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Effect of Uraemia and Anephric State on the Pharmacokinetics of Tenoxicam in the Rat

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Abstract—Renal alterations, uraemia and nephrotic syndrome induced in experimental animals caused a reduction in the plasma albumin concentration of 25 and 30%, respectively. As a result of this decrease, the unbound fraction of tenoxicam in uraemic rats (0·06 ± 0·02) and in anephric rats (0·11 ± 0·08) increased with respect to the control group (0·03 ± 0·004). The induced hypoalbuminaemia did not modify the blood to plasma concentration ratio. Both plasma clearance (CL) and apparent volume of distribution at steady-state (Vd_{ss}) rose significantly with the increase in the unbound fraction: (Vd_{ss} 55 ± 6 mL (control rats); 69 ± 12 mL (uraemic rats); 96 ± 30 mL (anephric rats); CL = 7 ± 1 mL h^{-1} (control rats); 12 ± 4 mL h^{-1} (uraemic rats); 15 ± 7 mL h^{-1} (anephric rats)). Tenoxicam elimination was found to be restrictive, with an extraction ratio less than 0·1 in the three groups. The induction of nephrotic syndrome was observed to have a significant effect on intrinsic metabolic activity, intrinsic clearance of tenoxicam being reduced by 30% in the anephric rats (161 ± 38 mL h^{-1}) with respect to the values obtained in the control group (228 ± 22 mL h^{-1}).

Tenoxicam is a new, non-steroidal anti-inflammatory agent whose characteristics have been studied from a pharmacokinetic viewpoint in various animal species (Guentert et al 1986). It has been described as a drug with high plasma protein binding (Day et al 1988), with a distribution volume less than the volume of physiological water (Guentert et al 1986), and very low clearance (Hartmann et al 1987). These characteristics led us to assume that small alterations in the degree of plasma protein binding may have a significant effect on the disposition of the drug.

This type of drug is frequently used where pathological alterations are present which cause changes in the intravascular protein concentration, chiefly albumin levels (rheumatoid arthritis, osteoarthritis) (Wanwimolruk et al 1982). It is quite common for these rheumatic conditions to be accompanied by other alterations, such as those affecting the liver and kidneys, which also produce a reduction in plasma albumin concentration (Gugler & Azarnoff 1976; Zini et al 1990).

Lin et al (1987) made the observation that, for this group of medicines, there were no studies correlating possible alterations in the unbound fraction or albumin concentration with variations in clearance and apparent distribution volume. Lin et al (1985 a, b) noted that an increase in the free fraction caused the clearance value of sulindac and diflunisal to rise. In the case of the oxicam group, no such study was available. Apart from this, non-steroidal anti-inflammatory agents of this type have the peculiarity that they present a high degree of patient-to-patient variability (Benveniste et al 1990). In the case of piroxicam and tenoxicam, their biological half-life values show broad variations (35–72 h; Schepper & Heynen (1985); 44–132 h; Nilsen et al (1988)). This means that although the values for clearance and distribution volume should be affected by changes in the unbound fraction in plasma, it is possible that the actual results obtained are disguised by the great interindividual variation.

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The present study has been designed to test whether changes in plasma protein binding have a significant effect on the pharmacokinetics of tenoxicam. The changes in protein binding were induced by means of renal alterations (uraemia and nephrotic syndrome). These alterations produce a significant drop in plasma albumin levels (Giacomini et al 1981).

Materials and Methods

Animals

Twenty four male Wistar rats, 220–250 g, were divided at random into three groups. The first was used as a control group; in the second, uraemia was induced; and in the third, nephrotic syndrome was produced.

Twelve hours before the experiment was started, the animals were anaesthetized with ether and the right jugular vein was cannulated for drug administration and blood sample collection. Following surgery, the animals were fasted, but had free access to water.

Experimental uraemia was induced by means of bilateral ligation of the ureters 24 h before the drug was administered using the model of Lin & Levy (1982). Experimental anephric state was produced by surgically exposing and isolating the renal artery and vein of both kidneys, placing two tight ligatures around each and cutting between the ligatures. Pharmacokinetic studies in anephric rats were carried out 16 h after surgery.

Drug administration and sample collection

Tenoxicam (5 mg kg^{-1}) was applied intravenously in a solution of 0·2 g in 10 mL of sodium bicarbonate buffer (pH 8; Tsai et al (1985a)) and 0·7 or 0·4 mL blood samples were taken at the following times: 15 min, 1, 2, 4, 6, 8, 10, and 12 h. Blood samples were centrifuged at 3500 rev min^{-1} for 20 min; the plasma obtained was frozen at −20°C until analysis.

Assay of tenoxicam

Levels of tenoxicam in the plasma, blood and buffer solution from the equilibrium dialysis were determined by HPLC, using a Waters liquid chromatograph with an ultraviolet detector 440-A, U6-K variable injection volume injector, and a double-piston pump (Macek & Wácha 1987) using a Nucleosil C18 (12·5 cm × 0·4 cm and 5 mm i.d.) column. The mobile component of the system consisted of acetonitrile, double-distilled water and acetic acid (58:38:4) (Tsai et al 1985b). Flow rate was 1 mL min^{-1} and detection was at 365 nm. Piroxicam was used as an internal standard.

To quantify the samples of the drug in blood and plasma, 0·3 mL of a solution of the internal standard in acetonitrile (1 g L^{-1}) was added to 0·15 mL of the biological fluid as a precipitating agent; the mixture was centrifuged at 3500 rev min^{-1} for 20 min and the supernatant obtained was ready for injection into the chromatograph. The volume injected in this study was 10 μL.

Plasma protein binding

Plasma protein binding was determined in the first two samples obtained from each of the rats in all three groups. The study was performed by the equilibrium dialysis method with the Dianorm system, using half-cells of capacity 0·25 mL. For each rat, one cell was utilized; 0·2 mL of extracted plasma and 0·2 mL of phosphate buffer solution (pH 7·4) were placed in the half-cells, separated by a dialysis membrane. These membranes had been pretreated by soaking in distilled water (20 min), isopropyl alcohol (20 min), distilled water (15 min), and the buffer solution (120 min).

The cells were placed in a thermostatically controlled water bath (37°C) for 6 h. The biological samples were then collected and stored frozen until analysis.

To quantify the samples of plasma and buffer solution obtained from equilibrium dialysis, 0·2 mL of the internal standard/acetonitrile solution was added to 0·1 mL of the biological sample. The mixture was centrifuged at 3500 rev min^{-1} for 20 min, and the supernatant was used for analysis.

Albumin concentration assay

The albumin concentration was determined by the bromocresol green method (Doumas et al 1971). Albumin was measured in each rat before surgery and 2 h after drug administration. Plasma (50 μL) was added to 5 mL of bromocresol green solution. Albumin levels were assessed by colorimetry at 640 nm.

Blood to plasma concentration ratio

The relationship between tenoxicam concentration in blood and plasma, was studied using 15 rats divided at random into three groups comprising healthy, uraemic and anephric rats. Tenoxicam (5 mg kg^{-1}) was administered to the rats intravenously and 20 min after the injection, a 6 mL sample of blood was extracted by cardiac puncture. Three mL blood was used to determine the drug concentration in blood; the remaining 3 mL were centrifuged at 3500 rev min^{-1} for 15 min, and the plasma was separated and kept in frozen storage before analysis of the plasma tenoxicam concentration.

Chemicals

Tenoxicam and piroxicam were supplied by Roche (Madrid) and Fides (Barcelona) respectively. The other reagents were provided by Scharlau (Barcelona).

Pharmacokinetic analysis

For analysis of individual kinetics in plasma and the assessment of the parameters, non-parametric models were used, based on the apparent volume of distribution at steady state and plasma clearance, calculated according to the following equations:

$$CL = D/AUC \quad (1)$$

$$Vd_{ss} = CL \times AUMC/AUC \quad (2)$$

where D is the dose and AUC and AUMC are the area under the curve and the area under the curve of the first moment, respectively. Both were calculated by the trapezoidal rule.

The well-stirred model was used to calculate apparent intrinsic clearance (CL_{int}) and the contribution of the unbound fraction in blood to the elimination of tenoxicam in the liver. For the purposes of these calculations, the elimination of tenoxicam in rats was assumed to occur only in the liver; Kwon & Bourne (1987) observed that the excretion of tenoxicam in urine amounted to less than 1% of the dose given, which means that equations for clearance need no adjustment for partial excretion in urine.

The extraction ratio (E) and unbound clearance (CL_u) were calculated by the quotient between blood clearance and hepatic flow, and between plasma clearance and the unbound fraction in plasma (f_u), respectively. Blood clearance was calculated by the quotient between CL and the blood to plasma concentration ratio.

Blood flow in the rat liver was taken to be 90 mL kg^{-1} min^{-1} as has been suggested by various authors (Øie & Yang 1988).

Statistical analysis

Comparison of data was performed using the *t*-test when two groups were being compared, and analysis of variance when more than two groups were involved, followed by the Mann-Whitney U-test, to determine which of the groups deviated from the others. Previously, Bartlett's test was used to calculate the homogeneity of variances. Statistical significance was set at $P < 0.05$.

Results

Using the chromatographic conditions described above, chromatograms were obtained with retention times of 1·5 and 2·2 min for tenoxicam and piroxicam, respectively. No type of interference from endogenous compounds within the rat plasma was observed, nor was any kind of metabolite of tenoxicam detected.

The calibration curves were prepared in distilled water from a stock solution of tenoxicam in acetonitrile (0·25 g L^{-1}, following the procedure used with the biological samples explained above). The calibration curves were obtained by linear regression, and were linear in all cases. The precision and accuracy of the analytical method were expressed as a coefficient of variation and relative error, whose respective values were found to be 6 and 7%. The limit of detection was established as 0·2 mg L^{-1}, and in all the samples assayed there was over 95% recovery of tenoxicam and internal standard.

Table 1. Tenoxicam unbound fraction in plasma and blood, blood to plasma concentration ratio and albumin plasma concentration in normal, uraemic and anephric rats.

	Rats		
	Control	Uraemic	Anephric
Unbound in plasma (f_u)	0·030±0·004	0·06±0·02	0·11±0·08
Unbound in blood (f_{us})	0·055±0·007	0·11±0·04	0·18±0·14
Albumin (%)	3·8±0·2	2·7±0·2	2·6±0·3
BPR	0·57±0·01	0·55±0·06	0·60±0·06

BPR, blood to plasma concentration ratio.

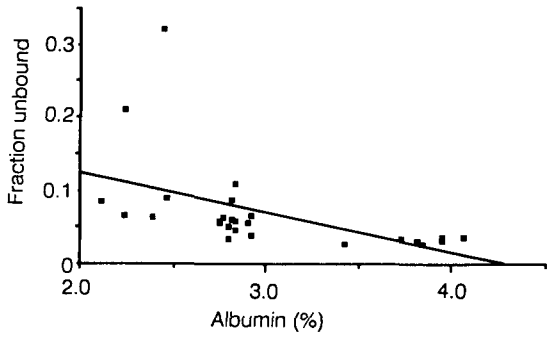

FIG. 1. Relationship between unbound fraction in plasma and albumin plasma concentration (r=0·53) ($P<0·05$).

Table 1 shows the f_u and f_{us} values. Both fractions were raised in hypoalbuminaemic rats compared with the control group ($P<0·001$). These rats were also found to have a significant reduction in the plasma albumin concentration when compared with the rats of the control group ($P<0·001$). No significant differences in the unbound fraction were detected between the uraemic rats and anephric rats ($P>0·05$), or in the blood to plasma concentration ratio found among the various groups ($P>0·05$).

Fig. 1 displays the relationship between the albumin concentration and the unbound fraction of the drug in plasma, showing a statistically significant correlation (r=0·53; $P<0·05$).

Statistically significant increases in the volume of distribution at steady-state and in total plasma clearance were observed in uraemic and anephric rats in comparison with the control group ($P<0·001$ and $P<0·003$, respectively)

Table 2. Pharmacokinetic parameters of tenoxicam in normal, uraemic and anephric rats.

	Rats		
	Control	Uraemic	Anephric
Vd_{ss} (mL)	55±6	69±12	96±30
CL (mL h^{-1})	7±1	12±4	15±7
CL_s (mL h^{-1})	12·5±2·0	22±7	23·5±11·0
CL_{int} (mL h^{-1})	228±22	206±21	161±38
E	0·010±0·002	0·017±0·006	0·02±0·01
CL_u (mL h^{-1})	223±23	201±24	157±40

Vd_{ss}, volume of distribution at steady-state.
CL, plasma clearance.
CL_s, blood clearance hepatic.
CL_{int}, apparent intrinsic clearance.
E, extraction ratio.
CL_u, unbound clearance.

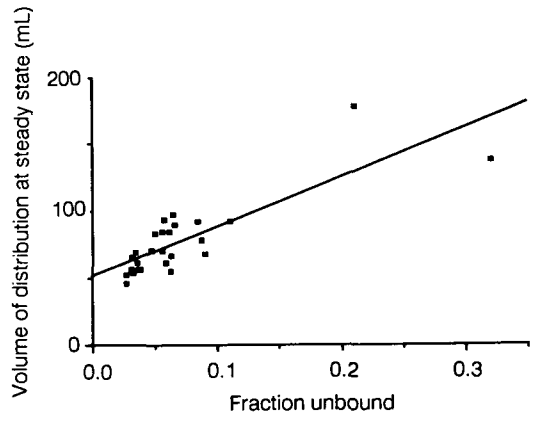

FIG. 2. Relationship between apparent volume of distribution at steady-state of tenoxicam and unbound fraction in plasma (r=0·81) ($P<0·0001$).

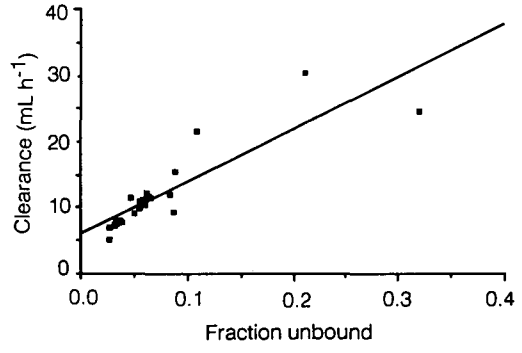

FIG. 3. Relationship between plasma clearance of tenoxicam and unbound fraction in plasma (r=0·86) ($P<0·0001$).

(Table 2). Figs 2 and 3 represent the relationships of Vd_{ss} and CL, respectively, with the unbound fraction in plasma. In both cases, a positive statistically significant correlation was observed between the kinetic parameters and f_u (r=0·81, $P<0·0001$ for Vd_{ss} and r=0·86, $P<0·0001$ for CL).

Values for the apparent intrinsic clearance and hepatic extraction ratio are given in Table 2. The reduction in the percentage of albumin had a statistically significant effect on the hepatic extraction ($P<0·01$) and intrinsic clearance ($P<0·001$).

Discussion

As can be seen from Table 1, tenoxicam has a high degree of plasma protein binding ($f_u=0·03$). The spectacular increase in the f_u value in the group of anephric rats is mainly due to 2 rats (numbers 4 and 7) which were found to have values of 0·2 and 0·3, respectively.

Most of the drug present in the blood was located in the plasma, as indicated by the blood to plasma concentration ratio. A reduction in the albumin concentration had a significant effect on the unbound fractions in plasma and blood, but not on this ratio.

The following expression relates the blood to plasma ratio (BPR) with the unbound fraction in plasma (Rowland & Tozer 1989):

$$BPR = \gamma.H.f_u + 1\text{-}H \quad (3)$$

where γ is the affinity of the blood cells for the drug and H the haematocrit value, which means that the BPR value will be influenced by changes in f_u, if γ and H remain constant. Modifications, however, may exist in both parameters, if there are pathological alterations.

For a drug with a small volume of distribution, the effect of an increase in f_u should be an increase, but not a proportional one, in Vd_{ss}. This study serves to illustrate this theoretical point: when the unbound fraction doubled (0·031 to 0·061) in the group of uraemic rats, the Vd_{ss} only increased by 20%. In the case of the anephric rats, the high value for the apparent average volume of distribution results from the finding that rats 4 and 7 of this group had Vd_{ss} values of 140 and 180 mL, respectively. If we take these two results into account, the increase in Vd_{ss} among the anephric rats was about 30%.

According to the predictions of Pang & Rowland (1977), in the case of drugs with a low extraction ratio (Table 2), clearance will be directly affected by changes in f_u. This hypothesis has been seen to be justified for tenoxicam; when the unbound fraction doubled, clearance rose significantly. However, the increase in clearance was not directly proportional to the rise in the unbound fraction, despite its low extraction ratio. This may be due to the reduction in intrinsic metabolic activity (CL_{int}), which was calculated as a decrease of 10% in the uraemic rats, and of 30% in the anephric rats (Table 2). The reduction in the intrinsic metabolic activity for tenoxicam should be attributed either to a secondary effect due to renal alteration or to a possible saturation of the metabolic pathways produced by an increase in the unbound drug concentration, since tenoxicam is a drug with restricted clearance, so elimination depends only on the unbound plasma concentration.

The effect of renal alterations on hepatic activity has been the subject of numerous studies. Decreases in metabolic activities have been observed for some drugs in chronic renal disease (Mezey & Tobon 1971; Leber & Schutterle 1972; Leber et al 1978) but there is some controversy surrounding these phenomena, and the mechanisms by which the liver metabolism is affected is not clear.

This study confirms the effects of changes in plasma protein binding on the disposition of tenoxicam. Additionally, the Vd_{ss} and CL values correlate significantly with an increase in the unbound fraction in plasma.

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