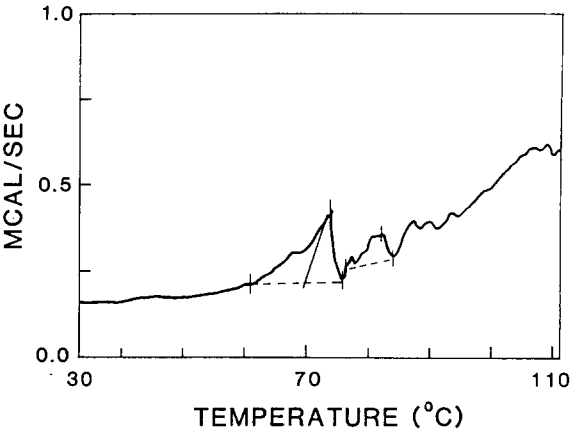

Fig. 2. Typical thermogram of isolated human stratum corneum obtained using a Perkin Elmer DSC 7.

and drying. Controls were treated in ethanol alone.

The effect of hydration was determined by treating SC with deionised water and measuring its birefringence as it dried. The birefringence of SC was also assessed before and after lipid stripping with a 2:1 chloroform:methanol mixture. The transmittance of each SC sample to unpolarized light between 655 and 665 nm was determined before and after each treatment.

Results

When desiccated SC samples were hydrated at 97% RH, their mass increased by 40 ± 20% (S.D., $n = 22$). Pretreatment with surfactant solutions did not significantly affect water uptake.

DSC analysis of SC that had been soaked in deionised water showed one large peak (0.4–0.8 cal/g) at 68 ± 2°C and another peak at 78 ± 3°C. A broad, poorly defined peak occurred above 95°C and no peaks were found between 30 and 45°C. A typical thermogram is shown in Fig. 2. Treatment of the SC in 5.4 mM SLS eliminated the two low temperature peaks although the peak beyond 95°C was still evident (Fig. 3). The effects of CTAB treatment were not significantly different from those of SLS. Treatment with B36T gave peaks at 55, 62, 67 and 100°C (Fig. 4) while in the case of DMS peaks were observed at 53

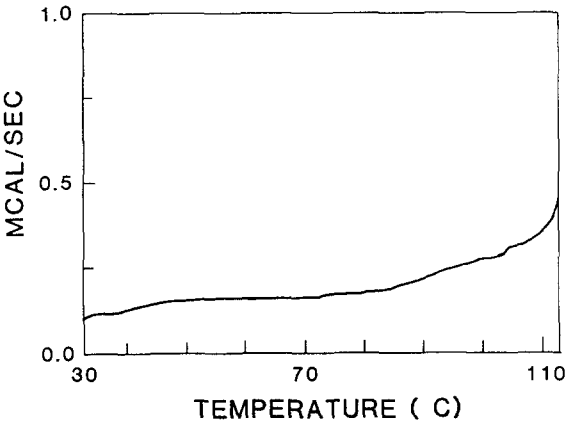

Fig. 3. Typical thermogram of human stratum corneum treated with 1.5% SLS for 1 h.

and 62°C but none between 67 and 80°C. PF-127 had no significant effect on the thermogram of SC and suspensions of the surfactants did not result in any peaks over the temperature range studied.

None of the surfactant treatments caused any significant change in the transmittance of SC over the frequency range studied. The birefringence of the SC was not significantly affected by its hydration state or treatment with B36T, Azone, PF-127 or ethanol. SLS increased the intensity of light by 46% indicating an increase in birefringence. CTAB caused a greater increase (by over 46%) and DMS treatment increased the intensity by

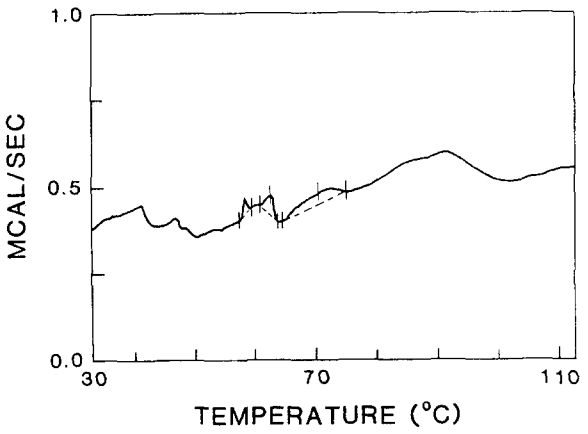

Fig. 4. Thermogram of human stratum corneum treated with 1.5% B36T for 1 h.

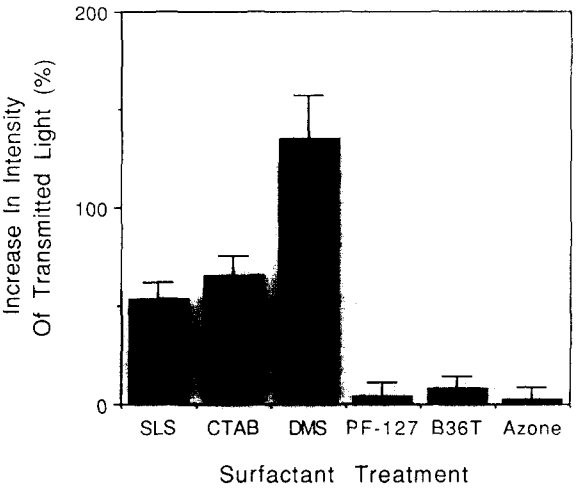

Fig. 5. Effect of different surfactant solutions on the birefringence of human stratum corneum assessed by the intensity of transmitted light. Results are the means of six determinations; standard deviations are indicated.

over 110% (Fig. 5). Removal of skin lipids from the SC failed to change its birefringence.

Discussion

DSC analysis of untreated SC gave poorly defined peaks at approx. 68 and 78°C which can be attributed to phase changes in the structure of intercellular lipid and lipid/protein conjugates, respectively (Elias, 1983; Potts et al, 1986). The broad peak observed beyond 100°C is probably caused by the denaturation of protein (Van Duzee, 1973). In agreement with the findings of Potts et al. (1986), no peaks were observed in normally hydrated SC near 40°C.

It has been generally found that cationic surfactants are more damaging to biological membranes than anionic surfactants which are more damaging than nonionic surfactants (Riegelman and Crowell, 1958). However, the reported rank order of skin irritation amongst the compounds studied in this work is DMS > CTAB > SLS ≫ B36T (Nater and DeGroot, 1988). This is the same order as that of their ability to alter the birefringence of the SC. This suggests that the ability of a compound to cause skin irritation is a

function of its ability to penetrate and interact with proteins of the SC and, presumably, viable tissues. The model proposed by Imokawa et al. (1975) using BSA solutions considers only protein interactions while that proposed by Middleton (1969) considers only lipid removal. Measurement of the birefringence of the SC may prove useful in the evaluation of potential penetration enhancers.

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