International Journal of Pharmaceutics, 89 (1993) 65–74

IJP 02938

The hydrolysis of azidoprofen esters: A model for a soft anti-inflammatory drug for topical application

A. Naik, W.J. Irwin and R.J. Griffin [1]

Drug Development Research Group, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET (UK)

(Received 23 March 1992)
(Accepted 3 June 1992)

Key words: Azidoprofen; Enzyme-mediated transesterification; Hydrolysis; Prodrug; Soft drug

Summary

A series of ester prodrugs of azidoprofen was investigated for susceptibility towards in vitro enzymatic and chemical hydrolysis. In each case, the parent acid was regenerated with the rate and extent of the process being dependent on the nature of the ester moiety. All esters, with the exception of the glycolamide derivative, exhibited much greater lability towards enzyme-mediated hydrolysis compared to chemical decomposition. In particular, the tetrahydropyranylmethyl ester showed the largest difference in sensitivity towards these two processes. This specificity is an important requirement in prodrug design and indicates the capacity to undergo rapid metabolic activation while exhibiting stability under conditions encountered during formulation and storage. The ability of skin to activate these prodrugs was demonstrated by hydrolysis of the methyl ester of azidoprofen on incubation with a hairless mouse skin homogenate. In addition, when enzymatic hydrolysis was monitored in aqueous alcoholic systems competitive hydrolysis and enzyme-mediated transesterification were observed. The rate of both processes increased with a longer chain ester and transesterification was the predominant reaction. Eventually, the new ester also underwent enzyme-mediated hydrolysis to yield azidoprofen.

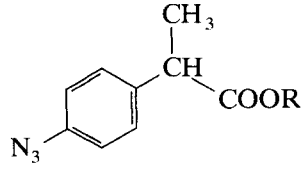
Introduction

In the preceding article (Naik et al., 1993), we have described the synthesis and reduction of azidoprofen (AZP) as a model for a soft antipsoriatic drug. This compound possesses an azido (N_3) function with the potential to undergo bioreduction to the inactive amino analogue, aminoprofen (AMP), as a means to minimise systemic effects. Physicochemical properties of such compounds may be modified by prodrug design where improved solubility, lipophilicity, chemical and enzymatic stability, taste and reduced irritability are required (Bundgaard, 1985). The skin is a highly active metabolic organ and cutaneous enzymes which can metabolise a wide range of endogenous and exogenous substances have been identified (Pannatier et al., 1978; Noonan and Wester, 1985; Täuber and Rost, 1987; Kappus, 1989). Consequently, various enzyme-labile derivatives which are able to utilise the wide range of cutaneous enzyme activities for bioactivation have been considered (Bucks, 1984; Hadgraft, 1985; Chan and Li Wan Po, 1989), and a variety of topical drugs have been the subject of such prodrug modifications in order to improve their penetration profile and/or localise drug action within the skin. For example, NSAIDs have been the

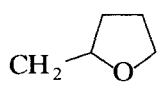
Correspondence to: W.J. Irwin, Drug Development Research Group, Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham B4 7ET, U.K.

[1] *Present address:* Dept of Chemistry, Bedson Building, The University, Newcastle upon Tyne NE1 7RU, U.K.

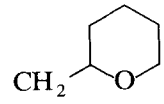
focus of bioreversible derivatisation in order to depress their gastro-intestinal side-effects (Jones, 1985) and temporary masking of the carboxylic acid function via esterification has been proposed as a promising means of reducing gastro-intestinal irritation (Whitehouse and Rainsford 1977). This is also a useful means of modifying the lipophilicity of prodrugs to optimise partitioning into the stratum corneum and maximising percutaneous transport. To provide a range of lipophilicities to enable modification of the percutaneous penetration profile we have made a series of ester prodrugs of AZP which include the methyl (**2**), ethyl (**3**), tetrahydrofuranylmethyl (THF, **8**), tetrahydropyranylmethyl (THP, **9**) and glycolamide (GA, **7**) derivatives. For these to be successful, the prodrug must undergo hydrolysis to the soft drug (AZP) after penetrating the epidermal barrier (Ando et al., 1977; Guy and Hadgraft, 1982, 1984); a bioactivation process which should be facilitated by the presence of skin esterases. Metabolism of the active entity to the inactive species (AMP) subsequent to exerting a pharmacological response must then follow. For this strategy to be satisfied, hydrolysis rates must exceed reduction. In the current study, the ester prodrugs of AZP were investigated for susceptibility to enzymatic and chemical hydrolysis using in vitro isolated enzyme systems and skin homogenates, in order to assess their prodrug potential.



R = H (**1**); CH_3 (**2**); C_2H_5 (**3**); C_3H_7 (**4**);
CH_2-CH(OH)-CH_3 (**5**);
CH-(CH_2OH)-CH_3 (**6**);
CH_2-$CONH_2$ (**7**, GA);



(**8**, THF);



(**9**, THP)

Experimental

Apparatus

HPLC analyses were performed using a system constructed from an Altex 100A dual reciprocating, solvent-metering pump which delivered mobile phases at a flow rate of 1 ml min^{-1} to a stainless steel column (10 cm × 4.6 mm) packed with 5 μm Hypersil-ODS (Shandon, U.K.) reversed-phase material. Samples were introduced through a Rheodyne 7120 injection valve fitted with a 10–100 μl loop as appropriate and UV detection was accomplished at 250 nm and 0.08–1.28 AUFS with a Pye Unicam LC3 variable wavelength UV detector equipped with an 8 μl flow cell. Chromatograms were recorded using either a JJ instruments CR452 or an Omniscribe D5000 chart recorder operated at chart speeds of 12.0 and 12.5 cm h^{-1}, respectively. Mobile phases comprised aqueous acetonitrile (50–65%) containing 0.1% diethylamine with the pH adjusted to 2.5 with orthophosphoric acid. Typical retention times were [MeCN (%), t_R (min)]: AZP (40, 4.5; 55, 2.6; 60, 2.2; 65, 1.9), methyl (55, 5.7), ethyl (60, 5.6), propyl (65, 6.2), THF (55, 6.1), THP (55, 10.2), glycolamide (40, 3.5). All pH measurements were undertaken using a Radiometer PHM 62 Standard pH meter (2 decimal place display) or a WPA CD 660 Digital pH meter (3 decimal place display) in conjunction with a Gallenkamp combination glass electrode calibrated with Colourkey buffer solutions (BDH Ltd).

Materials

Porcine liver esterase was a purified carboxylic ester hydrolase (Sigma Chemicals), suspended in 3.2 M ammonium sulphate solution adjusted to pH 8. Each mg of protein was equivalent to 260 units with each unit being capable of hydrolysing ethyl butyrate at a rate of 1 μl min^{-1} at 25°C (pH 8). The esterase solution contained 10.5 mg of protein per ml of solution and this was diluted with distilled water, as appropriate, prior to use. Concentrated Tris buffer solutions (0.5 M; pH 7.5 and 8.2) were prepared from Tris and HCl according to Perrin and Dempsey (1974) using a

10-fold salt concentration such that a constant ionic strength would be maintained on dilution.

Methods

Enzymatic hydrolysis

Porcine liver esterase studies (*In propylene glycol*): A reaction mixture consisting of concentrated Tris buffer (5 ml, pH 8.23), propylene glycol (PG, 5 ml), distilled water (34 ml) and porcine esterase solution (11 units per ml, 1 ml) was incubated in a reaction vessel at 37°C with constant stirring and protection from light. The reaction was initiated by the addition of AZP methyl ester stock solution (5 mM in PG, 5 ml). The composition of the reaction mixture was propylene glycol (20%) and Tris buffer solution (80%) with a final pH of 8.02. The concentrations of esterase and ester were 22 units per 100 ml and 0.5 mM, respectively. Control experiments, substituting distilled water for the esterase solution, were also conducted. Samples (0.5 ml) were withdrawn at appropriate intervals during the experiment and were diluted with internal standard solution (butyl paraben, 40 μg ml^{-1} in 20% PG, 0.5 ml) prior to analysis of 10 μl aliquots using a mobile phase composed of 50% aqueous acetonitrile. Concentrations of both the ester and AZP were calculated by interpolation of peak height ratios onto a calibration line prepared from standards chromatographed under the same conditions.

(*In alcohols*): Concentrated Tris buffer (pH 8.23, 2 ml), distilled water (15.8 ml) and porcine esterase solution (55 units ml^{-1}, 0.2 ml) were mixed together and equilibrated at 37°C. An aliquot (2 ml) of the ester stock solution (5 mM in 95% ethanol) was introduced to initiate the hydrolysis. The final composition was 0.5 mM in ester and 55 units 100 ml^{-1} in esterase in a solvent comprising ethanol (9.5% v/v, 2.06 M) with a pH of 8.06. Experiments were performed in duplicate together with controls. Samples (0.5 ml) were removed during the course of the reaction and these were diluted with an equal volume of solvent (10% aqueous EtOH) and internal standard solution (butyl paraben, 20 μg ml^{-1} in 10% EtOH, 1 ml). After HPLC analysis employing 50% aqueous acetonitrile concentrations of the reactant, the intermediate (in the case of transesterification) and the product (AZP) were determined from peak height ratios compared to calibration lines from standards assayed under identical conditions.

Enzymatic hydrolysis of the methyl (**2**), ethyl (**3**) and glycolamide (**7**) esters of AZP was similarly studied in aqueous methanolic solutions consisting of a methanol concentration equivalent in molar terms to the ethanol content in the above studies. The composition of the reaction mixture was thus MeOH (6.5% v/v, 2.03 M) in Tris buffer (final pH 8.02) with a final ester concentration of 0.5 mM and esterase level of 55 units 100 ml^{-1}. Samples (0.5 ml) were quenched by the addition of acetonitrile (1 ml) to denature the enzyme and stored at 0°C prior to analysis, at which time they were diluted with internal standard solution (butyl paraben, 25 μg ml^{-1} for methyl and ethyl esters, 1 ml; methyl paraben, 12.5 μg ml^{-1} for glycolamide ester, 1 ml) and 10 μl were analysed by HPLC.

(*In acetonitrile*): The alkyl (**2**,**3**), THF (**8**), THP (**9**) and GA (**7**) esters of AZP were investigated for hydrolytic decomposition in a solvent system composed of acetonitrile (10%) in Tris buffer (final pH 7.44), such that kinetic data could be compared without interference from transesterification reactions. The final concentrations of esterase and ester were 55 units 100 ml^{-1} and 0.5 mM, respectively. Samples (0.2 ml) were diluted with HCl (0.1 M, 0.4 ml) in MeCN (10%) and the mixtures were cooled in ice to quench reaction. Prior to analysis the mixtures were diluted (1 : 1) with internal standard (butyl paraben, 40 μg ml^{-1}) and 10 μl were analysed by HPLC.

Hairless mouse skin homogenate studies Two male hairless mice (type MF1/hr/hr/01a, supplied by Olac Ltd), each weighing approx. 20 g, were killed by cervical dislocation and the skin immediately dissected. Underlying subcutaneous tissues were gently removed, the skin was macerated into fine pieces and suspended in iced phosphate-buffered saline (PBS, comprising NaCl, 8.0 g; KCl, 0.2 g; KH_2PO_4, 0.2 g; $Na_2HPO_4 \cdot 2H_2O$, 1.44 g; H_2O to 1 l; 100 mg tissue per ml of buffer). The suspension was homogenised with a

French Press (Aminco), precooled to −4°C to minimise heat-induced denaturation of the cutaneous enzymes during processing. The homogenate was then centrifuged at 4000 rpm for 20 min, the supernatant was removed and an aliquot was assayed for protein content using Coomassie Brilliant Blue at 595 nm (Bradford, 1976) using bovine serum albumin as a reference. The supernatant from the homogenate was diluted with ice PBS to yield a protein concentration of 1.6 mg ml^{-1}, and either used immediately or stored at −15°C overnight.

A series of sample tubes containing either skin homogenate prepared as above or PBS (pH 7.4, 9.5 ml) as control together with methanol (0.3 ml) was incubated at 37°C. An aliquot of the ester stock solution (25 mM in methanol, 0.2 ml) was dispensed into each of the reaction vessels to initiate the reaction. Samples (0.5 ml) were withdrawn periodically and were vortexed with MeOH (1 ml) for 1 min. The incubation mixtures containing homogenate were centrifuged at 2500 × *g* for 5 min. An aliquot (0.5 ml) of the supernatant was diluted with an equal volume of internal standard solution (butyl paraben, 20 $\mu g\ ml^{-1}$ in distilled water) and 10 μl were assayed directly by HPLC. All incubations were performed in triplicate and protected from light for the duration of the experiment.

Chemical hydrolysis

Non-enzymatic hydrolyses of esters (0.5 mM) were conducted in solutions containing 10% acetonitrile and 90% Tris buffer (final pH 7.44) at 37 and 70°C. Samples were diluted with 0.1 M HCl, cooled in ice to quench the reaction and were diluted (1:1) with butyl parabens (15 $\mu g\ ml^{-1}$) prior to HPLC analysis (10 μl). Under these conditions, no reaction was observed during 3–4 h and experiments were also undertaken at pH 9.0 and 70°C.

Results and Discussion

The generation of azidoprofen from its ester prodrugs was examined by in vitro enzymatic hydrolysis using a commercially available, purified porcine liver carboxylic ester hydrolase. Due to the poor aqueous solubility of the esters, propylene glycol (PG) was employed as a cosolvent and enzymatic hydrolysis of the methyl ester was first followed in a 20% aqueous PG system. Fig. 1 shows a typical HPLC chromatogram of the disappearance of the methyl ester substrate together with the formation of azidoprofen as the hydrolysis product. In parallel with this product, a further component was produced which was not completely resolved but eluted between AZP and the internal standard. This latter product was not apparent in the control experiment, which was carried out under identical conditions in the absence of esterase, suggesting that its formation was an enzyme-mediated process. Moreover, the

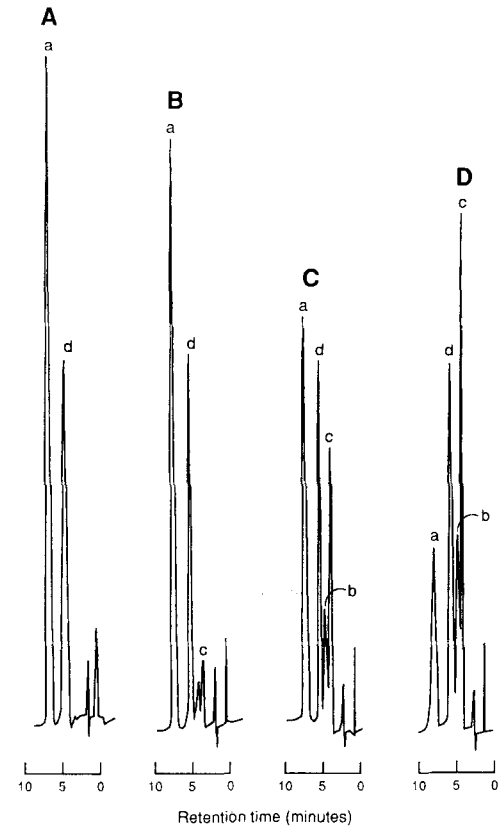


Fig. 1. HPLC chromatogram for the enzymatic degradation of AZP methyl ester in 20% propylene glycol at 37°C. (a) Methyl ester; (b) transesterified product; (c) AZP; (d) butyl paraben as internal standard. A, 0; B, 30; C, 120; D, 300 min.

component itself was unstable and disappeared with extended reaction times. That this species was not the methyl ester of aminoprofen nor AMP itself was confirmed by chromatography conducted with authentic compounds. In contrast to reactions in PG, the component was absent when enzymatic hydrolysis was performed in a solvent in which acetonitrile replaced PG, and complete mass balance of methyl AZP and AZP was achieved (Fig. 2).

A possible explanation for the presence of this intermediate was that an enzyme-mediated transesterification reaction occurred between the methyl ester and propylene glycol. Such a process would result in the formation of one or two glycol esters of AZP (**5,6**). To model this possibility, the stability of the methyl and ethyl esters was investigated in aqueous alcoholic solvents in which the alcohol function differed from the alkyl group of

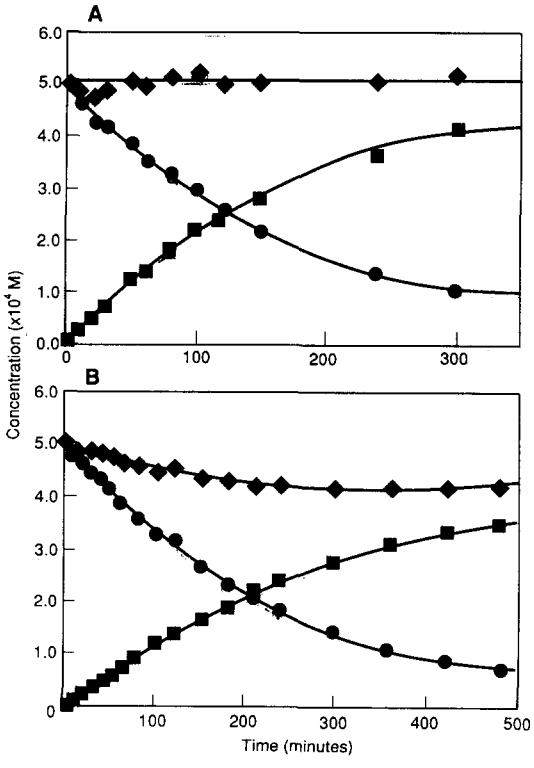


Fig. 2. Reaction profiles for the enzymatic hydrolysis of AZP methyl ester in (A) 20% propylene glycol and (B) 10% acetonitrile at 37°C. (●) Methyl ester; (■) AZP; (◆) mass balance, ester + AZP.

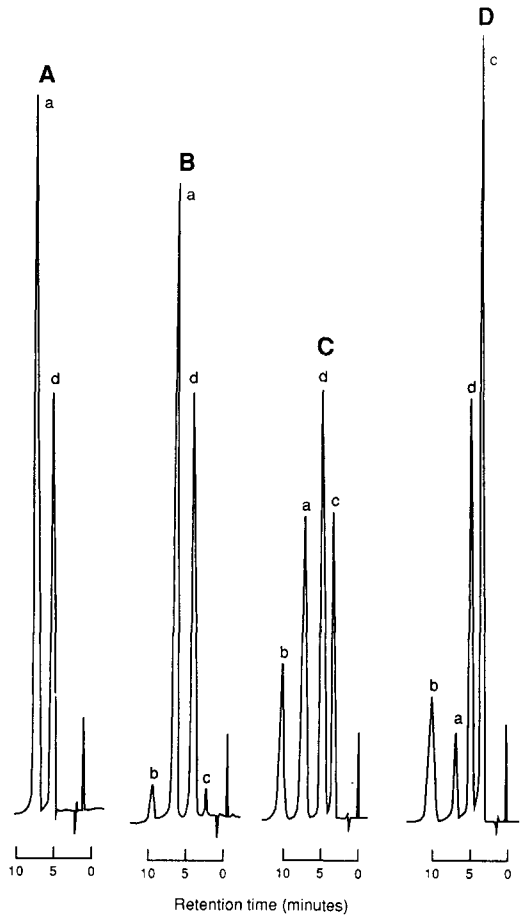


Fig. 3. HPLC chromatogram for the enzymatic degradation of AZP methyl ester in 10% ethanol at 37°C. (a) Methyl ester; (b) ethyl ester; (c) AZP; (d) butyl paraben as internal standard. A, 0; B, 30; C, 240; D, 660 min.

the ester. Fig. 3 illustrates an HPLC chromatogram following the fate of the methyl ester in 10% ethanolic buffer (pH 8.06), in the presence of esterase. The initial trace ($t = 0$ min) indicates the presence of the ester and internal standard alone. After 30 min, traces of the hydrolysis product (AZP) can be detected, but a further peak is also present. The ethyl ester of AZP was found to co-elute with this additional peak which suggests that transesterification is occurring under these conditions. In the absence of esterase the methyl ester remained unchanged. Formation of the ethyl ester intermediate can be clearly seen in Fig. 4 which displays the reaction profile showing the fate of the reactants and

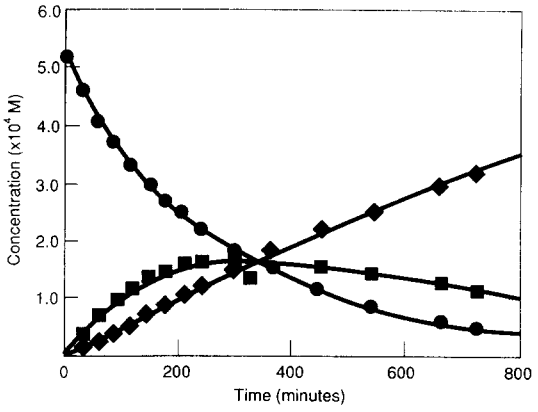
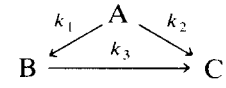


Fig. 4. Reaction profile for the enzymatic decomposition of AZP methyl ester in 10% ethanol-Tris buffer, pH 7.44, by 55 units % esterase at 37°C. (●) Methyl ester; (◆) AZP; (■) ethyl ester.

products. Both the esters eventually undergo hydrolysis to AZP. A similar profile was evident for the analogous transesterification of the ethyl ester in aqueous methanolic solution. In the event of transesterification of the methyl ester with propylene glycol two intermediates, the respective esters of the primary (**5**) and secondary (**6**) hydroxyl groups, may be expected. The ester for the primary alcohol (**5**) would be expected to predominate due to less steric hindrance in the transition state.

These glycol esters of AZP were synthesised in order to investigate whether one of these compounds was indeed the intermediate detected in aqueous propylene glycol. The ester involving the secondary OH (**6**) was characterised by a low field sextet in the ^{1}H NMR spectrum (δ, 5.05 ppm) whereas the primary analogue (**5**) displayed a more complex spectrum with no aliphatic absorptions lower than δ, 4.09. The experiment was repeated and the composition of the HPLC eluent adjusted from 50 to 45% aqueous acetonitrile in order to separate the unidentified peak. Co-elution of the transesterified species with the component identified as the primary ester of propylene glycol (**5**) from the synthetic sample suggested that this intermediate was this glycol ester of AZP, formed as a result of enzyme-mediated transesterification between the methyl ester and the co-solvent.

These events may be represented by the following kinetic model, proposed to describe the hydrolysis of an ester in an aqueous alcoholic solution, where the alcohol does not correspond to the ester function:



where A represents the original methyl ester of AZP undergoing transesterification to a second ester B. Both esters undergo hydrolysis to AZP (C). Reversibility may be excluded unless a mixed alcohol system is used to provide a significant concentration of the alcohol corresponding to the ester radical. The rates of change in concentration of species A, B and C are given by:

$$\frac{dA}{dt} = -(k_1 + k_2) \cdot [A] \quad (1)$$

$$\frac{dB}{dt} = k_1[A] - k_3[B] \quad (2)$$

$$\frac{dC}{dt} = k_2[A] + k_3[B] \quad (3)$$

Integration of these equations between time zero and the current time, according to Irwin et al. (1984a), enables expressions for the concentrations of each species at time t to be determined.

$$A_t = A_0 \cdot \exp[-(k_1 + k_2) \cdot t] \quad (4)$$

$$B_t = A_0 \cdot k_1 \cdot \left[\frac{\exp(-k_3 t) - \exp[-(k_1 + k_2) \cdot t]}{k_1 + k_2 - k_3}\right] \quad (5)$$

$$C_t = A_0 \cdot \left[1 - \{k_1 \cdot \exp(-k_3 t) + (k_2 - k_3) \cdot \exp[-(k_1 + k_2) \cdot t]\} \times \{k_1 + k_2 - k_3\}^{-1}\right] \quad (6)$$

Providing that $K_m \gg S_0$, enzyme reactions which are governed by Michaelis-Menten kinetics

approximate to a first-order model. In this case the rate constants for the transesterification reactions may be estimated by fitting the measured reaction profiles to Eqns 4–6 by non-linear regression (Irwin, 1990). The data are summarised in Table 1 and show that the sum of the degradation rate constants from the initial ester ($k_1 + k_2$) are generally in close agreement with the overall rate of disappearance obtained by first-order analysis. The hydrolysis rate of the ester with the same radical as the alcohol cosolvent could also be measured separately and values (k_3) are also recorded in Table 1. Transesterification (k_1) proceeds at a significantly faster rate than the corresponding hydrolysis reaction (k_2), and both of these reactions are favoured in the case of the ethyl ester, compared to the methyl ester. This may also be seen by a comparison of the hydrolysis rate constants k_2 and k_3; that for the hydrolysis of the ethyl ester is greater in each case. Although the competitive hydrolysis rate constant increases 12-fold the transesterification reaction is some 15 times faster. This may reflect the greater nucleophilicity of methanol compared to ethanol. Longer chain esters may also be expected to undergo more rapid enzyme-mediated hydrolysis, probably with an eventual parabolic dependence (Irwin and Belaid, 1988a,b), perhaps due to a more favourable hydrophobic interaction with the enzyme. Due to solubility problems it was not possible to confirm this using a homologous series of esters under a single set of conditions. However, preliminary studies did indicate that the propyl ester of AZP (**4**) was more vulnerable to enzymatic hydrolysis than either its methyl or ethyl homologues.

Transesterification is a well-documented reaction between esters and alcohols, where the ester radical differs from that of the alcohol. These reactions are invariably acid or base-catalysed and occur in aqueous and organic reaction media. In contrast, enzyme-catalysed transesterification has only recently begun to generate interest. The majority of the literature which exists in this field is concerned with reactions in anhydrous organic solvents (Kirschner et al., 1985; Bevinakatti and Banerji, 1988). Non-aqueous enzymology has been investigated with a view to mechanistic elucidation of the dependence of enzymatic properties on the nature of the solvent. Since the process of transesterification does not require water, as hydrolytic enzymes may also utilise alcohols as nucleophiles, it represents a model enzyme-mediated reaction for study in organic solvents (Zaks and Klibanov, 1988).

According to a detailed review on the physiological roles of carboxylic ester hydrolases by Leinweber (1987), information on the reversible nature of esterase action appears to be limited. Although isolated examples exist, the involvement of esterases in the synthesis of esters from acid and alcohol seems to be of minor physiological importance. In contrast, the pharmaceutical implications of non-enzymatic transesterification reactions have been identified (Irwin et al., 1984a,b, 1985) and illustrated with the reversible, base-catalysed transesterification of salicylate esters in aqueous alcoholic solutions. These esters

TABLE 1

Transesterification and hydrolysis rates for AZP esters at 37°C

System		Rate constant (min^{-1})($\times 10^3$)			
		k_1	k_2	k_3	$k_1 + k_2$
Methyl ester in ethanol	A	2.667	0.666	2.426	3.333
	B	–	–	2.214 [a]	3.224
Ethyl ester in methanol	A	40.719	7.994	5.739	48.713
	B	–	–	5.885 [b]	58.300

A, determined from non-linear regression; B, determined from first-order linear regression analysis.
[a] Hydrolysis of the ethyl ester in aqueous ethanol; [b] hydrolysis of the methyl ester in aqueous methanol.

are widely used as topical analgesics and preparations are commonly formulated with hydroxylic vehicles such as glycols. Formation of glycol esters may therefore be expected to affect the integrity of the pharmaceutical formulation. Similarly, in view of the esterase activity and drug metabolising capacity of the skin (Täuber and Rost, 1987), the potential of cutaneous transesterification, which may affect the availability and activity of the original ester should be considered. The possibility exists that such a modification may influence transdermal pharmacokinetics.

To allow a comparative examination of chemical and enzymatic hydrolysis between the various esters, an alcohol-free solvent system, to prevent transesterification reactions, was required. After preliminary studies with a range of solvents, a reaction medium consisting of 10% acetonitrile in buffer was employed. The alkyl (**2,3**), THF (**8**) and THP (**9**) esters were found to be hydrolysed quantitatively to the parent acid in the presence of porcine liver esterase, in 10% acetonitrile-buffer (pH 7.44) at 37°C. These enzyme-catalysed systems might be expected to follow Michaelis-Menten kinetics. The integrated form of this model is shown in Eqn 7 where S_0 is the initial substrate concentration, S_t represents its value at time t, K_m is the Michaelis constant and V_{max} denotes the maximum reaction rate.

$$\ln(S_t) + \frac{S_t}{K_m} = \ln(S_0) + \frac{S_0}{K_m} - \frac{V_{max}}{K_m} \cdot t \qquad (7)$$

At various concentrations (S_0') throughout the degradation profile the time for 50% reaction ($t_{1/2}$) may be calculated from the curve (Wharton and Szawelski, 1982; Nichols and Hewinson, 1987). This half-life is related to K_m and V_{max} by Eqn 8 which enables an estimate of these parameters to be made from a plot of $t_{1/2}$ vs S_0'.

$$t_{1/2} = \frac{0.6931 K_m}{V_{max}} + \frac{0.5 S_0'}{V_{max}} \qquad (8)$$

In the present experiments, this analysis showed a largely invariant value for $t_{1/2}$ throughout the reaction. This arises when $K_m \gg S_0'$ approximates the model to a first-order degradation when the degradation rate constant is given by $k = V_{max}/K_m$. First-order rate constants (k) and the corresponding half-lives ($t_{1/2}$) are summarised in Table 2.

In agreement with the rate data for hydrolysis in alcoholic media (Table 2), the process was favoured by the ethyl ester (**3**), compared to the methyl derivative (**2**). The enzymatic lability of the THP ester (**9**) was some 5-fold greater than that of the corresponding THF ester (**8**), and much higher than those of the sample alkyl esters (**2,3**). Under analogous non-enzymatic conditions, these esters proved to be highly stable. This indicates their potential ability to fulfill an important criterion for an ideal prodrug, that of providing metabolic lability while maintaining good chemical or aqueous stability. Kinetic data for comparative purposes were thus derived from a study of chemical hydrolysis under accelerated conditions, at pH 9.0 and 70°C. Reactions again followed first-order kinetics and kinetic data are also tabulated in Table 2. Of the esters studied, the methyl ester shows the greatest instability under these conditions, in contrast to its stability towards en-

TABLE 2

Kinetic data for the enzymatic and chemical hydrolysis of various esters of AZP in 10% acetonitrile-Tris buffer, pH 7.44, by 55 units % esterase at 37°C

Ester	Enzymatic hydrolysis		Chemical hydrolysis		$k_{enzymatic}/k_{chemical}$
	k (min^{-1})($\times 10^3$)	$t_{1/2}$ (min)	k (min^{-1})($\times 10^3$)	$t_{1/2}$ (min)	($\times 100$)
Methyl	2.583	268.3	46.32	15.0	5.6
Ethyl	7.598	91.2	20.71	33.5	36.7
THF	5.168	134.1	30.30	22.9	17.1
THP	26.77	25.9	29.14	23.8	91.9

zymatic hydrolysis. The reactivities of the THF and THP esters were not significantly different, in contrast to their susceptibilities towards enzymatic degradation. More importantly, if the ratios of the rate constants for the enzymatic and the chemical hydrolysis are compared for the series of esters ($k_{enzymatic}/k_{chemical}$), notwithstanding the vigorous reaction conditions used to quantify the latter process, the THP derivative exhibits the greatest promise as a potential prodrug.

The glycolamide ester (7), conversely, was significantly more susceptible to chemical hydrolysis and less labile to enzymatic hydrolysis than the corresponding alkyl, THF and THP esters. Typically, the first-order rate constant for chemical hydrolysis was 6.48×10^{-4} min^{-1} while enzymic degradation also followed first-order kinetics with a rate constant of 2.4×10^{-3} min^{-1}. In 10% MeCN-buffer, pH 7.4 and 37°C, the GA ester (7) was not subject to enzymatic hydrolysis yet underwent non-enzymatic degradation during the same period of study. Bundgaard and Nielsen (1988) have suggested glycolamide esters as useful prodrug functions for NSAIDs with carboxylic acid groups, combining high stability in aqueous solution with a high susceptibility towards enzymatic hydrolysis. The most important structural requirement for this profile appears to be the presence of two substituents on the amide nitrogen, *N*,*N*-disubstituted glycolamide esters being much more readily hydrolysed in plasma than either mono- or unsubstituted GA esters (Nielsen and Bundgaard, 1987, 1988). The unsubstituted nature of the GA ester of AZP in the present study, and its resultant lower lipophilicity, may therefore explain its stability to enzymatic attack.

The metabolic degradation of the methyl ester in the presence of a cutaneous homogenate was also investigated. The resulting reaction profile is presented in Fig. 5. First-order rate constants for the ester degradation and AZP formation were derived from linear transformation of these data up to 12 h and were 0.095 and 0.0816 h^{-1}, respectively. The formation of AZP was not observed in control experiments in which the skin homogenate was replaced by PBS. However, there was significant loss of the ester, possibly due to adsorption on to the reaction vessel. This loss was not evident in the skin experiment where good mass balance was achieved. Losses were undoubtedly due to adsorption onto glassware and such difficulties need to be overcome prior to studies with higher ester homologues. During these skin homogenate studies, reduction of the azido moiety was not apparent. This suggests that such a process is unlikely to occur during the delivery of AZP across the skin, an observation which parallels the behaviour of *m*-azidopyrimethamine, a soft dihydrofolate reductase inhibitor (Baker et al., 1991).

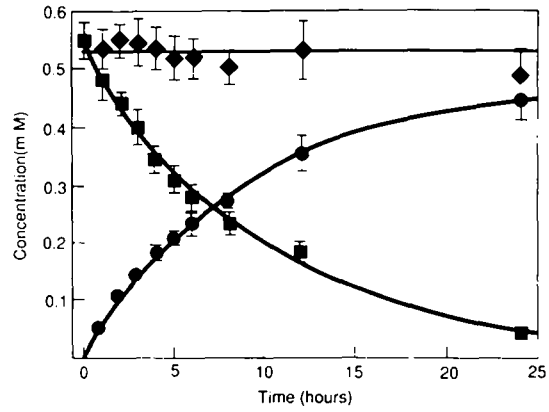


Fig. 5. Reaction profile for the cutaneous metabolism of AZP-methyl ester at 37°C. (■) Methyl ester; (●) AZP; (♦) mass balance, ester + AZP.

Acknowledgement

We are grateful to the Science and Engineering Research Council for the award of a Postgraduate Research Scholarship to A.N.

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