ELSEVIER

International Journal of Pharmaceutics 106 (1994) 115–123

international journal of pharmaceutics

Partitioning and differential scanning calorimetry studies of *N*-alkyllactame ester dermal prodrugs of indomethacin

F.P. Bonina [a,*], F. Castelli [b], L. Montenegro [a], G. Mazzone [a]

[a] *Istituto di Chimica Farmaceutica,* [b] *Dipartimento di Scienze Chimiche, Università di Catania, Catania, Italy*

(Received 13 September 1993; Modified version received 8 November 1993; Accepted 15 November 1993)

Abstract

The mechanism of indomethacin percutaneous absorption enhancement by *N*-alkyllactam ester prodrugs (esters I–III) was investigated by assessing stratum corneum partitioning and solubility together with diffusion parameters of both indomethacin and its prodrugs. Skin diffusional characteristics of indomethacin and its esters were evaluated on the basis of their apparent diffusion coefficients and their interaction, assessed by differential scanning calorimetry, with multilamellar liposomes of dipalmitoylphosphatidylcholine. From the results obtained, the enhancement ability of esters I and II could be attributed to their greater stratum corneum solubility associated with better diffusion characteristics compared to the parent drug, while the enhancement effect of ester III could be due only to its higher diffusion coefficient.

Key words: Indomethacin; Dermal prodrug; Partitioning; Diffusion; DSC

1. Introduction

In recent years much attention has been focused on the use of bioreversible derivatives of drugs, namely prodrugs, to improve their pharmacokinetic properties. The prodrug approach involves the chemical attachment of a suitable promoiety to the drug to impart the desired physicochemical properties.

Of the many problems addressed via the prodrug approach, incomplete or poor absorption through a biological membrane presents one of the most challenging problems to overcome. Since the skin membrane is a poorly permeable barrier for most drugs applied topically, the prodrug approach to enhance dermal and transdermal delivery has been increasingly used, as confirmed by a large number of papers (Bundgaard et al., 1983; Higuchi et al., 1983; Johansen et al., 1986), reviews (Chan and Li Wan Po, 1989; Sloan, 1989) and a book recently published (Sloan, 1992).

Notwithstanding the considerable interest in this topic, to date no guidelines have been well established about the choice of a suitable promoiety to impart the targeted physicochemical properties to a certain drug so as to improve its skin penetration.

On the basis of theoretical arguments, Guy and Hadgraft (1992) reported that in designing dermal prodrugs it is important to choose the promoiety so as to impart to the prodrug an

* Corresponding author.

SSDI 0378-5173(93)E0353-R

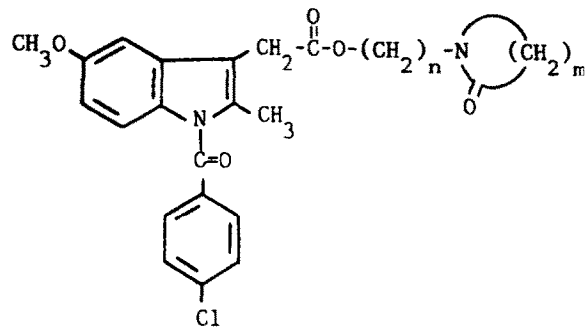
affinity for both lipophilic and hydrophilic environments. This derives from the assumption that the intercellular pathway represents the major route of transport across the stratum corneum and, because its lipid domain consists of multilamellar bilayers, the transporting species must be able to repetitively cross lipid-aqueous phase interfaces.

On the other hand, Sloan (1992) has extensively reviewed the literature regarding dermal prodrugs and, on the basis of his work and the data reported by others, he outlined that in a homologous series of prodrugs an increase in both lipid and water solubility with respect to the parent drug resulted in enhanced delivery of the parent drug through the skin.

Yet, it is well known that drug stratum corneum/vehicle partitioning and diffusional processes are the key parameters which control drug skin penetration (Hadgraft, 1989). Although there have been a large number of modes of action of penetration enhancers proposed in the literature (Barry, 1987) to elucidate their effects on drug partitioning and diffusion processes, no data have been reported on the enhancement mechanisms of dermal and transdermal prodrugs. We thought it noteworthy to study the influence of drug derivatization on diffusional and partitioning processes compared to the parent drug, since a better understanding of the prodrug enhancement mechanism could aid in the selection of suitable promoieties in the design of successful dermal and transdermal prodrugs relative to the partitioning and diffusional characteristics of the parent drug.

In this paper, in order to elucidate the mechanism of indomethacin percutaneous absorption enhancement by previously synthesized *N*-alkyllactam ester prodrugs (Bonina et al., 1991) (Fig. 1) which showed the preliminary required properties for dermal prodrugs (water stability, rapid enzymatic hydrolysis and in vitro drug skin penetration enhancement), we assessed stratum corneum/water partitioning and diffusion parameters of both indomethacin and its prodrugs.

Therefore, the stratum corneum/vehicle partition coefficient was measured using excised stratum corneum for both indomethacin and its pro-



I	n= 2	m= 3
II	n= 2	m= 4
III	n= 2	m= 5

Fig. 1. Chemical structure of esters I–III.

drugs. Since the effective diffusion coefficient cannot be measured directly at present (Kasting et al., 1992), to assess the skin diffusional characteristics of indomethacin and its esters we determined their apparent diffusion coefficients on the basis of previously determined in vitro fluxes through the skin (Bonina et al., 1991) and their experimental stratum corneum/water partition coefficient. Furthermore, since the interaction between stratum corneum structured lipid bilayers and the permeant is regarded as one of the main factors which affects drug permeation, we determined, by differential scanning calorimetry (DSC), the interaction between the tested compounds and multilamellar liposomes of dipalmitoylphosphatidylcholine (DPPC), which are regarded as a simple model of skin lipid bilayer (Beastall et al., 1988; Rolland et al., 1991). The results obtained from DSC studies were compared with the calculated apparent diffusion coefficients.

2. Materials and methods

2.1. Chemicals

Synthetic L-α-dipalmitoylphosphatidylcholine (DPPC) was obtained from Fluka Chemical Co.

(Buchs, Switzerland). Solutions of the lipid were chromatographically pure as assessed by two-dimensional thin-layer chromatography. Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile and water used in the HPLC procedures were of LC grade and were obtained from Carlo Erba (Italy). All other chemicals or solvents were of reagent grade.

1-Hydroxyethylazacycloalkan-2-one esters of indomethacin (esters I–III) used in this study were synthesized as previously described (Bonina et al., 1991).

2.2. Preparation of liposomes

DPPC multilamellar liposomes were prepared in the presence and absence of indomethacin and its esters I–III at a temperature above that of the gel-liquid crystalline phase transition. Chloroform-methanol (1 : 1, v/v) stock solutions of lipid and drug were mixed in order to obtain the chosen molar fraction of compound. The solvents were removed under nitrogen and the resulting film was kept overnight on a vacuum pump to remove the residual solvents.

Liposomes were prepared by adding 50 mM Tris buffer (pH 7.4) to the film, then heating at 60°C and vortexing three times for 1 min.

The samples were shaken for 1 h in a water bath at 60°C to homogenize the liposomes. Afterwards, aliquots of 120 μl (5 mg of lipid) were transferred to a 160 μl DSC aluminium pan and submitted to DSC analysis.

2.3. DSC

DSC was performed by using a Mettler TA 3000 system equipped with a DSC-30 cell and a TC-10 processor. The scan rate employed was 2°C/min in the temperature range 10–70°C after an initial isothermal period of 15 min. The sensitivity was 1.72 mW, and the reference pan was filled with Tris buffer solution. Temperature and enthalpy calibration was performed by using palmitic acid as reference. Enthalpy changes were calculated from peak areas by using the integration program of the Mettler processor.

The samples were cooled and heated four times to achieve reproducibility. After calorimetric scans, all the samples were extracted from the pan and aliquots were used to determine the amount of phospholipid by phosphorus assay (Bartlett, 1959).

2.4. Determination of stratum corneum / water partition coefficient

Samples of adult human skin (mean age 39 ± 7 years) were obtained from breast reduction operations. Sheets of stratum corneum (s.c.) were prepared in accordance with the method reported by Kligman and Christophers (1963). The s.c. sheets were dried and stored according to the method of Swarbrick et al. (1982). The s.c. samples were accurately weighed and then hydrated for 1 h in distilled water before starting the partitioning experiments. 2 ml of a saturated water solution of indomethacin or esters I–III was added to vials containing approx. 4–5 mg of hydrated s.c. and the mixture was allowed to equilibrate for 48 h at room temperature (25°C). The partitioning of indomethacin or its esters I–III between stratum corneum and water was determined by following the disappearance of the compound being tested from the aqueous solution of the incubation mixture. Samples of this solution were withdrawn and the content of indomethacin or esters I–III was determined by the HPLC method previously described (Bonina et al., 1991).

Partition coefficients were quantitated as (Oakley and Swarbrick, 1987):

$$K_{s.c./w} = (\mu g \text{ compound/mg unhydrated stratum corneum}) / (\mu g \text{ compound/mg water})$$

3. Results and discussion

The rationale for understanding the mechanism of prodrug topical delivery enhancement comes from an analysis of Fick's law:

$$J_{ss} = DK_{s.c./v}(dC/dx)$$

This law states that steady-state flux (J_{ss}) is proportional to the diffusion coefficient of the permeant through the skin barrier (D), the concentration (or more correctly, activity) gradient across the barrier (dC/dx), and the partition coefficient for the permeant between the vehicle and the stratum corneum ($K_{s.c./v}$). When the experiments are performed at the thermodynamic maximum of both drug and prodrug, skin penetration enhancement of prodrugs could be due to their diffusion and/or partition coefficient increase with respect to the parent drug.

In order to verify the effect of drug derivatization on the stratum corneum/water partition coefficient ($K_{s.c./w}$) it was measured both for indomethacin and its prodrugs, the results being listed in Table 1. As may be noted, only ester I appears to show an increase in $K_{s.c./w}$ compared to indomethacin, while the $K_{s.c./w}$ values for esters II and III are slightly lower than that of indomethacin. Since Guy and Hadgraft (1992) reported that drug stratum corneum solubility (S_m) may give a more valid indication of compounds which are likely to dissolve well in stratum corneum lipids, we calculated indomethacin and its prodrug S_m values using their previously determined water solubility (Bonina et al., 1991) from the following equation:

$$K_{s.c./v} = S_m/S_v$$

where S_m and S_v are the drug's saturation solubilities in stratum corneum and vehicle, respectively. As can be seen in Table 1, both esters I and II showed greater solubility in stratum corneum than indomethacin while ester III did not display any increase with respect to the parent drug.

Besides partitioning, the other skin permeation parameter which can be affected by drug derivatization to form prodrugs is diffusion.

Since most authors (Hadgraft, 1989) reported that for lipophilic molecules such NSAIDs the major route of skin penetration is the intercellular lipid pathway, we believe that interaction with lipid bilayers could be regarded as the most important parameter which affects the diffusional process for both indomethacin and esters I–III.

Recently, synthetic phospholipid liposomes have been used as a simple model for intercellular lipid lamellae of the stratum corneum to investigate bilayer fluidization by potential penetration enhancers (Beastall et al., 1988; Rolland et al., 1991). Physical changes in the characteristics of the lipid bilayers can be quantitatively assessed by a variety of biophysical techniques (French et al., 1993) among which DSC is one of the most commonly used.

In order to investigate how the lactamic ring promoiety in indomethacin esters could affect the prodrug interaction with the structured lipids in the intercellular channels with respect to indomethacin, we used multilamellar liposomes of DPPC as a model to study this interaction.

In Fig. 2A we report the calorimetric heating curves of DPPC liposomes in the presence of different molar fractions of indomethacin.

This drug is able to interact with DPPC liposomes causing, by increasing its molar fraction, a considerable shift in transition temperature (T_m) with a concomitant broadening of the calorimet-

Table 1
Water solubility (S_v), stratum corneum/water partition coefficient ($K_{s.c./w}$), stratum corneum solubility (S_m), flux through excised human skin and diffusion coefficient (D) of indomethacin and esters I–III

Compound	S_v [b] (μg/ml)	$K_{s.c./w}$	S_m (μg/mg)	Flux [b] ± S.D. ($\mu g\ cm^{-2}\ h^{-1}$)	$D \times 10^7$ ($cm^2\ h^{-1}$)
INDO [a]	4.03	170	685.1	0.083 ± 0.016	4.23
I	4.51	232	1046.3	0.287 ± 0.013	9.59
II	7.65	131	1002.1	0.343 ± 0.007	11.97
III	5.89	100	589.2	0.275 ± 0.011	16.10

[a] Indomethacin.
[b] Previously determined (Bonina et al., 1991).

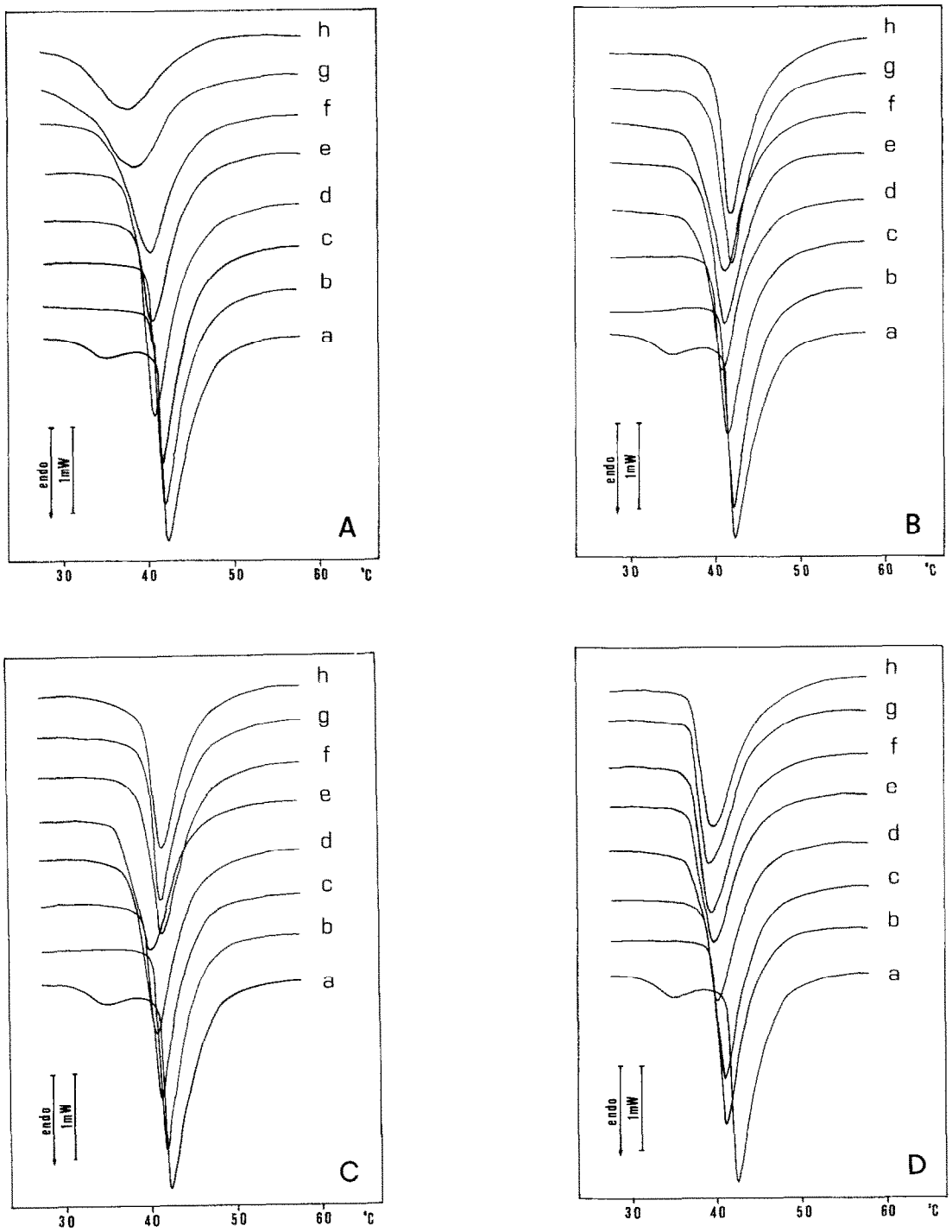


Fig. 2. DSC heating curves of hydrated DPPC liposomes in the presence of: (A) indomethacin; (B) ester I; (C) ester II; (D) ester III, at compound molar fraction: a = 0; b = 0.015; c = 0.03; d = 0.06; e = 0.09; f = 0.12; g = 0.18; h = 0.24.

ric curves associated with the well known gel-liquid crystal phase transition typical for DPPC multilayers (Fig. 2A and Table 2).

The enthalpy changes (ΔH), related to the peak area, remained nearly constant (data not reported).

Such interaction between indomethacin and DPPC liposomes was studied earlier by DSC (Hwang and Shen, 1981; Lasonder and Weringa, 1990) and our calorimetric results agree with the literature, where it was reported that the T_m and the cooperativity of the phase transition of DPPC liposomes were decreased by the presence of indomethacin.

This behaviour is similar to that shown by the interaction between lipophilic or amphipathic drugs and DPPC liposomes, largely studied (Cater et al., 1974; Estep et al., 1978; Castelli et al., 1992) and explained in terms of a 'fluidifying' effect due to the introduction of lipophilic drug molecules into the ordered structure of the lipid bilayer. The drug molecules can intercalate between the flexible acyl chains of lipid as 'interstitial impurities', causing T_m variations without ΔH changing (Jorgensen et al., 1991), according to the temperature depression of the melting points of ideal solutions (Guggenheim, 1952; Lee, 1977).

Fig. 2A and 3A reveal, by the linear decrease in T_m with the increase in molar fractions, that indomethacin is well solubilized in the lipid bilayer at all tested concentrations, interacting with DPPC vesicles. In Fig. 2B–D we report typical heating curves of DPPC liposomes containing different molar fractions of prodrugs ($X_{prodrug}$) I–III. As already observed for indomethacin, an interaction between the prodrugs being tested and the model membrane occurs. Plotting T_m values vs $X_{prodrug}$ (Fig. 3B–D), an almost linear decrease in T_m at low molar fractions ($X_{prodrug} < 0.06$) may be noted while at higher molar fractions a different trend is observed. We believe that low prodrug molar fractions could be the most suitable for predicting changes in skin diffusional characteristics, since low permeant concentrations in the stratum corneum occur during the skin permeation process.

Table 2
Main transition temperature (T_m, °C) of DPPC liposomes at different molar fractions of indomethacin and esters I–III

Molar fraction	Compound			
	Indomethacin	I	II	III
0.00	42.2	42.2	42.2	42.2
0.015	41.8	42.0	41.8	41.4
0.03	41.6	41.4	41.4	40.9
0.06	41.1	40.7	40.7	40.2
0.09	40.4	40.9	40.1	39.9
0.12	39.9	41.0	40.7	40.1
0.18	39.0	41.6	41.6	39.9
0.24	37.8	41.6	41.6	38.5

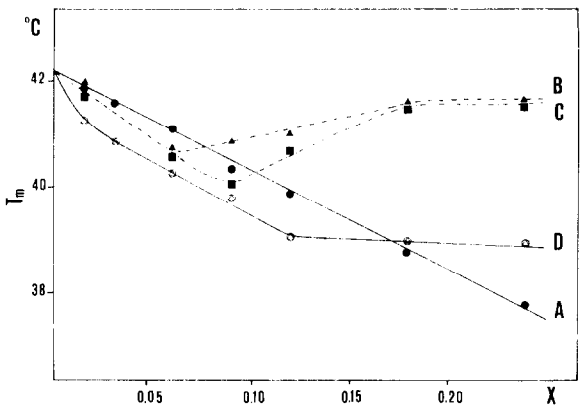


Fig. 3. Transition temperature (T_m, °C) values (average of at least four runs), in heating mode, as a function of molar fraction of: (A) indomethacin; (B) ester I; (C) ester II; (D) ester III.

Regarding the low molar fractions ($0 < X_{prodrug} < 0.06$) T_m decreases as the prodrug molar fraction increases and this effect is greater than that observed for the corresponding indomethacin molar fractions. These results suggest a greater interaction of prodrugs I–III with the lipid bilayer compared to indomethacin. This greater interaction should reflect an increase in the prodrug diffusion coefficient compared to indomethacin because of the increased permeant mobility. As may be noted in Fig. 3, for low molar fractions this interaction is greater for ester III, which contains ethylcaprolactam as promoiety, followed by esters II and I, containing ethylvalerolactam and ethylpyrrolidone promoieties, respectively. This increasing interaction could be attributed to

an increase in both prodrug lipophilicity and the ring size of the promoiety.

The higher molar fractions were studied in order to gain a better understanding of prodrug behaviour under extreme conditions of membrane solubility. Therefore, while in the case of indomethacin a regular T_m decrease was observed as the molar fraction increased, for prodrugs I–III an increased (I and II) or constant (III) T_m was observed (Fig. 3). This different behaviour with respect to indomethacin could be explained by considering the possibility that at high molar fractions the prodrug molecules aggregated inside the membranes, because of their more lipophilic character (Jain, 1988). The consequence of this process could be a withdrawal of prodrug molecules from the homogeneous lipid-prodrug dispersions, causing an increase in T_m towards the value observed for pure DPPC, or at least a nearly constant T_m. This inversion in the calorimetric effect takes place in the following order: ester I < II < III and could be attributed to the different size of the promoiety group and/or to the different lipophilicity of esters I–III.

As already observed for indomethacin, the presence of increasing molar fraction of prodrugs did not produce any ΔH change in the gel-liquid crystal phase transition.

To ensure that the calorimetric effects are due to prodrug interactions with lipid bilayers, we also evaluated the interaction between pure promoieties and DPPC liposomes. Since no interaction was detected under our experimental conditions (data not reported), it was possible to exclude any effect of the free promoieties on the calorimetric results.

In order to validate the suitability of DPPC multilamellar liposomes as a model of the structured intercellular lipids of the stratum corneum we calculated the apparent diffusion coefficients of indomethacin and its prodrugs and compared them with the calorimetric results (T_m) (Fig. 4).

Indomethacin and its prodrugs' apparent diffusion coefficients were calculated, on the basis of the partition coefficient values reported in this paper and their flux through excised human skin from saturated water solutions previously determined (Bonina et al., 1991), using the following equation:

$$D = J_{ss} h / K_{s.c./w} S_v$$

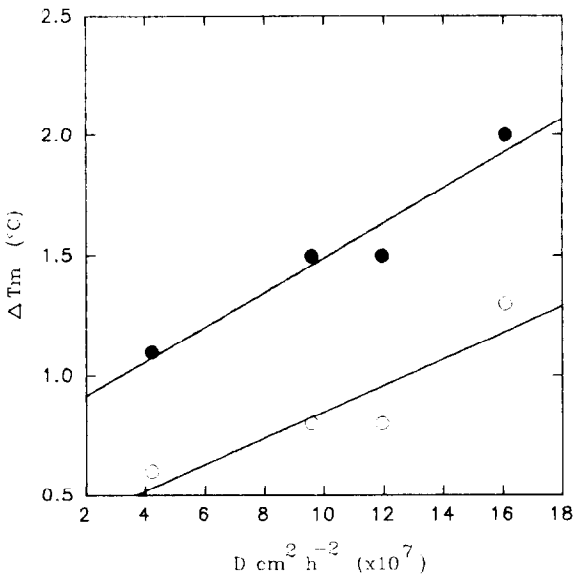


Fig. 4. Relationship between the apparent diffusion coefficient and ΔT_m values of indomethacin and esters I–III at molar fraction: (○) 0.03; (●) 0.06.

A nominal 35 μm skin thickness (h) was used and tortuosity effects were ignored (Kadir and Barry, 1991).

As may be noted in Fig. 4, the linear relationship between the indomethacin and prodrug apparent diffusion coefficients and their ΔT_m values ($T_{mDPPC} - T_{mcompound}$) at different molar fractions (0.03 $r = 0.91$; 0.06 $r = 0.97$) indicates that, in our case, DSC of DPPC multilamellar liposomes could be regarded as a good in vitro test to predict drug and prodrug diffusional characteristics.

On the basis of the results obtained in stratum corneum/water partitioning and diffusion studies, we attempted to elucidate the mechanism by which prodrugs I–III enhanced in vitro indomethacin flux through human skin (see Table 1). Regarding esters I and II, respectively containing ethylpyrrolidone and ethylvalerolactam as promoieties, their enhancement ability could be attributed to their greater stratum corneum solubility associated with a stronger interaction with

the lipid bilayer and hence better diffusion characteristics compared to the parent drug.

As regards ester III, containing ethylcaprolactam as promoiety, the skin enhancement ability of this prodrug could be attributed to its greatest interaction with the lipid bilayer (greatest D value) but not to an increase in stratum corneum solubility compared to indomethacin. In future, it might be noteworthy to validate N-ethyllactam promoiety enhancement mechanism by studying partitioning and the diffusional properties of N-ethyllactam esters of drugs with different physicochemical properties. The results of these studies could therefore be useful in assessing the suitability of these promoieties in the design of dermal prodrugs to improve diffusional and/or partitioning characteristics of the parent drug.

4. Acknowledgements

This work was partially supported by the Italian M.U.R.S.T. (60% and 40%). We would like to thank Professor N.A. Mancini (CUMEC) for his kind permission to use the Mettler TA 3000 system.

5. References

Barry, B.W., Mode of action of penetration enhancers in human skin. *J. Controlled Release*, 6 (1987) 85–97.

Bartlett, G.R., Phosphorous assay in column chromatography. *J. Biol. Chem.*, 234 (1959) 466–468.

Beastall, J.C., Hadgraft, J. and Washington, C., Mechanism of action of Azone as a percutaneous penetration enhancer: lipid bilayer fluidity and transition temperature effects. *Int. J. Pharm.*, 43 (1988) 207–213.

Bonina, F.P., Montenegro, L., De Capraris, P., Bousquet, E. and Tirendi, S., 1-Alkylazacycloalkan-2-one esters as prodrugs of indomethacin for improved delivery through human skin. *Int. J. Pharm.*, 77 (1991) 21–29.

Bundgaard, H., Hoelgaard, A. and Mollgaard, B., Leaching of hydrolytic enzymes from human skin in cutaneous permeation studies as determined with metronidazole and 5-fluorouracil prodrugs. *Int. J. Pharm.*, 15 (1983) 285–292.

Castelli, F., Puglisi, G., Giammona, G. and Ventura, C.A., Effect of the complexation of some nonsteroidal anti-inflammatory drugs with β-cyclodextrin on the interaction with phosphatidylcholine liposomes. *Int. J. Pharm.*, 88 (1992) 1–8.

Cater, B.R., Chapman, D., Hawes, S.M. and Saville, J., Lipid phase transition and drug interactions. *Biochim. Biophys. Acta*, 363 (1974) 54–69.

Chan, S.Y. and Li Wan Po, A., Prodrugs for dermal delivery. *Int. J. Pharm.*, 55 (1989) 1–16.

Estep, T.N., Mountcastle, D.B., Biltonen, R.L. and Thompson, T.E., Studies on the anomalous thermotropic behaviour of aqueous dispersions of dipalmitoylphosphatidylcholine-cholesterol mixtures. *Biochemistry*, 17 (1978) 1984–1989.

French, E.J., Pouton, C.W. and Walters, K.A., Mechanism and prediction of nonionine surfactant effects on skin permeability. In Walter, K.A. and Hadgraft, J. (Eds), *Pharmaceutical Skin Penetration Enhancement*, Dekker, New York, 1993, 113–143.

Guggenheim, E.A., *Thermodynamics*, North-Holland, Amsterdam, 1952.

Guy, R.H. and Hadgraft, J., Percutaneous penetration enhancement: physicochemical considerations and implications for prodrug design. In Sloan, K.B. (Ed.), *Prodrugs – Topical and Ocular Drug Delivery*, Dekker, New York, 1992, pp. 1–16.

Hadgraft, J., Formulation of anti-inflammatory agents. In Lowe, N.J. and Hensby, C.N. (Eds), *Nonsteroidal Anti-inflammatory Drugs. Pharmacol Skin*, Karger, Basel, 1989, Vol. 2, pp. 21–43.

Higuchi, W.I., Gordon, N.A., Fox, J.L. and Ho, N.F.H., Transdermal delivery of prodrugs. *Drug Dev. Ind. Pharm.*, 9 (1983) 691–706.

Hwang, S.B. and Shen, T.Y., Membrane effects of antiinflammatory agents: 2. Interaction of nonsteroidal anti-inflammatory drugs with liposomes and purple membranes. *J. Med. Chem.*, 24 (1981) 1202–1211.

Jain, M.K., Order and dynamics in bilayers and Solute in bilayers. In Jain, M.K. (Ed.), *Introduction to Biological Membranes*, Wiley, New York, 1988, pp. 122–165.

Johansen, M., Mollgaard, B., Wotton, P.K., Larsen, C. and Hoelgaard, A., In vitro evaluation of dermal prodrug delivery – transport and bioconversion of a series of aliphatic esters of metronidazole. *Int. J. Pharm.*, 32 (1986) 199–206.

Jorgensen, K., Ipsen, J.H., Mouritsen, O.G., Bennet, D. and Zuckermann, M.J., A general model for the interaction of foreign molecules with lipid membranes: drugs and anaesthetics. *Biochim. Biophys. Acta*, 1062 (1991) 227–238.

Kadir, R. and Barry, B.W., α-Bisabolol, a possible safe penetration enhancer for dermal and transdermal therapeutics. *Int. J. Pharm.*, 70 (1991) 87–94.

Kasting, G.B., Smith, R.L. and Anderson, B.D., Prodrugs for dermal delivery: solubility, molecular size, and functional group effects. In Sloan, K.B. (Ed.), *Prodrugs – Topical and Ocular Drug Delivery*, Dekker, New York, 1992, pp. 117–161.

Kligman, A.M. and Christophers, E., Preparation of isolated sheets of human skin. *Arch. Dermatol.*, 88 (1963) 702–705.

Lasonder, E. and Weringa, W.D., An NMR and DSC study of the interaction of phospholipid vesicles with some anti-inflammatory agents. *J. Colloid Interface Sci.*, 139 (1990) 469–478.

Lee, A.G., Lipid phase transition and phase diagrams: II. Mixture involving lipids. *Biochim. Biophys. Acta*, 472 (1977) 285–344.

Oakley, D.M. and Swarbrick, J., Effect of ionization on the percutaneous absorption of drugs: partitioning of nicotine into organic liquids and hydrated stratum corneum. *J. Pharm. Sci.*, 76 (1987) 866–871.

Rolland, A., Brzokewicz, A., Shroot, B. and Jamoulle, J.C., Effect of penetration enhancers on the phase transition of multilamellar liposomes of dipalmitoylphosphatidylcholine. A study by differential scanning calorimetry. *Int. J. Pharm.*, 76 (1991) 217–224.

Sloan, K.B., Prodrugs for dermal delivery. *Adv. Drug Del. Rev.*, 3 (1989) 67–101.

Sloan, K.B., *Prodrugs – Topical and Ocular Drug Delivery*, Dekker, New York, 1992.

Swarbrick., J., Brom, J. and Lee, G., Drug permeation through human skin: I. Effect of storage conditions of skin. *J. Invest. Dermatol.*, 78 (1982) 63–66.