International Journal of Pharmaceutics, 77 (1991) 21–29

ADONIS 037851739100363Z

IJP 02530

1-Alkylazacycloalkan-2-one esters as prodrugs of indomethacin for improved delivery through human skin

F.P. Bonina [1], L. Montenegro [1], P. De Capraris [2], E. Bousquet [1] and S. Tirendi [1]

[1] *Institute of Pharmaceutical Chemistry, University of Catania, Catania (Italy)*
and [2] *Department of Pharmaceutical Chemistry, University of Napoli, Napoli (Italy)*

(Received 16 April 1991)
(Accepted 14 May 1991)

Key words: Indomethacin; Ester prodrug; Percutaneous absorption; In vitro absorption; Enzymatic hydrolysis; Human skin

Summary

1-Alkylazacycloalkan-2-one esters of indomethacin were synthesized and assayed to determine their stability in aqueous media, their susceptibility to undergoing in vitro enzymatic hydrolysis and their flux through excised human skin. 1-Methylazacycloalkan-2-one esters of indomethacin (I–IV) proved unstable in aqueous media while 1-ethylazacycloalkan-2-one esters (V–VIII) were much more stable. Esters V–VIII were readily hydrolyzed in vitro by porcine esterase and penetrated excised human skin better than the parent drug. The lipophilic index was determined by means of HPLC for esters I–VIII. Plotting the flux through the skin of esters V–VIII against their lipophilic index resulted in a parabolic relationship, the maximum being ester VI.

Introduction

Several papers (Nowack et al., 1985; Chien et al., 1988; Catz and Friend, 1989) and patents (Sato et al., 1986; Sakamaki et al., 1987), which have recently appeared in the literature, prove that indomethacin, a potent anti-inflammatory drug used in topical and systemic therapy, is now considered a good candidate for transdermal delivery. Transdermal delivery of indomethacin is regarded as an interesting and desirable administration route in reducing the dose frequency and gastric irritation associated with high plasma levels after oral administration.

Since indomethacin accumulates predominantly in the synovial fluid, Chien et al. (1988) assert that an indomethacin transdermal device could be particularly useful in the treatment of rheumatoid arthritis.

A major problem in producing an indomethacin transdermal device is the low skin permeability of indomethacin, therefore alternative strategies to increase the skin permeability of this drug need to be developed. To achieve this goal, penetration enhancers like Azone (Ogiso et al., 1986), cyclohexanone derivatives (Akitoshi et al., 1988), limonene (Okabe et al., 1989), alkyl esters (Catz and Friend, 1989) and other chemi-

Correspondence: F.P. Bonina, Institute of Pharmaceutical Chemistry, University of Catania, Catania, Italy.

cals have been extensively used to increase in vitro indomethacin skin permeability, although toxicity and skin irritation have generally limited the practical application of these promoters in transdermal or dermal delivery systems.

The prodrug approach represents an alternative and very promising method of enhancing the skin permeability of drugs. The prodrug concept involves the chemical modification of a drug into a bioreversible form in order to change its pharmaceutical and pharmacokinetic properties and thus enhance its delivery. Regeneration of the parent drug occurs in vivo by either enzymatic or simply by chemical processes. Since the skin is a highly active metabolic organ (Pannatier et al., 1978; Bickers and Kappas, 1980) this approach has been increasingly used in the past few years to optimize the dermal and transdermal delivery of drugs (for reviews, see Chan and Li Wan Po, 1989; Sloan, 1989).

Recently, the prodrug approach has been used to increase dermal (Sloan et al., 1984) and transdermal (Milosovich et al., 1989) delivery of indomethacin: *N*,*N*-dialkylhydroxylamine indomethacin derivatives and 2-(*N*,*N*-diethylamine)ethylindomethacin ester · HCl were synthesized in order to enhance dermal and transdermal delivery, respectively, of this drug. The above-mentioned indomethacin prodrugs were able to increase in vitro indomethacin flux through the skin but being poorly stable in water their potential practical use in hydrophilic vehicles could be limited.

In order to obtain indomethacin transdermal prodrugs with increased water stability and better skin penetration than the parent drug, we synthesized 1-alkylazacycloalkan-2-one esters of indomethacin (I–VIII) (Fig. 1). 1-Alkylazacycloalkan-2-ones were chosen as pro-moieties, since they are regarded as skin penetration enhancers (Barry, 1983; Quan et al., 1990). It should be noted that lactamic rings are present in some of the most effective skin penetration enhancers, such as Azone and *N*-methylpyrrolidone. These derivatives were assayed to determine their water stability, their susceptibility in undergoing in vitro enzymatic cleavage and their flux through excised human skin.

Materials and Methods

Apparatus

Melting points were recorded on a Büchi 510 capillary melting-point apparatus and are uncorrected. The IR spectra were measured on a Perkin Elmer model 281 spectrometer utilizing potassium bromide discs.

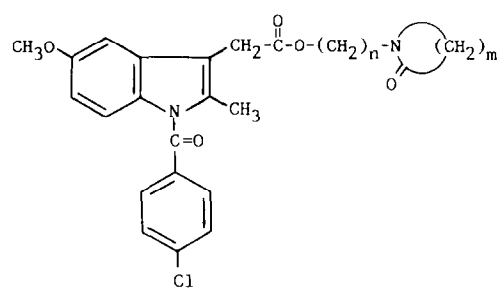
^{1}H-NMR spectra were recorded with a Brüker model AC 250, using $CDCl_3$ as solvent and TMS as internal standard.

Elemental analysis was performed on a Carlo Erba model 1108 elemental analyzer.

The HPLC system consisted of a Waters model 510 pump with a model 490 E UV-Vis detector, an automatic sample injection module (Wisp model 712) a Waters C_{18} μBondapack, 4.6 mm × 30 cm reverse-phase column, and an NEC AP-CIV computer.

Chemicals

Indomethacin was obtained from Sigma. 2-Pyrrolidinone, δ-valerolactam, ϵ-caprolactam and 2-azacyclooctanone were purchased from Aldrich.



I	n= 1	m= 3
II	n= 1	m= 4
III	n= 1	m= 5
IV	n= 1	m= 6
V	n= 2	m= 3
VI	n= 2	m= 4
VII	n= 2	m= 5
VIII	n= 2	m= 6

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

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.

All 1-hydroxymethylazacycloalkan-2-ones used in this study were prepared by heating each lactam with paraformaldehyde for 3 h at 120°C using standard procedures previously described in detail (Bohme et al., 1961).

All 1-(2-hydroxyethyl)azacycloalkan-2-ones were prepared by refluxing each lactam sodium salt with 2-bromoethanol in anhydrous xylene for 5 h as described in the literature (Sidel'kovskaya et al., 1965).

TABLE 1

Yield, melting point, IR and ^{1}H-NMR data for esters I–VIII

Compound	Yield (%)	m.p. (°C)	IR (KBr)(cm^{-1})			^{1}H-NMR (δ)
			$-CH_2-\underset{\displaystyle C=O}{\overset{\displaystyle |}{N}}-C=O$	$>N-\underset{\displaystyle |}{C}=O$	$-O-\underset{\displaystyle |}{C}=O$	
I	58.5	98–99	1700	1670	1740	3.68 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 5.36 (s, 2, $-O-CH_2-N<$);
II	37.4	93–94	1685	1660	1750	3.67 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 5.38 (s, 2, $-O-CH_2-N<$);
III	54.1	112–113	1680	1655	1745	3.66 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 5.45 (s, 2, $-O-CH_2-N<$);
IV	48.3	108–109	1690	1645	1745	3.68 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 5.47 (s, 2, $-O-CH_2-N<$);
V	61.1	110–111	1680	1660	1750	3.67 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 4.24 (t, 2, $-O-\underline{CH_2}-CH_2-N<$); 3.50 (t, 2, $-O-CH_2-\underline{CH_2}-N<$);
VI	24.6	111–112	1685	1640	1750	3.65 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 4.27 (t, 2, $-O-\underline{CH_2}-CH_2-N<$); 3.56 (t, 2, $-O-CH_2-\underline{CH_2}-N<$);
VII	21.5	103–104	1690	1645	1745	3.66 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 4.26 (t, 2, $-O-\underline{CH_2}-CH_2-N<$); 3.54 (t, 2, $-O-CH_2-\underline{CH_2}-N<$);
VIII	35.8	107–108	1680	1655	1750	3.68 (s, 2, $-CH_2-\underset{\displaystyle |}{C}=O$); 4.25 (t, 2, $-O-\underline{CH_2}-CH_2-N<$); 3.52 (t, 2, $-O-CH_2-\underline{CH_2}-N<$);

Preparation of indomethacin acid 1-alkylazacycloalkan-2-one esters (I–VIII)

A mixture of *N*-hydroxyalkylazacycloalkan-2-one (0.15 mol), indomethacin (0.15 mol) and neutral alumina (10 g) in xylene (50 ml) was refluxed under azeotropic conditions until the stoichiometric amount of water was removed. The alumina was then filtered off and the filtrate was washed with a 5% aqueous solution of sodium carbonate and water, dried over anhydrous sodium sulphate and evaporated in vacuo. The residue obtained solidified within a few days and was crystallized from benzene-petroleum ether (compounds I–IV) or chromatographed through a column of silica gel using ethyl acetate-cyclohexane (10:90) (compounds V–VIII). The melting points, yields, IR data and ^{1}H-NMR chemical shifts of esters I–VIII are listed in Table 1. Elemental analyses (C, H, N) were within $\pm 0.3\%$ of the theoretical value.

Determination of chemical stability and solubilities

The rate of hydrolysis of ester derivatives I–VIII was studied in a solution of isotonic phosphate buffer, pH 7.4, with an ionic strength (μ) of 0.5 at 32°C. The disappearance of the ester was followed by HPLC analysis. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual indomethacin esters against time. The solubilities of indomethacin esters V–VIII were determined in duplicate in water and isopropyl myristate (ipm) by stirring an excess of each derivative in 2 ml of the solvent with a magnetic stirrer for 24 h at room temperature. Thereafter, the mixtures were filtered and the concentrations of the compounds in their saturated solutions were determined by the HPLC method described below. The solubilities of indomethacin esters I–IV were not determined, due to their poor stability in water and ipm.

HPLC analysis of indomethacin acid and its esters

Indomethacin and its esters were determined by HPLC using a mobile phase consisting of acetonitrile and 0.1 M acetic acid (60:40). The chromatograph was run at ambient temperature at a flow rate of 1.8 ml/min. The column effluent was monitored continuously at 250 nm with a 0.05 aufs. Under these conditions, indomethacin and its esters showed the following retention times: indomethacin, 4.3 min; I, 5.5 min; II, 6.2 min; III, 7.2 min; IV, 8.2 min; V, 4.9 min; VI, 5.7 min; VII, 6.4 min; VIII, 7.3 min. Quantifying the compounds was performed by measuring the peak areas in relation to those of standards chromatographed under the same conditions.

Determination of apparent lipophilic index of indomethacin esters

The lipophilic indices of indomethacin esters were determined by the reverse-phase HPLC method. Using this method, the lipophilic index (log K) is calculated from the equation:

$$\log K = \log[(t_r - t_0)/t_0]$$

where t_r is the retention time of a retained peak, and t_0 is the retention time of an elution solvent.

Esterase hydrolysis

Hydrolysis of esters V–VIII was determined using the procedure described by Wong et al. (1989). Porcine esterase (obtained from Biochemica) was diluted 1000 times with the isotonic phosphate buffer and used to hydrolyze esters V–VIII. The ester solutions were prepared by dissolving an aliquot of the compound in methanol to give a concentration of about 10^{-4} M. A volume of 50 μl of this solution was diluted with 3 ml of isotonic phosphate buffer pre-thermostated at 37°C. The solution was then thermostated at 37°C and 100 μl of the esterase solution were added. The concentration of the ester in the solution was monitored by the HPLC method reported above.

Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual indomethacin esters against time.

Permeability studies using excised human skin

Samples of adult human skin (mean age 36 $\pm$ 8 years) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at

60 ± 1°C for 2 min (Kligman and Christophers, 1963), after which stratum corneum and epidermis (SCE) were removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in a desiccator at approx. 25% RH. Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in Franz-type diffusion cells supplied by LGA (Berkeley, CA).

The exposed skin surface area was 0.75 cm^2 and the receptor volume was 3.2 ml. The receiving compartment contained ethanol/water 50:50 for ensuring pseudo-sink conditions by increasing the solubility of indomethacin and its esters in the receptor phase. Other authors (Mueller, 1988; Touitou and Fabin, 1988) have used ethanol and water in the receptor compartment to ensure solubility of lipophilic compounds in in vitro percutaneous absorption studies. Indomethacin acid and its esters V–VIII were applied to the skin surface as suspensions (400 μl). The suspensions were stirred for 24 h prior to use. Samples of the receiving solution were withdrawn at intervals and replaced with fresh solutions. The samples were analyzed for indomethacin and ester content by HPLC as described above. In the case of esters V–VIII, intact ester was found in the receptor phase together with a variable amount of indomethacin acid. Therefore, the fluxes through the skin of these esters were obtained by plotting the cumulative amount of indomethacin acid equivalents permeated vs time and dividing the slopes of the steady-state portions of the graphs by the area of the diffusion cells.

Results and Discussion

Chemical stability, solubility and lipophilicity data

From the chemical stability data in phosphate buffer of esters I–VIII, reported in Table 2, the marked lability of esters I–IV is evident whereas substituting the methyl for an ethyl group more stable esters (V–VIII) were obtained.

Recently, Wong et al. (1989), reporting on biodegradable esters as skin penetration enhancers, have pointed out a similar increase in chemical stability of the ester group as its distance from the cyclic urea ring increases. As can be seen from Table 2, azacycloalkanone ring size was not as important as the ester group to ring distance in determining the chemical stability of esters I–VIII. The chemical instability of esters I–IV in aqueous media does not allow us to determine their solubility; solubility data of esters V–VIII are reported in Table 2.

Esters V–VIII were more soluble than indomethacin in isopropyl myristate and their solubility was enhanced as the azacycloalkanone ring size increased.

The water solubilities of esters V and VIII were close to that of indomethacin while com-

TABLE 2

Solubility in water and ipm, half-life, lipophilic index and flux through excised human skin for indomethacin and esters I–VIII

Compound	Solubility		$t_{1/2}$ [a]		Log K	Flux ± S.D. [b] (μg/cm^2 per h)
	Water (μg/ml)	ipm (mg/ml)	pH 7.40 buffer	Esterase (1.3 U/ml)		
Indomethacin	4.03	2.79	–	–	0.125	0.083 ± 0.016
I	–	–	33 min	–	0.298	–
II	–	–	25 min	–	0.373	–
III	–	–	6 min	–	0.460	–
IV	–	–	119 min	–	0.539	–
V	4.51	2.81	20 days	5.63 h	0.221	0.287 ± 0.013
VI	7.65	3.54	20 days	3.05 h	0.326	0.343 ± 0.007
VII	5.89	9.42	18 days	3.29 h	0.392	0.275 ± 0.011
VIII	3.76	14.03	16 days	3.70 h	0.468	0.068 ± 0.028

[a] $t_{1/2}$ was calculated from the equation: $t_{1/2} = \ln 0.5/k^1$, k^1 being the pseudo-first-order rate constant.
[b] Flux at steady-state $n = 3$.

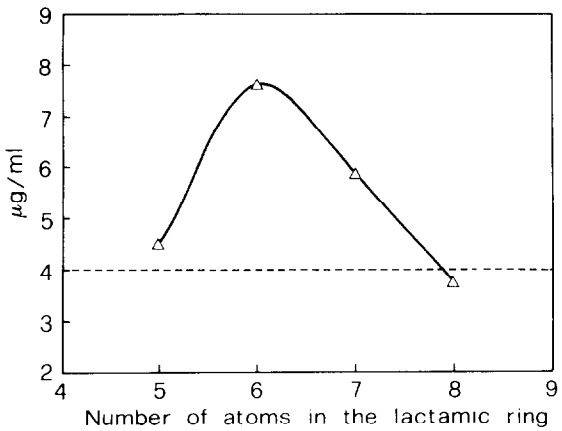


Fig. 2. Relationship between water solubility of the esters V–VIII and number of atoms in the lactamic ring. Indomethacin water solubility is shown as a horizontal dashed line.

pounds VI and VII proved more soluble than the parent drug: a parabolic relationship profile seems to exist between lactamic ring size and water solubility with compound VI exhibiting the maximum solubility (Fig. 2).

Using the HPLC method, we investigated the lipophilic indices of esters I–VIII. Plotting the lipophilic index vs the number of atoms in the 1-methylazacycloalkanone ring, we observed a linear relationship ($r = 0.999$) with a slope of 0.081 (Fig. 3). The plot of the lipophilic index and number of atoms of 1-ethylazacycloalkanone ring showed a similar relationship ($r = 0.994$) and the same slope (0.081) (Fig. 3). The parallelism indicates that the lipophilicity is proportional to the azacycloalkanone ring size for both series of esters.

Enzymatic hydrolysis

Generally, bioreversible manipulation of the physicochemical properties of drugs to increase their skin permeability has two distinct objectives: optimization of systemic delivery and/or delivery to the dermis. An essential prerequisite for success in the use of prodrugs is that prodrug reconversion into the parent drug occurs in the skin. Since the skin is a highly active metabolic organ containing esterases, several dermal and transdermal prodrugs, reported in the literature, possess an ester linkage capable of undergoing enzyme-catalyzed cleavage to the parent drug (Chan and Li Wan Po, 1989).

Since the preparation of skin homogenates may present some problems due to the tenacious and elastic nature of the outermost layer of the skin (Johansen et al., 1986), different models have been developed to mimic skin esterase activity and to assess the susceptibility of ester prodrugs in undergoing bioconversion in the skin. Thus, Johansen et al. (1986) and Bundgaard et al. (1989) reported the possibility of using human plasma to assess the hydrolysis rates of ester prodrugs for dermal delivery. Cheung et al. (1985), studying cutaneous biotransformation of topical corticosteroids, have shown that skin esterase and liver esterase are very similar in their activity and have concluded that cutaneous esterases are well modelled by liver esterases.

Recently, Wong et al. (1989) used porcine liver esterase to assess the biodegradability of some transdermal penetration enhancers. To evaluate the susceptibility of esters V–VIII in undergoing skin esterase enzymatic hydrolysis, we used porcine liver esterase as an enzymatic model. The half-lives of esters V–VIII in the presence of porcine esterase are reported in Table 2. All the esters were readily hydrolyzed by porcine es-

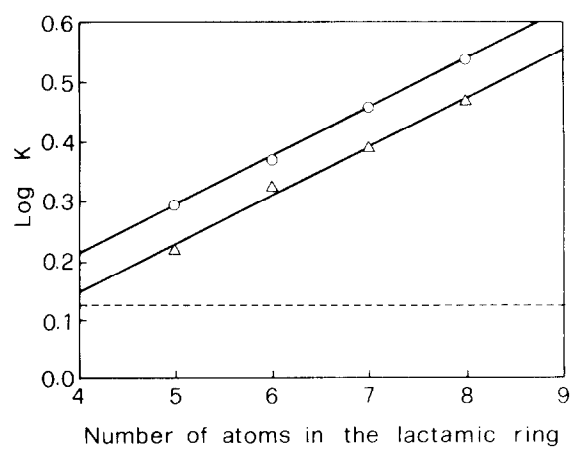


Fig. 3. Relationship between lipophilic indices of esters I–VIII and number of atoms in the lactamic ring. Esters I–IV (○); esters V–VIII (△). Indomethacin lipophilic index is shown as a horizontal dashed line.

terase. No difference in hydrolysis rate was observed as the ring size of *N*-ethyllactams increased.

Skin permeability

SCE membranes were used to assess skin permeation of indomethacin acid and ester prodrugs V–VIII. Suspensions of the compounds in water were applied to the skin in order to ensure a constant driving force while providing maximum thermodynamic activity. In the case of esters V–VIII, intact ester was found in the receptor phase together with a variable amount of indomethacin acid ranging between 10 and 12% with respect to the amount of intact ester permeated. The observed hydrolysis should be ascribed to the residual enzymatic activity of the skin samples we used given that the simple chemical hydrolysis would not justify the degree of hydrolysis observed.

Several researchers in in vitro dermal prodrug studies through full thickness human skin have observed high enzymatic activity of excised human skin (Bundgaard et al., 1983, 1989). Probably, the poor enzymatic activity that we found in our in vitro permeation studies is due to the use of SCE membranes obtained by means of a thermal separation technique: heating is known to damage enzymatic systems. Carrying out some preliminary in vitro permeation experiments using full thickness human skin, we obtained much lower fluxes (almost undetectable) for both indomethacin and esters V–VIII although the esters were almost completely reconverted into the parent drug within 72 h (unpublished data). These lower fluxes may be explained on the basis of the evidence reported by others (Scheuplein and Blank, 1973; Bronaugh and Stewart, 1984) that the dermis in vitro can act as a significant additional artificial barrier to the absorption of lipophilic compounds.

Plotting the cumulative amount of indomethacin acid or total indomethacin acid equivalents permeated against time we obtained typical plots similar to that reported in Fig. 4. Steady-state fluxes of indomethacin acid and esters V–VIII are reported in Table 2. Esters V–VII proved to permeate the skin better than indomethacin while ester VIII presented a steady-

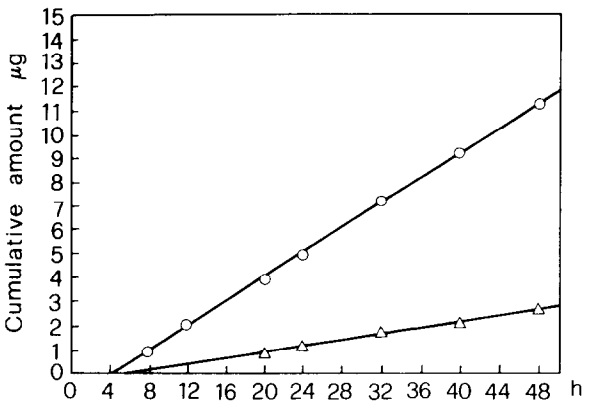


Fig. 4. Plot of cumulative μg of indomethacin penetrated through excised human skin from a suspension of indomethacin in water (△) and a suspension of ester VI in water (○) vs time.

state flux value similar to that for indomethacin. Plotting indomethacin flux values obtained from esters V–VIII against the lipophilic index of these esters (Fig. 5), the trend is a parabolic relationship with a maximun at log $K = 0.326$ (ester VI). Such a profile was also reported by Nghiem et al. (1987) in studying the in vitro penetration of a series of acetaminophen prodrugs through snake skin plotting flux vs lipophilicity. It is interesting to note that the highest indomethacin flux is obtained from ester VI which proved the most soluble in water. These results agree well with the theory, reported by Sloan (1989), that for a ho-

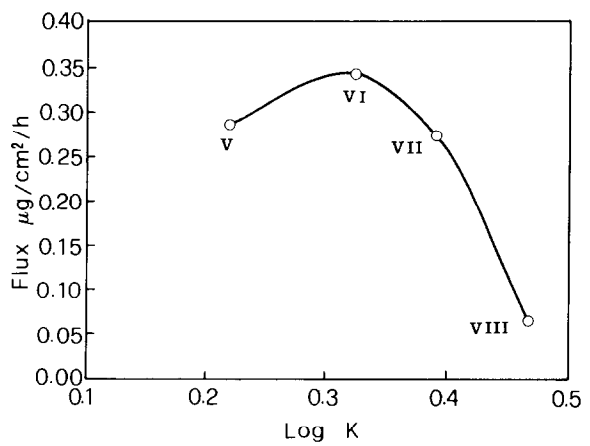


Fig. 5. Relationship between lipophilic index and steady-state flux through excised human skin of esters V–VIII.

mologous series of prodrugs an increase in lipid solubility almost always results in enhanced delivery of the parent drug through the skin, and optimum delivery is achieved using the members of the series which are more water soluble than the parent drug or that are the more water soluble members of the series.

In conclusion, 1-ethylazacycloalkan-2-one esters of indomethacin proved fairly stable in aqueous media, were readily hydrolyzed by esterases and were able to penetrate through excised human skin better than the parent drug. Further in vivo studies are planned to investigate the potential use of these esters as dermal or transdermal prodrugs of indomethacin.

Acknowledgement

We thank Assessorato Beni Culturali-Regione Sicilia for financial support.

References

Akitoshi,Y., Takayama, K., Machida, Y. and Nagai, T., Effect of cyclohexanone derivatives on percutaneous absorption of ketoprofen and indomethacin. *Drug Des. Del.*, 2 (1988) 239–245.

Barry, B.W., *Dermatological Formulations*. Dekker, New York, 1983, pp. 160–172.

Bickers, D.R. and Kappas, A., The skin as a site of chemical metabolism. In Gram, T.E. (Ed.), *Extrahepatic Metabolism of Drugs and other Foreign Compounds*. MTP, Lancaster, 1980, pp. 295–318.

Bohme, H., Driesen, G. and Schunemann, D., Reactions of *N*-hydroxymethyl and *N*-chloromethyl lactams. *Arch. Pharmacol.*, 294 (1961) 344–348.

Bronaugh, R.L. and Stewart, R.F., Methods for in vitro percutaneous absorption studies. III. Hydrophobic compounds. *J. Pharm. Sci.*, 73 (1984) 1255–1258.

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.

Bundgaard, H., Mork, N. and Hoelgaard, A., Enhanced delivery of nalidixic acid through human skin via acyloxymethyl ester prodrugs. *Int. J. Pharm.*, 55 (1989) 91–97.

Catz, J. and Friend, D.R., Alkyl esters as skin permeation enhancers for indomethacin. *Int. J. Pharm.*, 55 (1989) 17–23.

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

Cheung, Y.W., Li Wan Po, A. and Irwin, W.J., Cutaneous biotransformation as a parameter in the modulation of the activity of topical corticosteroids. *Int. J. Pharm.*, 26 (1985) 175–189.

Chien, Y.W., Xu, H., Chiang, C. and Huang, Y., Transdermal controlled administration of indomethacin. I. Enhancement of skin permeability. *Pharm. Res.*, 5 (1988) 103–106.

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.

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

Milosovich, S.M., Hussain, A.A., Hussain, M. and Dittert, L., The utilization of prodrugs to enhance transdermal absorption of testosterone, deoxycorticosterone and indomethacin. *Prog. Clin. Biol. Res.*, 292 (1989) 272–277.

Mueller, L.G., Novel anti-inflammatory esters, pharmaceutical compositions and methods for reducing inflammation. *UK Patent, GB 2 204 869 A*, 23 Nov. 1988.

Nghiem, B.T., Wong, O., Masaki, K., Kuehnoff, J., Konishi, R., and Higuchi, T., Effects of esterase activity in snake skin on ester prodrugs of acetaminophen, results presented at the Japan Unites States Congress of Pharmaceutical Sciences, Dec. 1987, Honolulu, HI, poster N-04-w-53.

Nowack, H., Marin, U. Reger, R., Boehmee, H., Schriever, K.H., Bocionek, P., Elbers, R and Kampffmeyer, H.G., Cutaneous absorption of indomethacin from two topical preparations in volunteers. *Pharm. Res.*, 5 (1985) 202–206.

Ogiso, T., Ito, Y., Iwaki, M. and Atago, H., Absorption of indomethacin and its calcium salt through rat skin: effect of penetration enhancers and relationship between in vivo and in vitro penetration. *J. Pharmacobio-Dyn.*, 9 (1986) 517–525.

Okabe, H., Takayama, K., Ogura, A. and Nagai, T., Effect of limonene and related compounds on the percutaneous absorption of indomethacin. *Drug Des. Del.*, 4 (1989) 313–321.

Pannatier, A., Jenner, P., Testa, B. and Etter, J.C., The skin as drug-metabolizing organ. *Drug Metab. Rev.*, 8 (1978) 319–343.

Quan, D., Higuchi, R.I., Takayama, K., Higashiyama, K. and Nagai, T., Enhancing effect of piperidone derivatives on the percutaneous absorption of indomethacin. *Drug Des. Del.*, 6 (1990) 61–71.

Sakamaki, Y., Noguchi, T., Kyobashi, Y., Inagi, T. and Muramatsu, T., Improved transdermal absorption of indomethacin, *Jap. Patent JP 62 61,918*, 18 Mar. 1987.

Sato, S., Matsumoto, K., Sakai, I. and So, I., Gel pharmaceuticals for transdermal adsorption, *Jap. Patent JP 61, 254, 519*, 12 Nov. 1986.

Scheuplein, R.J. and Blank, I.H., Mechanism of percutaneous absorption. IV. Penetration of non-electrolytes (alcohols) from acqueous solution and from pure liquid. *J. Invest. Dermatol.*, 60 (1973) 286–296.

Sidel'kovskaya, F.P., Kolodkin, F.L. and Shiroyan, F.R., Synthesis of *N*-β-hydroxyethyl lactams and their reaction with thionyl chloride. *Izv. Akad. Nauk SSSR, Ser. Khim.*, 2 (1965) 374–376.

Sloan, K.B., Selk, S., Haslam, J., Caldwell, L. and Shaffer, R., Acyloxyamines as prodrugs of anti-inflammatory carboxylic acids for improved delivery through skin. *J. Pharm. Sci.*, 73 (1984) 1734–1737.

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

Touitou, E. and Fabin, B., Altered skin permeation of a highly lipophilic molecule: tetrahydrocannabinol. *Int. J. Pharm.*, 43 (1988) 17–22.

Wong, O., Tsuzuki, N., Nghiem, B., Kuehnhoff, J., Itoh, T., Masaki, K., Huntington, J., Konishi, R., Rytting, J.H. and Higuchi, T., Unsaturated cyclic ureas as new non-toxic biodegradable transdermal penetration enhancers. II. Evaluation study. *Int. J. Pharm.*, 52 (1989) 191–202.