ELSEVIER

International Journal of Pharmaceutics 168 (1998) 17–22

international journal of pharmaceutics

Differential scanning calorimetry of human and animal stratum corneum membranes

S.M. Al-Saidan [a], B.W. Barry [b,*], A.C. Williams [b]

[a] *Department of Pharmaceutical and Medical Sciences, College of Health Sciences, Public Authority for Applied Education and Training (PAAET), P.O. Box 14281, Faiha, 72853, Kuwait*

[b] *Postgraduate Studies in Pharmaceutical Technology, The School of Pharmacy, University of Bradford, Bradford, BD7 1DP, UK*

Received 21 January 1998; accepted 2 February 1998

Abstract

Thermal transitions in desiccated stratum corneum membranes of neonatal rats, mice and rabbits and adult abdominal human skin were investigated using differential scanning calorimetry (DSC). Four endothermic transitions at 39–45 (T1), 55–58 (Tx), 68–74 (T2) and 77–86°C (T3) were observed. T1, T2 and T3 were attributed to phase changes in the intercellular lipid bilayers. Tx was a weak transition, the exact nature of which is unknown. A fifth transition at 48°C (Ty) was only observed with neonatal rabbit stratum corneum and was attributed to lipid melting. The DSC results indicated that the temperatures of lipid transitions of human and animal tissues were different although the patterns of their thermograms were similar. The temperatures of T2 and T3 of human stratum corneum were higher than those of the animals. DSC of neonatal rabbit stratum corneum showed a lipid transition not seen in stratum corneum of human or other animal species.

Keywords: Skin; Animal species; Thermal analysis; Lipids

1. Introduction

The outermost layer of mammalian skin, the stratum corneum, acts as the principal barrier to diffusion of most substances (Scheuplein and Bronaugh, 1983). It essentially consists of flattened keratinocytes embedded in a matrix of multi-lamellar lipid bilayers (Elias et al., 1977, 1983).

Thermal analysis techniques such as differential scanning calorimetry (DSC) and differential thermal analysis (DTA) have been used to study thermal transitions in mammalian stratum corneum. Typically, thermal transitions occur at 35–42, 60–77, 70–90 and 95–120°C and are referred to as T1, T2, T3 and T4, respectively. T1, T2 and T3 are attributed to phase changes in the

* Corresponding author. Tel.: +44 1274 384760; fax: +44 1274 384769.

0378-5173/98/$19.00
PII S0378-5173(98)00078-7

intercellular lipid bilayers and T4 is associated with protein denaturation (Barry and Williams, 1995). In human stratum corneum, a fifth transition at 51–55°C has also been reported and attributed to the covalently bound lipids of the corneocyte envelope (Gay et al., 1994; Cornwell et al., 1996; Naik and Guy, 1997).

Biological factors, such as species variations, are generally recognized to have impact on skin permeability. In general, skin permeability increases in the order: human, pig, guinea pig, rat, and rabbit, but the ranking is not consistent with change of penetrant (Barry, 1983; Sato et al., 1991; Walters, 1993). Only a few studies, however, have investigated the effect of species differences on the thermal behaviour of stratum corneum (Rehfeld et al., 1980; Rehfeld and Elias, 1982; Knutson et al., 1985; Golden et al., 1987; Hirvonen et al., 1991). These workers showed different results, probably due to variations including heating rate, degree of hydration of samples and sampling methods. In our work, using identical experimental conditions, we investigated and compared the DSC thermograms from neonatal rabbits, neonatal rats, neonatal mice and adult human abdominal stratum corneum. Dry samples were used in this comparison to avoid any discrepancies that might arise due to variations in the degree of hydration of samples. In the present study, we hoped to detect variations among thermograms of the different species that may correlate with the reported differences in skin permeability among these species (Barry, 1983; Sato et al., 1991; Walters, 1993).

2. Experimental

2.1. Preparation of stratum corneum

Neonatal rabbits (offspring of F1, New Zealand/California cross), neonatal rats (albino outbred, Sprague–Dawley) and neonatal mice (albino outbred, OLAC MF1) were used within 24 h of birth. Stratum corneum sheets were obtained by placing the full thickness skin onto cotton wool moistened with phosphate buffer solution (potassium dihydrogen phosphate and disodium hydrogen phosphate; BDH Laboratory Supplies, Poole, UK), pH 7.4, containing 0.025% trypsin (type III, bovine; Sigma, St. Louis, MO) and incubating for 10–12 h at 37°C. After this treatment, stratum corneum sheets were removed from the skin and the digested material was separated from the stratum corneum by agitation, in a conical flask, with distilled water. The cleaning process was repeated three times, with fresh distilled water. The cleaned sheets were then soaked, overnight, in distilled water at room temperature. Finally, the membranes were rinsed with fresh distilled water and dried, overnight, on wire meshes under ambient conditions. Human abdominal skin was obtained postmortem and stored at −20°C. Human stratum corneum membranes were prepared as for animal tissue except that the skin was dermatomed (Citenco, Borehamwood, Herts, UK) into 400 μm thick layers, before digestion with trypsin. All samples were stored in a desiccator, over silica gel, for at least 3 days before thermal analysis. Samples of lipid-extracted stratum corneum were obtained by soaking the dried membranes in chloroform-methanol (2:1 v/v) for 3 h, at room temperature, followed by overnight soaking in fresh chloroform-methanol (2:1 v/v). The delipidized samples were desiccated, as above, before thermal analysis.

2.2. Differential scanning calorimetry

Desiccated stratum corneum samples were folded and hermetically sealed into 75 μl stainless-steel capsules and heated from 0 to 140°C at 10°C/min, using a DSC7 Differential Scanning Calorimeter (Perkin-Elmer, USA). After the first heating run, samples were cooled, in their hermetically sealed pans, to 0°C and then immediately reheated again to 140°C at 10°C/min as before. Sample weight was about 4 mg for mouse stratum corneum and 10 mg for the other species. Because of difficulties in accurately measuring onset temperatures, transition temperatures reported from all thermograms are taken from the peak maxima values.

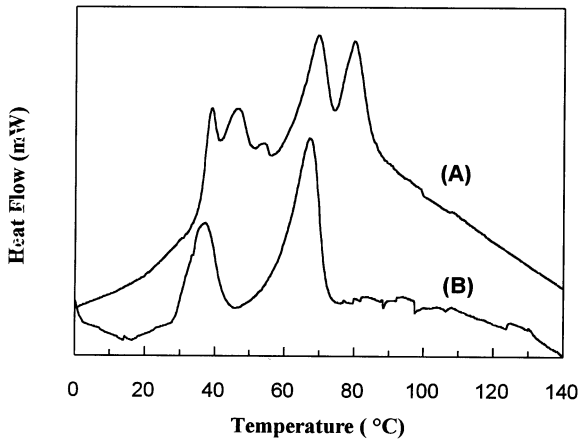

Fig. 1. DSC thermogram of (A) desiccated neonatal rabbit stratum corneum and (B) reheated sample.

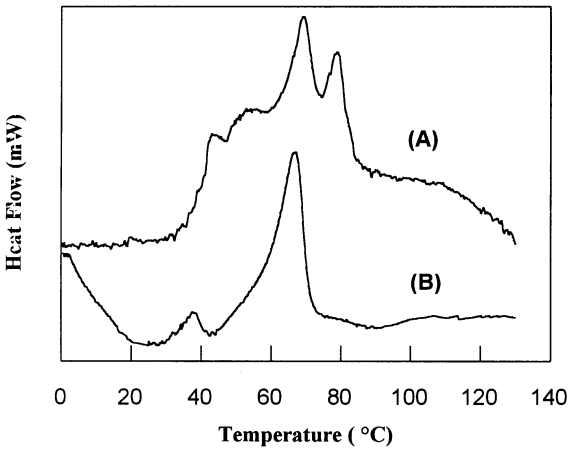

Fig. 3. DSC thermogram of (A) desiccated neonatal mouse stratum corneum and (B) reheated sample.

3. Results and discussion

DSC analysis of dry stratum corneum samples of neonatal rabbits, neonatal rats, neonatal mice and human abdomen showed three major thermal transitions, T1, T2 and T3 that occurred at 39–45, 68–74 and 77–86°C, respectively (Figs. 1–4). Similar transition values were reported by Van Duzee (1975), Goodman and Barry (1986), Golden et al. (1987), Bouwstra et al. (1989), and several other workers (Barry and Williams, 1995; Naik and Guy, 1997).

On the second heating, endotherm T3 disappeared in the samples of the different species. However, two endothermic transitions developed at 37–40°C and 66–73°C, for the animal stratum corneum membranes, and only one transition at about 72°C for the human samples. T1, T2 and T3 were removed by lipid extraction with chloroform-methanol (2:1 v/v), and are thus attributed to lipid phase changes (Winfield and Tylor, 1990; Cornwell et al., 1996). The transition T1 of human stratum corneum was minor, difficult to distinguish and not apparent in all the samples. It was also lost on the second heating cycle (Fig. 4). This behaviour agrees with the results of other workers (Van Duzee, 1975; Cornwell et al., 1996).

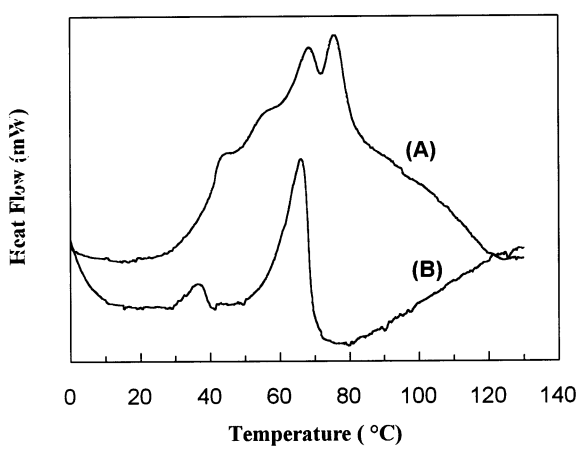

Fig. 2. DSC thermogram of (A) desiccated neonatal rat stratum corneum and (B) reheated sample.

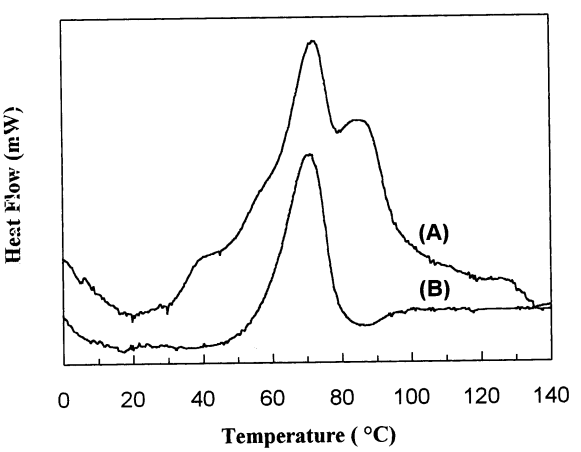

Fig. 4. DSC thermogram of (A) desiccated human abdominal stratum corneum and (B) reheated sample.

Table 1
The transition midpoint temperatures observed in desiccated stratum corneum membranes from human and neonates of different animal species (means ± S.D.)

Species	n[a]	First heating					Second heating	
		T1	Ty	Tx	T2	T3	T1	T2
Rabbit	6	39.8 ± 0.6	48 ± 1.6	56.9 ± 2.7	69.5 ± 1.0	78.6 ± 1.6	39.5 ± 1.3	67.9 ± 1.3
Rat	8	44.3 ± 0.6	—	55.1 ± 1.0	70.2 ± 0.6	77.2 ± 0.6	39.8 ± 0.7	68.1 ± 1.0
Mouse	8	43.2 ± 1.4	—	55.2 ± 0.7	68.6 ± 1.0	78.3 ± 1.1	37.9 ± 1.4	66.7 ± 1.2
Human	7	41.1 ± 1.5	—	57.2 ± 1.6	73.4 ± 0.3	86.1 ± 1.2	—	72.3 ± 0.8

[a] Number of replicates (T1 of human stratum corneum was apparent in only three replicates).

Endotherm T1 of neonatal rabbit stratum corneum was considerably pronounced compared with those of the other species (Fig. 1). The transition temperature of T1 ranged from 39°C to 45°C among the different species tested and on the second heating this transition occurred at a similar temperature. The reason for this variation in the transition temperatures is not understood and requires further investigation. However, further preliminary studies (data not shown) indicated the involvement of free sterols, particularly cholesterol, in the T1 endotherm.

In the present work, a weak transition at 55–57°C was also observed with the animal and human abdominal stratum corneum samples. The intensity of this transition varied inter- and intra-species. Gay et al., 1994 and Cornwell et al., 1996 also reported this transition and attributed it to structural changes in the covalently bound lipids present on the outside of the corneocyte envelope. However, the exact nature of this transition and its role in the barrier function of the tissue are unknown.

The thermogram from samples of rabbit stratum corneum showed an unreported transition at 48°C (Ty) (Fig. 1). This transition was irreversible on the second heating and thus probably arose from melting of lipids; its importance will be discussed later. In their DSC studies, Hirvonen et al. (1991) reported only two lipid transitions, at 44°C and 68°C, from rabbit pinna stratum corneum. However, comparison between their work and the present study is difficult. Hirvonen et al. used hydrated samples (40%), prepared stratum corneum membranes by a heat separation method (at 60°C for 2 min), which might have eliminated Ty, and used mature rabbit tissue. Also they did not observe T3 in the rabbit pinna stratum corneum.

A direct comparison of the DSC of desiccated samples from the different species shows four endothermic transitions; however, the transition temperatures varied between the species (Table 1). The transition temperatures T2 and T3 were higher from the human samples than from those of the animals. This may imply that human lipid molecules are more highly ordered and closely packed, resulting in a greater barrier stability and impermeability. Also the appearance of a lipid transition at low temperature (48°C) in neonatal rabbit stratum corneum may suggest that the lipid is 'softer' than those of human and the other animal species investigated in our work. These observations correlate with the fact that for most penetrants so far tested, human skin is less permeable than those of the animals, and rabbit skin is the most permeable of those we investigated.

Although stratum corneum of human and animals displays similar classes of lipids (such as ceramides, free sterols, free fatty acids and sterol esters) there are some differences in the distribution of long-chain fatty acids (C_{24} and greater) in the lipid classes (Elias et al., 1977, 1983). Several authors have suggested that these long-chain saturated free fatty acids can form a relatively stable hydrophobic barrier (Elias et al., 1977, 1983; Wertz and Downing, 1982).

It is not known whether the variations observed in the lipid transitions of the different species are related to the saturated free fatty acids. Also, do

the variations in the thermal transitions, due to subtle variations within the lipid bilayers structure of these species, explain the inter-species differences in percutaneous penetration? The answers to these questions require further investigations to establish a possible correlation between lipid thermal transitions and skin permeability of the different species.

Variations in skin permeability among species have also been attributed to several factors, such as degree of hairiness, variability of growth pattern, differences in sebaceous mechanisms, sweating and tissue layer thickness (McCreesh, 1965). However, some of these factors have been questioned because the physical characteristics of skin vary widely between man and animals, which may affect the rate of transdermal permeation. For example, skins of rabbits, rats and mice lack sweat glands but abound in hair follicles (Idson, 1975). However, rabbit skin usually shows higher permeability than other species, even though neither epidermal structure nor appendageal frequency differs appreciably from species that are more resistant to penetration (Winkelmann, 1969). Dupuis et al. (1984) attributed species variations to differences in the number of cell layers and intercellular volume. Variations in skin permeability have also been attributed to the quantity of intercellular lipids (Elias et al., 1983; Lampe et al., 1983), to the quality and quantity of stratum corneum lipids (Smith et al., 1982), or to the amount of skin surface lipids (Sato et al., 1991). Nevertheless, all these authors have studied the effect of species variations from a particular angle, presenting reasonable justifications for their explanations. These explanations may, however, not necessarily hold for a wider range of penetrants or animal species. Hence, the effect of species variations on skin permeability requires further investigation using different techniques concurrently, such as thermal analysis, lipid analysis and permeability studies, with the consideration of the structure of the stratum corneum. This also will enable us to gain more knowledge and understanding of the nature of the skin permeability barrier.

Acknowledgements

As a visiting academic, S.M. Al-Saidan thanks Professor Barry for his invitation to work in his laboratories, The School of Pharmacy, University of Bradford, which supported the work and PAAET, Kuwait, for the travel grant.

References

Barry, B.W., 1983. Dermatological Formulations: Percutaneous Absorption. Marcel Dekker, New York.

Barry, B.W., Williams, A.C., 1995. Permeation enhancement through skin. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology, vol. 11. Marcel Dekker, New York, pp. 449–493.

Bouwstra, J.A., Peschier, L.J.C., Brussee, J., Boddeé, H.E., 1989. Effect of *N*-alkyl-azocycloheptan-2-ones including azone on the thermal behaviour of human stratum corneum. Int. J. Pharm. 52, 47–54.

Cornwell, P.A., Barry, B.W., Boustra, J.A., Gooris, G.S., 1996. Modes of action of terpene penetration enhancers in human skin; differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. Int. J. Pharm. 127, 9–26.

Dupuis, D., Rougier, A., Roguet, R., Lotte, C., Kalopissis, G., 1984. In vivo relationship between horny layer reservoir effect and percutaneous absorption in human and rat. J. Invest. Dermatol. 82, 353–356.

Elias, P.M., Goerke, J., Friend, D.S., 1977. Mammalian epidermal barrier layer lipids: composition and influence on structure. J. Invest. Dermatol. 69, 535–546.

Elias, P.M., Grayson, S., Lampe, M.A., Williams, M.L., Brown, B.E., 1983. The intercorneocyte space. In: Marks, R., Plewig, G. (Eds), Stratum Corneum. Springer, Berlin, pp. 53–67.

Gay, C.L., Guy, R.H., Golden, G.M., Mak, V.H.W., Francoeur, M.L., 1994. Characterization of low-temperature (i.e. $< 65°C$) lipid transitions in human stratum corneum. J. Invest. Dermatol. 103, 233–239.

Golden, G.M., Guzek, D.B., Kennedy, A.H., McKie, J.E., Potts, R.O., 1987. Stratum corneum lipid phase transitions and water barrier properties. Biochemistry 26, 2382–2388.

Goodman, M., Barry, B.W., 1986. Differential scanning calorimetry of human stratum corneum: effect of penetration enhancers azone and dimethyl sulphoxide. Anal. Proc. 23, 397–398.

Hirvonen, J., Rajala, R., Laine, E., Paronen, P., Urtti, A., 1991. Penetration enhancers dodecyl *N*,*N*-dimethylamino acetate and azone alter the structure of the skin — a DSC study. In: R.C. Scott, R.H. Guy, J. Hadgraft (Eds.), Prediction of Percutaneous Penetration: Methods, Measurements, Modelling, vol. 2. IBC Technical Services, London, p. 350.

Idson, B., 1975. Percutaneous absorption. J. Pharm. Sci. 64, 901–924.

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

Lampe, M.A., Burlingame, A.L., Whitney, J., Williams, M.L., Brown, B.E., Roitman, E., Elias, P.M., 1983. Human stratum corneum lipids: characterization and regional variations. J. Lipid Res. 24, 120–130.

McCreesh, A.H., 1965. Percutaneous toxicity. Toxicol. Appl. Pharmacol. 7 (Suppl. 2), 20–26.

Naik, A., Guy, R.H., 1997. Infrared spectroscopic and differential scanning calorimetric investigations of the stratum corneum function. In: Potts, R.O., Guy, R.H. (Eds.), Mechanisms of Transdermal Drug Delivery. Marcel Dekker, New York, pp. 87–162.

Rehfeld, S.J., Elias, P.M., 1982. Mammalian stratum corneum contains physiologic lipid thermal transitions. J. Invest. Dermatol. 79, 1–3.

Rehfeld, S.J., Lampe, M.A., Elias, P.M., 1980. Novel physiologic thermal transitions due to lipid in mammalian keratinizing epithelia (abstract). J. Cell. Biol. 87, CD134.

Sato, K., Sugibayashi, K., Morimoto, Y., 1991. Species differences in percutaneous absorption of nicorandil. J. Pharm. Sci. 80, 104–107.

Scheuplein, R.J., Bronaugh, R.L., 1983. Percutaneous absorption. In: Goldsmith, L.A. (Ed.), Biochemistry and Physiology of the Skin, vol. 2. Oxford University Press, New York, pp. 1255–1295.

Smith, W.P., Christensen, M.S., Nacht, S., Gans, E.H., 1982. Effect of lipids on the aggregation and permeability of human stratum corneum. J. Invest. Dermatol. 78, 7–11.

Van Duzee, B.F., 1975. Thermal analysis of human stratum corneum. J. Invest. Dermatol. 65, 404–408.

Walters, K.A., 1993. Veterinary application of skin penetration enhancers. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement, vol. 59. Marcel Dekker, New York, pp. 348–353.

Wertz, P.W., Downing, D.T., 1982. Glycolipids in mammalian epidermis: structure and function in the water barrier. Science 217, 1261–1262.

Winfield, A.J., Tylor, P.M., 1990. Thermal analysis as a screen test for potential skin penetration enhancers. In: Scott, R.C., Guy, R.H., Hadgraft, J. (Eds.), Prediction of Percutaneous Permeation; Methods, Measurements, Modelling. IBC Technical Services, London, pp. 412–416.

Winkelmann, R.K., 1969. The relationship of the structure of the epidermis to percutaneous absorption. Br. J. Dermatol. 81 (Suppl. 4), 11–22.