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Naproxen 1-alkylazacycloalkan-2-one esters as dermal prodrugs: in vitro evaluation

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Summary

1-Alkylazacycloalkan-2-one esters of naproxen were synthesized and assayed to determine their stability in phosphate buffer and isopropyl myristate, susceptibility to undergoing in vitro enzymatic hydrolysis and flux through excised human skin. 1-Methylazacycloalkan-2-one esters of naproxen (I–IV) proved poorly stable both in aqueous media and isopropylmyristate while 1-ethylazacycloalkan-2-one esters (V–VIII) were much more stable. Esters V–VIII were readily hydrolyzed in vitro by porcine esterase and esters V–VI penetrated excised human skin better than the parent drug from aqueous suspensions. On the basis of the results obtained, 1-ethylpyrrolidone and 1-ethylvalerolactam appear to be suitable promoieties for obtaining naproxen dermal prodrugs.

Introduction

The therapeutic efficacy of drugs following topical administration is strongly affected by their ability to penetrate the skin. Several papers deal with different strategies to increase drug percutaneous absorption (Woodford and Barry, 1986; Sloan, 1989; Loth, 1991). Penetration enhancers have been extensively used to achieve this goal although they show some potential disadvantages (Hadgraft, 1989). Their inclusion within a formulation may increase the absorption of components other than the drug, which can provoke skin damage and irritancy. 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 enhancing its delivery.

Recently, both penetration enhancers and the prodrug approach have been used to increase the percutaneous absorption of non-steroidal anti-inflammatory agents (NSAIs) (Hadgraft, 1989). Naproxen is one of the most potent anti-inflammatory agents that has been used for both topical and systemic therapy (Hart and Huskisson, 1984). Yano et al. (1986), investigating the percu-

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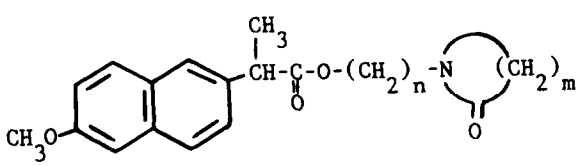
taneous absorption of a range of NSAIs in humans, reported that the percentage of naproxen absorbed after 4 h was only 1.3%. Penetration enhancers such as surfactants (Chowhan et al., 1978) and *n*-alkanoic *N,N*-dimethylamides (Irwin et al., 1990) have successfully been used to increase the percutaneous absorption of naproxen through rat skin. Nevertheless, the use of rat skin may be misleading when considering human percutaneous absorption, since rat skin has been reported to be more permeable than human skin (Wester and Noonan, 1980).

In a recent patent, Mueller (1988) reported naproxen ethoxycarbonyloxyethyl esters as prodrugs to increase the skin permeability of this drug after topical application. These prodrugs penetrated human skin better than naproxen in in vitro experiments, however, no information was given on their chemical stability and susceptibility to undergoing enzymatic hydrolysis.

In order to obtain naproxen dermal prodrugs with suitable water stability and better skin penetration than the parent drug, we synthesized 1-alkylazacycloalkan-2-one esters of naproxen (I–VIII) (Fig. 1). 1-Alkylazacycloalkan-2-ones were chosen as promoieties for two reasons: (a) they are regarded as skin penetration enhancers (Barry, 1983; Quan et al., 1990) and lactamic rings are present in some of the most effective skin penetration enhancers like Azone and *N*-methylpyrrolidone; (b) in a previous paper (Bonina et al., 1991), using 1-alkylazacycloalkan-2-ones as promoieties, we prepared indomethacin esters which had the requisites to be regarded as indomethacin (trans)dermal prodrugs.



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 strucures of esters I–VIII.

Naproxen derivatives were assayed to determine their water and isopropyl myristate stability, susceptibility to undergoing in vitro enzymatic cleavage and flux through excised human skin.

Materials and Methods

Apparatus

Melting points were taken on a Buchi 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 Bruker model WP 80, 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 600 pump with a model 486 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 an NEC APCIV computer.

Chemicals

Naproxen was obtained from Sigma. 2-Pyrrolidinone, δ-Valerolactam, ϵ-caprolactam and 2-azacyclooctanone were purchased from Aldrich. Methanol and water used in the HPLC procedures were of LC grade and were bought from Carlo Erba (Italy). All other chemicals and solvents were of reagent grade.

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

Naproxen esters I–VIII (Fig. 1) were synthesized following the same procedure previously described for indomethacin 1-alkylazacycloalkan-2-one esters (Bonina et al., 1991).

Esters I–IV were crystallized from benzene-petroleum ether while esters V–VIII were chromatographed through a column of silica gel using ethyl acetate-cyclohexane (10:90). 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 and enzymatic stability

The hydrolysis rate of ester derivatives I–VIII in solution of isotonic phosphate buffer, pH 7.4 ($\mu = 0.5$), and in isopropyl myristate (IPM) was determined at 32°C following the disappearance of the ester by HPLC analysis. Enzymatic hydrolysis of esters V–VIII was determined as previously described (Bonina et al., 1991).

Briefly, porcine esterase (Biochemica) was diluted 1000-fold with isotonic phosphate buffer prior to use. 50 μl of methanolic ester solution (10^{-4} M) was diluted with 3 ml of isotonic phosphate, 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 the HPLC method reported below.

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)
			$-CH_2-N-C=O$	$-O-C=O$	
I	48.2	61–62	1710	1745	1.58 (d,3H,$-CH_3$); 3.88 (q,1H,$-\underline{CH}-$); 5.35 (s,2H,$-O-CH_2-N<$);
II	37.5	65–66	1670	1740	1.58 (d,3H,$-CH_3$); 3.88 (q,1H,$-\underline{CH}-$); 5.41 (d,1H,$-O\underline{CH}-N<$, J = 9.7 Hz); 5.47 (d,1H,$-O-\underline{CH}-N<$, J = 9.7 Hz)
III	49.7	75–77	1670	1745	1.55 (d,3H,$-CH_3$); 3.86 (q,1H,$-\underline{CH}-$); 5.42 (d,1H,$-O-\underline{CH}-N<$, J = 9.8 Hz); 5.44 (d,1H,$-O-\underline{CH}-N<$, J = 9.8 Hz)
IV	40.2	110–12	1665	1740	1.60 (d,3H,$-CH_3$); 3.84 (q,1H,$-\underline{CH}-$); 5.32 (d,1H,$-O-\underline{CH}-N<$, J = 9.9 Hz); 5.55 (d,1H,$-O-\underline{CH}-N<$, J = 9.9 Hz)
V	38.5	70–71	1690	1740	1.62 (d,3H,$-CH_3$); 3.48 (t,2H,$-O-CH_2-\underline{CH_2}-N<$, J = 5.4 Hz); 3.88 (q,1H,$-\underline{CH}-$); 4.18 (t,2H,$-O-\underline{CH_2}-CH_2-N<$, J = 5.4 Hz)
VI	11.5	65–67	1680	1745	1.60 (d,3H,$-CH_3$); 3.52 (t,2H,$-O-CH_2-\underline{CH_2}-N<$, J = 5.3 Hz); 3.86 (q,1H,$-\underline{CH}-$); 4.15 (t,2H,$-O-\underline{CH_2}-CH_2-N<$, J = 5.3 Hz)
VII	25.5	70–71	1675	1740	1.62 (d,3H,$-\underline{CH}-$); 3.50 (t,2H,$-O-CH_2-\underline{CH_2}-N<$, J = 5.4 Hz); 3.86 (q,1H,$-\underline{CH}-$); 4.25 (t,2H,$-O-\underline{CH_2}-CH_2-N<$, J = 5.4 Hz)
VIII	4.1	98–100	1680	1740	1.58 (d,3H,$-CH_3$); 3.54 (t,2H,$-O-CH_2-\underline{CH_2}-N<$, J = 5.3 Hz); 3.89 (q,1H,$-\underline{CH}-$); 4.26 (t,2H,$-O-\underline{CH_2}-CH_2-N<$, J = 5.3 Hz)

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

HPLC analysis of naproxen acid and its esters

Naproxen and its esters were determined by HPLC using a mobile phase consisting of methanol and phosphate buffer, pH 2.0 (65 : 35). The chromatograph was run at ambient temperature at a flow rate of 1.4 ml/min. The column effluent was monitored continuously at 271 nm.

Under these conditions naproxen and its esters showed the following retention times: naproxen, 6.33 min; I, 7.48 min; II, 9.07 min; III, 11.68 min; IV, 15.11 min; V, 7.25 min; VI, 8.87 min; VII, 11.48 min; VIII, 14.70 min. The compounds were quantified by measuring the peak areas compared to those of standards chromatographed under the same conditions.

Solubility and apparent lipophilic index of naproxen esters

The solubilities of naproxen esters V–VIII were determined in duplicate in water and IPM by stirring an excess of each derivative in 2 ml of the solvent for 24 h at room temperature. The stirring time was reduced to 6 h for determining the solubility of esters I–IV. Thereafter, the mixtures were filtered and the concentrations of the compounds in their saturated solutions were determined by the HPLC method described above. The solubilities of naproxen esters I–IV in water were not determined, due to their poor stability.

The lipophilic indices of naproxen esters were determined by the reverse phase HPLC method as previously reported (Bonina et al., 1991).

Permeability studies using excised human skin

Samples of adult human skin (mean age 36 ± 8 years) were obtained from breast reduction operations. Stratum corneum and epidermis membranes (SCE) were prepared, stored and rehydrated as described in a previous paper (Bonina and Montenegro, 1992). Permeation experiments were performed using Franz-type diffusion cells supplied by LGA (Berkeley, CA). The exposed skin surface area was 0.75 cm^2 and the receptor phase (3.2 ml) consisted of ethanol/water 50 : 50 for ensuring pseudo-sink conditions (Mueller, 1988; Touitou and Fabin, 1988). Naproxen acid and its esters V–VIII were applied to the skin surface as water or IPM suspensions (400 μl). The suspensions were stirred for 24 h prior to use. Each experiment was run for 72 h since Mueller (1988), studying in vitro skin permeation of naproxen ester prodrugs, reported that a steady-state flux was achieved only after 24–48 h. Samples of the receiving solution were withdrawn at intervals and replaced with fresh solutions. The samples were analyzed for naproxen and ester content by HPLC as described above. The fluxes through the skin of esters V–VIII were determined by plotting the cumulative amount of naproxen 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 and enzymatic stability

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 produces more stable esters (V–VIII).

A similar trend was observed on evaluation of the stability of esters I–VIII in IPM even though esters I–IV proved more stable in IPM than in phosphate buffer.

TABLE 2

Half-lives of chemical and enzymatic hydrolysis of esters I–VIII

Compound	$t_{1/2}$		
	Buffer (pH 7.4)	IPM	Esterase (1.3 U/ml)
I	26.2 min	86.6 h	–
II	35.4 min	40.8 h	–
III	20.1 min	133.5 h	–
IV	48.5 min	63.0 h	–
V	16 days	9.3 days	3.85 h
VI	10 days	9.7 days	2.31 h
VII	8 days	10.3 days	2.26 h
VIII	3 days	9.9 days	2.91 h

As may be noted in Table 2, the azacycloalkanone ring size was less important than the ester group-to-ring distance in determining the chemical stability of esters I–VIII in both phosphate buffer and IPM.

Since an essential prerequisite for the successful use of prodrugs is their reconversion into the parent drug within the skin, we assessed the enzymatic cleavage of esters V–VIII using porcine liver esterases which are regarded as a good model for skin esterase enzymatic activity (Cheung et al., 1985; Wong et al., 1989). From the data reported in Table 2, it can be noted that all the esters were readily hydrolyzed by porcine esterase and no significant difference in hydrolysis rate was observed as the ring size of *N*-ethyllactams increased ($p > 0.05$).

Lipophilicity and solubility data

Regarding the lipophilicity indices of esters I–VIII, we observed, as already noted for analogue indomethacin esters (Bonina et al., 1991), a linear increase ($r > 0.99$) of this parameter as the size of the azacycloalkanone ring for each series of derivatives increased (I–IV and V–VIII; see Table 3).

The solubility data of esters I–VIII in water and IPM are reported in Table 3. The chemical instability of esters I–IV in aqueous media did not allow us to determine their water solubility. Esters V and VI were much more water soluble than the parent drug while compounds VII and VIII respectively showed a solubility similar to or lower than naproxen. As may be noted in Table 3, the solubilities of esters V–VIII were much greater in IPM than in water. The derivatives (IV and VIII) with the highest melting point within each series showed the lowest solubility in IPM. A similar trend was reported by Waranis and Sloan (1987), studying the vehicle effects and the skin penetration of a homologous series of 6,9-bisacyoxymethyl-6-mercaptopurine prodrugs for enhancing 6-mercaptopurine dermal delivery. Notwithstanding the fact that the lipoidal character of the derivatives regularly increased as the number of atoms in the lactamic ring increased, the IPM solubility decreased for 1-alkylazacyclooctan-2-one esters (IV and VIII). A similar trend between the lipoidal character and IPM solubility has been reported for a homologous series of 6-mercaptopurine prodrugs (Waranis and Sloan, 1987).

Skin permeability

SCE membranes were used to assess skin permeation of naproxen acid and ester prodrugs V–VIII since, as reported by others (Scheuplein and Blank, 1973; Bronaugh and Stewart, 1984), the dermis in vitro can act as a significant artificial barrier to the absorption of lipophilic compounds. Each compound was applied to the skin as a suspension in order to ensure a constant driving force while providing maximum thermodynamic activity.

TABLE 3

Water and IPM solubilities, lipophilic indices and fluxes through excised human skin for naproxen and esters I–VIII

Compound	Solubility		Log *K*	Flux ± S.D. [a]	
	Water (μg/ml)	IPM (mg/ml)		Water	IPM
Naproxen	10.28	5.40	0.171	1.171 ± 0.064	16.501 ± 2.230
I	–	7.98	0.286	–	–
II	–	7.67	0.407	–	–
III	–	6.99	0.553	–	–
IV	–	3.24	0.693	–	–
V	121.08	7.22	0.265	3.178 ± 0.123	16.132 ± 1.998
VI	88.52	6.68	0.394	2.046 ± 0.058	17.024 ± 2.067
VII	11.97	6.18	0.544	0.918 ± 0.287	17.369 ± 2.462
VIII	4.36	2.93	0.678	0.642 ± 0.103	8.713 ± 1.056

[a] $n = 3$.

On the basis of the short half-lives of esters I–IV in both phosphate buffer (20–50 min) and IPM (40–80 h), we decided not to evaluate their in vitro skin permeability since these experiments would need to last 72 h due to the long lag time of naproxen. After 72 h, no naproxen could be detected in the receptor phase following application to the skin of esters V–VIII in both water and IPM. The same lack of enzymatic activity was found in previous studies on analogue indomethacin esters (Bonina et al., 1991) and was attributed to the use of SCE membranes obtained by means of a thermal separation technique.

Plotting the cumulative amount of naproxen acid or total naproxen acid equivalents permeated vs time, we obtained typical plots similar to that reported in Fig. 2. Steady-state fluxes of naproxen acid and esters V–VIII from both water and IPM suspensions are reported in Table 3. As regards water suspensions, esters V and VI proved to permeate the skin better than naproxen while esters VII and VIII presented a steady-state flux value similar to that for the parent drug. As already observed for indomethacin 1-ethylazacycloalkan-2-one esters (Bonina et al., 1991), in this naproxen prodrug series, using aqueous suspensions, the highest flux was obtained from the ester (V) which proved the most water soluble. These results agree well with the theory, reported by Sloan (1989), that for a homologous series of prodrugs with increased lipid solubility, the optimum delivery of the parent drug through the skin 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. The flux values of naproxen and esters V–VIII from IPM were notably higher than those obtained from aqueous suspensions, however, esters V–VII did not show any flux increase compared to the parent drug while that of ester VIII decreased considerably.

Since the thermodynamic activity of a drug is constant in a state of suspension independent of the vehicle (Higuchi, 1960), the same fluxes from both water and IPM would have been expected. The higher flux values obtained from IPM supensions indicate that this vehicle affects the skin permeability by acting as a penetration enhancer. Loth (1991) reported that IPM enhanced indomethacin permeation through excised human skin by increasing its diffusion coefficient (Loth, 1991). Other authors (Sato et al., 1988) pointed out that IPM could act by increasing drug diffusivity in the stratum corneum and/or drug partition coefficients. The lack of increase in naproxen skin permeability using esters V–VIII in IPM suspensions could be attributed to the strong interaction of IPM with the stratum corneum which did not allow us to demonstrate the different promoting effects of these prodrugs which were observed from water suspensions. Furthermore, as regards the IPM enhancement mecha-

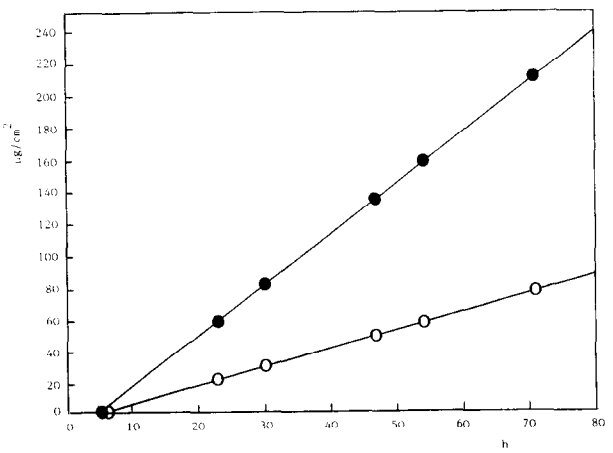


Fig. 2. Plot of cumulative amount (μg) of naproxen penetrated through excised human skin from a suspension in water of naproxen (○) and of ester V (●) vs time.

nism, we believe that IPM could predominantly increase the partitioning of naproxen and its prodrugs into the skin, due to the considerable solubility of these esters in IPM. This could explain the lowest naproxen flux obtained from ester VIII in IPM suspensions, since this ester showed the lowest IPM solubility.

In conclusion, 1-ethylazacycloalkan-2-one promoieties provided naproxen ester prodrugs that were fairly stable in aqueous media and IPM, and readily hydrolyzed by esterases. Compounds V and VI increased naproxen skin permeation from aqueous vehicle but were unable to enhance naproxen flux through the skin from IPM vehicle. Therefore, on the basis of the previous studies on indomethacin 1-ethylazacycloalkan-2-one esters (Bonina et al., 1991) and the results obtained in this work, 1-ethylpyrrolidone and 1-ethylvalerolactam appear to be suitable promoieties for obtaining dermal prodrugs of these drugs. In vivo evaluations of *N*-alkyllactamic esters of NSAIs are currently in progress to validate the suitability of these promoieties in the design of anti-inflammatory dermal prodrugs.

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