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In vitro evaluation of *N*-acyllactam esters of indomethacin as dermal prodrugs

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Abstract

Indomethacin *N*-acyllactam esters 1–6 were synthesized and assayed to determine their water and isopropylmyristate (IPM) stability, susceptibility to undergoing in vitro enzymatic hydrolysis and flux through excised human skin. Esters 1–6 showed poor stability in phosphate buffer pH 7.4 while they were stable in IPM over a period of 48 h. All the prodrugs, apart from derivative 6, were readily hydrolyzed by porcine esterase. Derivative 6, which possessed the greatest stability in phosphate buffer, was slowly hydrolyzed by esterases. Since esters 1–6 were poorly stable in water their skin permeation was determined using IPM as a vehicle. Esters 1–3 proved to permeate the skin better than indomethacin while esters 4–6 provided smaller (4 and 5) or similar indomethacin cumulative amount permeated through the skin over 24 h compared to the parent drug. Therefore, in this investigation only the derivatives (1–3) which showed increased lipophilicity and water solubility compared to the parent provided a moderate enhancement of in vitro indomethacin skin permeation.

Keywords: Indomethacin; Dermal prodrug; Skin permeation; Human skin

1. Introduction

Among the different strategies used to increase drug skin permeation, the prodrug approach, in addition to the use of penetration enhancers, is one of the most promising. The prodrug approach consists in making a new transient derivative which imparts suitable physicochemical drug properties for the better penetration of the skin and especially the stratum corneum (SC) which is regarded as the main barrier in the permeation process. At the same time, the prodrug should be able to readily enzymatically regenerate the parent drug in the target tissues (viable epidermis and/or dermis). In a recent book on topical prodrugs (Sloan, 1992), some guidelines have been outlined for a more rational dermal prodrug design. Hence, for a successful dermal prodrug approach, Sloan (1992) suggested that prodrugs should possess both increased lipophilicity and hydrophilicity compared

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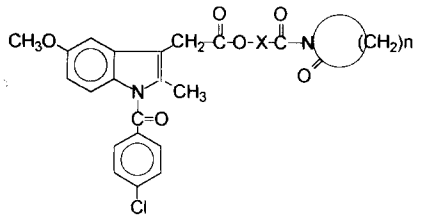

1) X = $-CH_2-$ N = 3

2) X = $-CH_2-$ N = 4

3) X = $-CH_2-$ N = 5

4) X = $-\underset{CH_3}{CH}-$ N = 3

5) X = $-\underset{CH_3}{CH}-$ N = 4

6) X = $-\underset{CH_3}{CH}-$ N = 5

Fig. 1. Chemical structures of esters 1–6.

to the parent drug. Furthermore, Guy and Hadgraft (1992) outlined that drug derivatization with a promoiety which possesses inherent enhancing ability would be a promising strategy.

On the basis of similar considerations, we previously prepared indomethacin and naproxen dermal prodrugs (Bonina et al., 1991, 1993) using *N*-alkyllactams, which are regarded as effective skin penetration enhancers (Barry, 1983), as promoieties.

Some of these prodrugs showing good water stability, rapid enzymatic hydrolysis and increased flux through excised human skin can be regarded as interesting indomethacin and naproxen dermal prodrugs.

In this paper, we synthesized indomethacin *N*-acyllactam esters 1–6 (Fig. 1) in order to evaluate the effect of introducing a carbonyl group between the lactamic ring and the alkyl chain on both the physicochemical properties and skin permeation of the prodrugs. A further rationale for designing these prodrugs was that Bundgaard and Nielsen (1987) patented *N*-acylamides, among which *N*-acylpyrrolidone, as useful promoieties for obtaining topical and oral prodrugs of drugs containing one carboxylic acid function. However, in this patent, no information was reported about the ability of these prodrugs to penetrate the skin to justify their topical use.

Indomethacin derivatives 1–6 were assayed to determine their water stability, susceptibility to undergoing in vitro enzymatic cleavage and flux through excised human skin.

2. Materials and methods

2.1. Apparatus

Melting points were recorded in open capillary tubes with a Buchi apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer 282 spectrometer (KBr discs for solids or for liquid films). ^{1}H-NMR spectra were recorded with a Brucker model Aspect 3000 (300 MHz) instrument by using $CDCl_3$ as solvent. Chemical shifts are given in δ values downfield from Me_4Si as internal standard. Elemental analyses were carried out with a Carlo Erba model 1106 analyzer and results were within 0.40% of the theoretical values.

The HPLC system consisted of a Waters model 600 pump, a model 490 E UV-Vis detector, a Wisp model 712 automatic sample injection module, a Waters C_{18} μBondapack, 4.6 mm × 30 cm reverse-phase column and a NEC PowerMate SX Plus computer.

2.2. Chemicals

Indomethacin was obtained from Sigma (Milan, Italy). 2-Pyrrolidinone, δ-valerolactam and ϵ-caprolactam were purchased from Aldrich (Italy). Acetonitrile and water used in the HPLC procedures were of LC grade and were bought from Carlo Erba (Milan, Italy). All other chemicals or solvent were of reagent grade.

All *N*-(2-chloroacyl)lactams and *N*-(3-chloropropionyl)lactams were prepared following the method described in the literature (Moore and Mathias, 1986).

2.3. Synthesis of indomethacin N-acyllactam esters 1–6

To a solution of indomethacin (3.6 g, 10.08 mmol) in dry, ethanol-free chloroform (15 ml), triethylamine (1.5 ml, 10.8 mmol), sodium iodide (0.3 g, 1 mmol) and the appropriate *N*-(2-chloroacyllactam (10 mmol) were added. The mixture was refluxed for 10–30 h, poured into water (50 ml) and then extracted with ethyl acetate (2 × 50 ml). The combined extracts, washed with 2% aqueous solution of thiosulphate, 2% sodium carbonate and water, were dried over sodium sulphate and evaporated in vacuo. The resulting residue was purified by column chromatography on silica gel (light petroleum ether/ethyl acetate, 7:3 v/v as eluent) to give indomethacin *N*-acyllactam esters 1–6 in 15–36% yield. Attempts to prepare indomethacin *N*-propionyllactam esters, following the procedure employed for compounds 1–6 and starting from *N*-(3-chloropropionyl)-lactams, failed since *N*-propenoyllactams, together with the recovered indomethacin, were obtained.

2.4. Determination of chemical and enzymatic hydrolysis rates

The hydrolysis rate of esters 1–6 was determined in a solution of isotonic phosphate buffer, pH 7.4 (μ = 0.5), at 32° C and in IPM. The disappearance of the ester was followed by the HPLC method reported below. Enzymatic hydrolysis of esters 1–6 was determined as previously described (Bonina et al., 1991). Porcine esterase was diluted 1000-fold with isotonic phosphate buffer, pH 7.4, prior to use. 30 μl of an ester solution in acetonitrile (10^{-3} M) was added to 3 ml of isotonic phosphate buffer, thermostated at 37° C and then 100 μl of the esterase solution was added. The concentration of the ester in the solution was monitored by HPLC analysis.

2.5. HPLC analysis of indomethacin and esters 1–6

Indomethacin and esters 1–6 were determined by HPLC using a mobile phase consisting of acetonitrile and 0.1 M acetic acid (60:40) at a flow rate of 1.8 ml/min, at room temperature. The effluent was monitored continuously at 250 nm. Indomethacin and esters 1–6 demonstrated the following retention times: indomethacin, 4.06 min; 1, 6.68 min; 2, 7.77 min; 3, 10.04 min; 4, 7.55 min; 5, 9.30 min; 6, 11.69 min. The compounds were quantified by measuring the peak areas compared to those of standards cromatographed under the same analytical conditions.

2.6. Solubility and apparent lipophilicity indices of indomethacin and esters 1–6

The solubility of indomethacin and esters 1–6 in isopropyl myristate (IPM) was determined in duplicate by stirring an excess of each compound in 2 ml of solvent for 24 h at room temperature. Then the mixtures were filtered, suitably diluted with acetonitrile, and analyzed by the HPLC method described above to determine the concentrations of indomethacin and esters 1–6.

The lipophilicity indices (log K) of indomethacin and esters 1–6 were determined by the reverse-phase HPLC method as previously described (Bonina et al., 1991).

2.7. In vitro skin permeation experiments

Samples of adult human skin (mean age 38 ± 10 years) were obtained from breast reduction operations. Stratum corneum and epidermis membranes (SCE) were prepared, stored, and rehydrated as previously described (Bonina and Montenegro, 1992). Skin permeation experiments were carried out using Franz diffusion cells (LGA, Berkeley, CA) whose exposed skin surface area and receptor volume were 0.75 cm^2 and 4.5 ml, respectively. The receiving compartment contained ethanol/water 50:50 for ensuring sink conditions (Mueller, 1988; Touitou and Fabin, 1988). The receiving solution was stirred and maintained at 35 ± 1° C throughout the experiments. Indomethacin and esters 1–6 were applied to the skin surface as IPM saturated solution (400 μl) and the experiment was run for 24 h. Samples of the receiving solution (50 μl) were withdrawn at

Table 1
Physical and spectral data of compounds 1–6

Compound	m.p. (°C)	IR (cm^{-1})	^{1}H-NMR $\delta(CDCl_3)$
1	121–122	1740, 1710, 1680	3.79 (t,2H,CH_2NCO) 5.16 (s,2H,OCH_2)
2	86–87	1740, 1700, 1690	3.68–3.72 (m,2H,CH_2NCO) 5.13 (s,2H,OCH_2)
3	111–112	1740, 1700	3.80–3.90 (m,2H,CH_2NCO) 5.10 (s,2H,OCH_2)
4	180–181	1740, 1690	3.70–3.80 (m,2H,CH_2NCO) 5.81 (q,1H,OCH)
5	145–146	1740, 1710, 1690	3.67–3.72 (m,2H,CH_2NCO) 5.81 (q,1H,OCH)
6	119–120	1740, 1710, 1690	3.60–3.70 (m,2H,CH_2NCO) 5.70 (q,1H,OCH)

24 h and analyzed for indomethacin or ester 1–6 content by the HPLC method described above.

3. Results and discussion

3.1. Synthesis of indomethacin N-acyllactam esters 1–6

The physical and spectral data of indomethacin *N*-acyllactam esters 1–6 are reported in Table 1. The low yields observed are more likely due to the facile and spontaneous polymerization of *N*-(2-chloroacyl)lactams at room or higher temperature (Moore and Mathias, 1986). Support for this contention arises from the fact that the highest yield was observed in the synthesis of compound 1 which requires the *N*-(2-chloroacetyl)lactam not prone to polymerization (Moore and Mathias, 1986).

3.2. Chemical and enzymatic hydrolysis

As reported in Table 2, esters 1–6 showed a poor stability in phosphate buffer pH 7.4 while they were stable in isopropyl myristate over 48 h. As may be noted, no significant difference ($p > 0.05$) in the hydrolysis rate was observed as the ring size of lactams increased in both series of derivatives (1–3 and 4–6).

Since an essential prerequisite for the successful use of dermal prodrugs is their reconversion into the parent drug within the skin, we assessed the enzymatic cleavage of esters 1–6 using porcine liver esterases which are regarded as a good model for skin esterase enzymatic activity (Cheung et al., 1985; Wong et al., 1989). As shown in Table 2, all the prodrugs, apart from derivative 6, were readily hydrolyzed by porcine esterase and no significant difference in the hydrolysis rate was observed as the ring size of lactams increased ($p > 0.05$). It worth noting that derivative 6, which was slowly hydrolyzed by porcine esterase, showed the greatest stability in phosphate buffer. Compared to previously synthesized indomethacin *N*-alkyllactam esters (Bonina et al., 1991), derivatives 1–6 were less stable in phosphate buffer than *N*-ethylactam derivatives but much more stable than *N*-methylactam esters. Unfortunately, no comparison could be made between indomethacin *N*-propionyllac-

Table 2
Half-lives ($t_{1/2}$) of chemical and enzymatic hydrolysis of esters 1–6

Compound	$t_{1/2}$ (h)	
	Buffer (pH 7.4)	Esterase (1.3 U/ml)
1	10.84	1.16
2	25.38	1.46
3	38.50	2.91
4	5.70	3.52
5	8.85	2.51
6	54.32	11.54

tam esters and previously synthesized *N*-ethyllactam esters since, as mentioned above, all attempts to synthesize the *N*-propionyllactam derivatives failed.

3.3. Solubility and lipophilicity

Since the horny layer is regarded as the main barrier in drug skin permeation and is basically a lipophilic barrier, drug lipophilicity is considered as one of the key parameters which controls drug skin permeation. A number of authors (Sloan, 1989; Guy and Hadgraft, 1992) have reported that more lipophilic drug derivatives could show better partitioning and solubility into the SC which could result in enhanced skin permeation. Regarding esters 1–6 synthesized in this work, esterification of the indomethacin carboxylic group gave, as expected, esters with increased lipophilicity compared to the parent drug (see Table 3).

As shown in Fig. 2, the lipophilicity indices of esters 1–6 increased as the size of the lactamic ring increased, for both series of derivatives. A similar pattern was observed for their IPM solubility (Table 3).

On the basis of theoretical considerations and experimental data, several authors (Guy and Hadgraft, 1992; Sloan, 1992) have pointed out that dermal prodrug water solubility may play a role as important as lipophilicity in the skin permeation process, especially for very lipophilic drug.

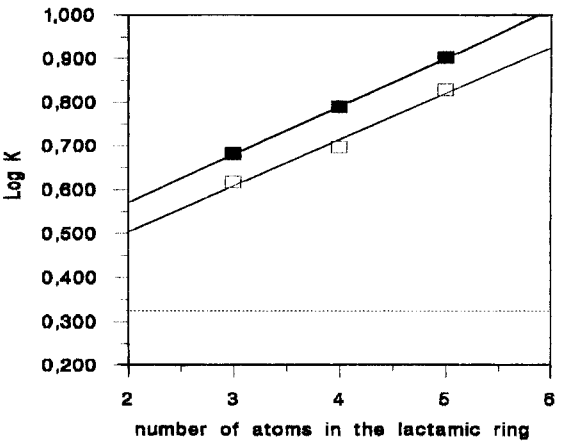

Fig. 2. Relationship between lipophilicity indices of esters 1–6 and number of atoms in the lactamic ring. (□) Esters 1–3; (■) esters 4–6. The lipophilicity index of indomethacin is represented as a horizontal dashed line.

Since esters 1–6 showed poor water stability, we could not experimentally measure their water solubility. Therefore, we calculated this parameter for esters 1–6 by using the theoretical method reported by Yalkowsky and Valvani (1980) and Yalkowsky et al. (1983). According to this method, which has already been used in similar evaluations (Osborne and Lambert, 1992), the water solubility of solid compounds was calculated based on Eq. 1:

$$\log S_w = -\log P - 0.01 \cdot MP + 1.05 \quad (1)$$

where S_w, MP and log P represent the water solubility, melting point and *n*-octanol/water partition coefficient, respectively.

Table 3
Calculated water solubility (S_w) and experimentally determined IPM solubility (S_i), lipophilicity indices (log K), calculated partition coefficient (log P_c) and cumulative amount permeated through excised human skin after 24 h (Q) of indomethacin and esters 1–6

Compound	S_w ($\mu g/cm^2$)	S_i ($\mu g/ml$)	Log K	Log P_c	$Q \pm$ S.D. [a] ($\mu g/cm^2$)
Indomethacin	82	–	0.326	3.10	0.732 ± 0.144
1	136	1717	0.617	3.39	1.367 ± 0.231
2	261	3454	0.697	3.47	1.393 ± 0.188
3	112	3225	0.828	3.60	1.299 ± 0.131
4	31	284	0.682	3.45	0.364 ± 0.050
5	56	1788	0.789	3.56	0.595 ± 0.123
6	81	2309	0.903	3.67	0.886 ± 0.168

[a] All the experiments were run in duplicate on three different donors.

McCall (1975) reported that the lipophilicity index (log K) is linearly related to log P, and proposed a HPLC method as a useful alternative to 1-octanol-water partition measurements. Since this HPLC method was recommended for partitioning studies of compounds unstable in solution, we applied this method for calculating log P of esters 1–6 using Eq. 2:

$$\log P = \log K' + \log K \quad (2)$$

Log K' was determined from the elution times of standards of known log P (indomethacin and naproxen).

The water solubilities of indomethacin and esters 1–6 are reported in Table 3.

Although the water solubility of indomethacin calculated using Eq. 1 did not agree well with that previously and experimentally determined by us (Bonina et al., 1991), it could be useful to compare the water solubility values of esters 1–6 calculated using these equations to that of indomethacin and to evaluate the effect of this parameter on their in vitro skin permeation.

As shown in Table 3, the water solubility of esters 1–3 was greater compared to that of indomethacin, while this parameter was lower (esters 4 and 5) or similar (ester 6) for esters 4–6 compared to the parent drug. Furthermore, no linear relationship was observed between water solubility and the lactamic ring size, for both series of derivatives (1–3 and 4–6).

3.4. Skin permeability

Since esters 1–6 were poorly stable in water we used isopropyl myristate (IPM) as a vehicle to apply these compounds to the skin. Saturated IPM solutions of esters 1–6 were used to ensure the maximum thermodynamic activity, thus obtaining a constant driving force. After having applied esters 1–6 to the skin, no intact ester was observed in the receptor phase after 24 h apart from ester 6. The complete hydrolysis of esters 1–5 observed in our in vitro skin permeation experiments could be attributed both to the chemical instability of these derivatives and to the enzymatic activity of SCE samples used in this study. A residual enzymatic activity of SCE samples has been already observed in previous studies under similar experimental conditions (Bonina et al., 1991, 1993). In the case of ester 6, small amounts of this derivative (about 30% of the total amount of indomethacin equivalent permeated) were detected in the receptor phase together with indomethacin. This finding could be explained with a greater chemical and enzymatic stability of ester 6 compared to the other derivatives. Thus, in vitro skin permeability results were expressed as indomethacin or its equivalent cumulative amount penetrated through human skin after 24 h and are reported in Table 3. Esters 1–3 proved to permeate the skin better than the parent drug while esters 4–6 provided smaller (4 and 5) or similar (6) indomethacin cumulative amounts permeated through the skin compared to indomethacin.

Lipophilicity and water solubility are regarded by some authors (Guy and Hadgraft, 1992; Sloan, 1992) as the key parameters for a successful dermal prodrug design.

Since the skin is regarded as a lipophilic rate-limiting barrier, enhancement of prodrug lipophilicity seems to be important to improve both diffusional characteristics and solubility of the parent drug in the stratum corneum. The enhancement of water solubility, especially for very lipophilic drugs, is equally important for at least two reasons (Guy and Hadgraft, 1992): (a) at the molecular level, because the lipid domain of the SC consists of multilamellar bilayers, the transporting species must be able to repetitively cross lipid-aqueous phase interfaces; (b) lipophilic derivatization to obtain prodrugs increases partitioning into the SC, forming a reservoir, but the subsequent transport into the aqueous milieu beneath may be limited by both prodrug aqueous solubility and the ability of epidermal enzymes to convert the prodrug into a more polar metabolite. In this way, viable epidermis, rather than the stratum corneum, would be the rate-limiting membrane (Friend et al., 1988) and, therefore prodrugs should possess suitable water solubility.

Regarding our in vitro skin permeation results, water solubility seems to be a more important parameter than lipophilicity in determining an increase of indomethacin percutaneous absorp-

tion. Therefore, while all the esters showed increased lipophilicity with respect to indomethacin only derivatives 1–3, which presented greater water solubility than the parent drug, provided increased indomethacin skin permeation. A similar trend was observed for previously prepared indomethacin and naproxen *N*-alkylactam esters (Bonina et al., 1991, 1993). However, for esters 1–3 no relationship was found between their skin permeation and their water solubility.

In conclusion, although indomethacin *N*-acyllactam esters showed an increased water stability compared to the chemical analogue *N*-alkylactam esters previously reported, they were not stable enough to be formulated in practical aqueous vehicles. Regarding the enzymatic hydrolysis rate, all the derivatives, apart from ester 6, were readily hydrolyzed by porcine esterase. Only derivatives 1–3, which showed increased lipophilicity and water solubility compared to the parent drug, provided a moderate enhancement of in vitro indomethacin skin permeation.

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