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Physiological pharmacokinetic model for the distribution and elimination of tenoxicam

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Summary

A physiologically based pharmacokinetic model for tenoxicam distribution and excretion in the rat was developed. The drug concentrations in plasma and all the tissues except testis were simulated using flow-limited equations, while testis concentrations were calculated using a membrane-limited passive diffusion equation. The elimination of tenoxicam was described in the model by renal and hepatic (metabolic and biliary) excretion with gastro-intestinal secretion and reabsorption. In order to validate the model, 15 tissue samples, plasma (for free and total concentration), urine and feces samples were collected and assayed by HPLC after i.v. injection of tenoxicam (4.5 mg/kg). Good agreements between simulation and experimental data over a 24-h period following drug administration were obtained for plasma and tissues. The terminal half-life of tenoxicam was 8.8 h in plasma and ranged in tissues from 6.1 h in intestine to 10.6 h in brain. The fraction of free tenoxicam in plasma ranged from 1.2 to 2.1% of the total tenoxicam concentration (5.7–21.9 μg/ml).

Introduction

Tenoxicam (Ro 12 0068; Fig. 1) is a new non-steroidal, anti-inflammatory and analgesic drug (Kirchheimer et al., 1982). It inhibits prostaglandin synthetase and has a long plasma half life of about 50 h in man (Bird et al., 1983), which allows the drug to be given in once-daily dosage. Conventional compartmental models based on plasma concentration–time curve analysis have been adopted to describe the pharmacokinetics of tenoxicam in rat and human (Pickup and Lowe, 1981; Dixon and Lowe, 1984; Joly, 1984). However, such compartmental analysis was unable to clarify the time course of tenoxicam concentrations in tissues. Physiological pharmacokinetic models have been used to define drug distribution into tissues and to estimate the interspecies relationship for drug kinetics (Ichimura et al., 1983, 1984). Such models are also useful as a predictive tool when drug is administered to subjects with altered physiological state (Farris et al., 1985). This study describes such a physiologically based pharmacokinetic model for tenoxicam incorporating drug distribution and elimination involving plasma, gut lumen and 15 tissues of the rat. Protein binding of tenoxicam to plasma protein was also examined.

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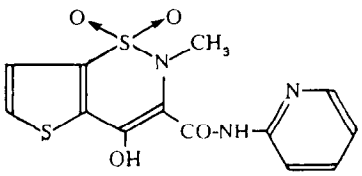


Fig. 1. Structure of tenoxicam (Ro 12-0068).

Materials and Methods

Subjects, injection and sampling

Tenoxicam (Hoffman La Roche, Switzerland), 4.5 mg/kg, was administered i.v. via the tail vein of male albino Wistar rats (230–250 g) after an overnight fast with free access to water. At predetermined times the rats were sacrificed by collection of the blood sample from the heart under ether anesthesia. Whole brain, heart, lung, stomach, spleen, pancreas, intestine, gut contents, liver, kidney and testis were obtained by dissection for the measurement of drug concentration and organ weight. About 1 g of muscle, fat, skin with fur and bone with marrow were also collected for drug assay. Five rats were used for each sampling time; 15, 30 min, 1, 1.5, 3, 6, 12, 18 and 24 h. From a further group of 5 rats, urine and feces were separated with a metabolism cage, and collected at 6, 24 and 48 h for urine and at 48 h for feces. One ml of each plasma sample was immediately transferred to an ultrafiltration set for protein binding determination. All other samples were stored at $-20\,^{\circ}C$ until analysis. To determine the tissue-to-plasma partition coefficients of the drug, frequent i.v. injections were given for a total of 8 times at 0, 12, 18, 24, 27, 30, 32, and 34 h in a group of 5 rats. Based on simulations using single-dose parameter values this regimen was expected to produce equilibration between plasma and tissues. 6 mg/kg tenoxicam was injected as an initial dose and 3 mg/kg was injected at each subsequent dose. Plasma and tissue samples were obtained 1 h after the last dose.

Protein binding study

The centrifugal ultrafiltration membrane technique was used to determine the in vivo binding of tenoxicam to plasma protein. The plasma samples collected were placed into microporous filters fitted with an ultrafiltration membrane (Amicon, type YMT, Danvers, MA, U.S.A.), and centrifuged at 1000 *g* for 10 min at $36\,^{\circ}C$. The concentration of drug in the filtrate was determined by HPLC as described below. Drug binding to the apparatus was slight with less than 10% of the free drug concentration bound in the absence of plasma protein.

Drug assay

Each tissue sample (0.5 g) was mixed and homogenized with 0.5 ml perchloric acid (0.4 M), 0.5 ml phosphate buffer (1 M, pH 1.5) and 0.5 ml Ro 13-9297 (5 or 10 μg/ml in 0.01 M Na_2HPO_4 solution) as an internal standard. The homogenized mixture was extracted with 7 ml of ether for 30 min. After centrifugation, the organic phase was transferred to a clean tube and evaporated to dryness at $40\,^{\circ}C$. The residue was dissolved in 300 μl of methanol, and 20 μl of this solution was injected into the liquid chromatograph. Tissue standards were prepared by adding measured amounts of tenoxicam in a small volume to the homogenized tissue. All other procedures were the same as for the unknown samples. Plasma samples (0.5 ml) were mixed with internal standard and 0.5 ml acetonitrile and centrifuged. 20 μl of the supernatant was injected onto the HPLC system. Plasma filtrate (20 μl) from the protein binding study was injected directly. The mobile phase consisted of methanol : cetrimide (2 mmol) : phosphate buffer (0.1 M, pH 6) : distilled water (68 : 10 : 10 : 12). The samples were separated on a reverse-phase column (μ-Bondapack C 18, Waters Associates, MA, U.S.A.) with a flow rate of 1.5 ml/min and detected using a UV detector (Model 440, 365 nm, Waters Associates). Standard curves were linear ($r > 0.997$) in the range of 0.5–20 μg/ml. The measured retention times for tenoxicam and internal standard were 3 min and 5 min, respectively, and the sensitivity of the assay was 0.1 μg/ml for plasma and tissue and 0.05 μg/ml for plasma filtrate. Measured recoveries from plasma and tissues were greater than 90%.

Physiological model development and calculations

The physiological pharmacokinetic model

utilized in this study is shown in Fig. 2. The model was constructed on the basis of the following assumptions: (1) each tissue is a well-stirred compartment; (2) the drug distribution is flow-limited except for the testis; (3) tissue to plasma partition coefficients are concentration- and time-independent. The carcass serves as a residual compartment, and includes tissues and organs not otherwise incorporated into the model. The model includes 4 drug clearances: metabolic, kidney, bile and feces clearance. The blood flow to the liver was defined as the sum of flow from stomach, spleen, pancreas, intestine and direct flow from the hepatic artery. Gut absorption (K_{GI}) and excretion (K_{IG}) rate constants are included between the intestine and gut lumen. Drug distribution for testis were simulated as a membrane-limited passive diffusion model. The following mass-balance equations describe the drug concentration in each compartment of the physiological model.

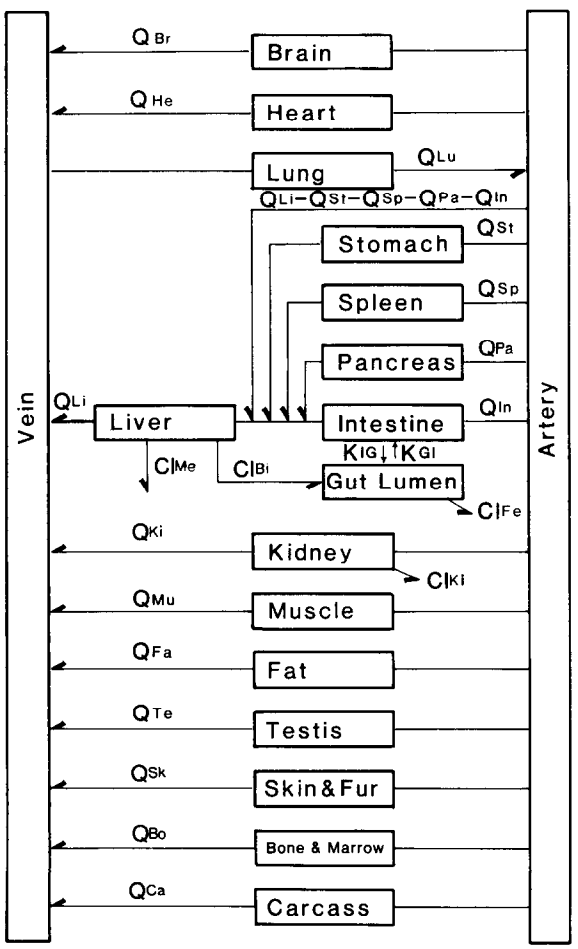


Fig. 2. Physiological pharmacokinetic model of tenoxicam disposition in the rat.

Vein

$$V_{Ve} \cdot dC_{Ve}/dt = Q_{Br} \cdot C_{Br}/R_{Br} + Q_{He} \cdot C_{He}/R_{He} + Q_{Li} \cdot C_{Li}/R_{Li} + Q_{Ki} \cdot C_{Ki}/R_{Ki} + Q_{Mu} \cdot C_{Mu}/R_{Mu} + Q_{Fa} \cdot C_{Fa}/R_{Fa} + Q_{Te} \cdot C_{Te}/R_{Te} + Q_{Sk} \cdot C_{Sk}/R_{Sk} + Q_{Bo} \cdot C_{Bo}/R_{Bo} + Q_{Ca} \cdot C_{Ca}/R_{Ca} - Q_{Ve} \cdot C_{Ve}$$

Artery

$$V_{Ar} \cdot dC_{Ar}/dt = Q_{Lu} \cdot C_{Lu}/R_{Lu} - Q_{Ar} \cdot C_{Ar}$$

Testis

$$V_{Te} \cdot dC_{Te}/dt = H_{Te}(C_{Ar} - C_{Te}/R_{Te})$$

Lung

$$V_{Lu} \cdot dC_{Lu}/dt = Q_{Ve} \cdot C_{Ve} - Q_{Lu} \cdot C_{Lu}/R_{Lu}$$

Liver

$$V_{Li} \cdot dC_{Li}/dt = C_{Ar}(Q_{Li} - Q_{St} - Q_{Sp} - Q_{Pa} - Q_{In}) + Q_{St} \cdot C_{St}/R_{St} + Q_{Sp} \cdot C_{Sp}/R_{Sp} + Q_{Pa} \cdot C_{Pa}/R_{Pa} + Q_{In} \cdot C_{In}/R_{In} - Q_{Li} \cdot C_{Li}/R_{Li} - Cl_{Li} \cdot C_{Ar}$$

Kidney

$$V_{Ki} \cdot dC_{Ki}/dt = Q_{Ki}(C_{Ar} - C_{Ki}/R_{Ki}) - Cl_{Ki} \cdot C_{Ar}$$

Intestine

$$V_{In} \cdot dC_{In}/dt = Q_{In}(C_{Ar} - C_{In}/R_{In}) + K_{GI} \cdot V_{GU} \cdot C_{GU} - K_{IG} \cdot V_{In} \cdot C_{In}$$

Gut lumen

$$V_{Gu} \cdot dC_{Gu}/dt = Cl_{Bi} \cdot C_{Ar} + K_{IG} \cdot V_{In} \cdot C_{In} - K_{GI} \cdot V_{Gu} \cdot C_{Gu} - Cl_{Fe} \cdot C_{Gu}$$

Non eliminating organs: stomach, spleen, pancreas, muscle, fat, brain, skin and fur, bone and marrow, and carcass

$$V_i \, dC_i/dt = Q_i(C_{Ar} - C_i/R_i)$$

Urine

$$dC_{Ur}/dt = Cl_{Ki} \cdot C_{Ar}$$

Where

V_i = tissue volume (ml)
C_i = drug concentration (μg/ml)
Q_i = plasma flow (ml/h)
R_i = tissue to plasma partition coefficient
Cl_i = clearance (ml/h)
H_i = mass transfer coefficient (membrane diffusion parameter, ml/h)
K_{GI} = first order rate constant for gut secretion (ml/h)
K_{IG} = first order rate constant for gut absorption (ml/h)

The subscripts are as follows: Ve, venous; Ar, artery; Br, brain; He, heart; Li, liver; Ki, kidney;

TABLE 1

Physiological parameters used in the model for 240-g Wistar rats

Organ	Volume [a] (V, ml)	Plasma flow rate (Q, ml/h)	Linear binding constant [a] (R)	Membrane diffusion rate constant [b] (H, ml/h)	Clearance [c] (Cl, ml/h)
Vein	7.0	1800 [d]	–	–	–
Artery	3.5	1800 [d]	–	–	–
Brain	1.54	27 [e]	0.014	–	–
Heart	0.65	48 [d]	0.18	–	–
Lung	0.98	1800 [d]	0.31	–	–
Stomach	2.11	72 [f]	0.14	–	–
Spleen	0.36	27 [f]	0.07	–	–
Pancreas	0.52	32 [f]	0.12	–	–
Intestine	8.4	420 [f]	0.25	–	–
Gut lumen	4.5	–	–	–	–
Liver	5.24	686 [f]	1.12	–	5.48
Kidney	1.38	438 [d]	1.01	–	0.023
Muscle	108 [d]	216 [d]	0.08	–	–
Fat	16.8 [e]	60 [d]	0.03	–	–
Testis	2.88	22.6 [f]	0.09	0.27	–
Skin and fur	45.2 [c]	144 [d]	0.15	–	–
Bone [h]	14.8 [e]	76.8 [g]	0.10	–	–
Carcass	16.1 [c]	81.6 [c]	0.09 [c]	–	–
Bile clearance (Cl_{Bi})					0.52
Feces clearance (Cl_{Fe})					1.20
Gut absorption and excretion rate constant ($K_{IG} \cdot K_{GI}$)					0.05 [b]

[a] Experimental data.
[b] Estimated from experimental data.
[c] Calculated from data.
[d] Tsuji et al., 1983.
[e] Gerlowski and Jain, 1983.
[f] Jain et al., 1981.
[g] Ichimura et al., 1983.
[h] Bone and marrow.

Mu, muscle; Fa, fat; Te, testis; Sk, skin and fur; Bo, bone and marrow; Ca, carcass; Lu, lung; St, stomach; Pa, pancreas; In, intestine; Gu, gut lumen; Bi, bile; Fe, feces; Ur, urine; Me (Fig. 2), metabolic. The 19 differential equations of the model were solved simultaneously using the program MULTI-FORTE (Bourne, 1986; Gear, 1969) on a Macintosh 512K desktop computer.

Determination of model parameters

The physiological parameters used in the model are summarized in Table 1. The partition coefficients (R value) and the volume of most organs were determined experimentally as a mean of 5 measurements. The volume of plasma, muscle, fat, skin and fur, bone and marrow were taken from literature values (Tsuji et al., 1983; Gerlowski and Jain, 1983), with an assumed tissue density of 1 g/ml. Plasma flow rates were also taken from literature (Tsuji et al., 1983; Gerlowski and Jain, 1983; Jain et al., 1981; Ichimura et al., 1983) and scaled to a body weight of 240 g when necessary. The R value of carcass was calculated as a volume proportional mean of the non-eliminating organs, and the volume of carcass was calculated by subtracting the sum of all other tissues and plasma from the total rat weight of 240 g. The plasma flow rate of carcass was calculated as the difference between venous flow rate and sum of output tissue plasma flow rate to venous. The diffusion parameters into testis (H_i), gut absorption (K_{GI}) and excretion (K_{IG}) rate constants were adjusted to obtain a reasonable simulation of the data. The total body clearance and renal clearance (Cl_{Ki}) values were calculated from the data as dose/AUC and $K_u \times V_1$ respectively, where AUC is the area under the plasma drug concentration vs time curve, K_u is the excretion rate constant into urine, and V_1 is the volume of distribution of the central compartment for a two-compartment pharmacokinetic model. The hepatic clearance term (Cl_{Li}) was calculated as the difference between the total clearance and the renal clearance values. The bile clearance (Cl_{Bi}) was estimated from the amount of drug in the gut lumen, and the feces clearance (Cl_{Fe}) was estimated from the amount of drug excreted into feces.

Results and Discussion

Validation of the physiological model

The physiological model developed herein simulates the disposition and elimination of tenoxicam in plasma, urine, feces, gut lumen and 15 other tissue compartments of the male rat. The model was used to obtain the solid lines shown in Fig. 3, with the experimental results represented by the symbols and error bars (mean ± S.D.). Good correlations were obtained over the 24-h simulation period for 15 tissues and plasma. The great majority of drug concentrations in tissues and plasma were simulated within 20% of the experimental determinations except for some scattered data points. The model also simulates the sum of urine excretion and gut lumen contents within about 10% and 15% of the experimental determinations, respectively. Brain, testis, and spleen were initially calculated using membrane-limited passive diffusion equations because of the delay in the peak time for these well-perfused tissues. However, the 'best fit' values for the membrane perfusion coefficients were not different from the plasma flow rates to the brain or spleen. Thus, only the testes were calculated with a membrane-limited passive diffusion equation. With a fitted value for the mass transfer coefficient there is good agreement between the model generated and the experimental data.

An analysis of the model parameters which were obtained for 240 g rats (Table 1) shows that the linear binding constant (R) for tenoxicam ranges from 0.014 for brain to 1.12 for liver. The blood to plasma ratio was measured separately and found to be 0.73 with a hematocrit value of 0.43. Tenoxicam tissue concentrations were lower than in plasma with the exception of liver and kidney, suggesting reduced distribution into all tissues except liver and kidney. The major route of tenoxicam elimination was metabolic clearance in the liver which accounts for over 90% of the total clearance. The terminal half life and AUC of plasma and tissues are shown in Table 2.

The terminal half life of tenoxicam in experimental data ranged from 6.1 h for intestine to 10.6 h for brain, and the AUC ranged from 2.7 $\mu g \cdot h/ml$ for brain to 232.3 $\mu g \cdot h/ml$ for liver. The

terminal half life of tenoxicam from rat plasma (8.8 h) was about 6-fold less than the results in humans (Bird et al., 1983; Pickup and Lowe, 1981). The maximum observed tenoxicam concentrations in most of the tissues occurred at the first data point (0.25 h), and at 0.5 h for brain, stomach, muscle and fat, and at 1 h for skin and fur, testis and spleen.

Plasma protein binding study

The extent of in vivo protein binding of tenoxicam to rat plasma (Fig. 4) was 97.9% to

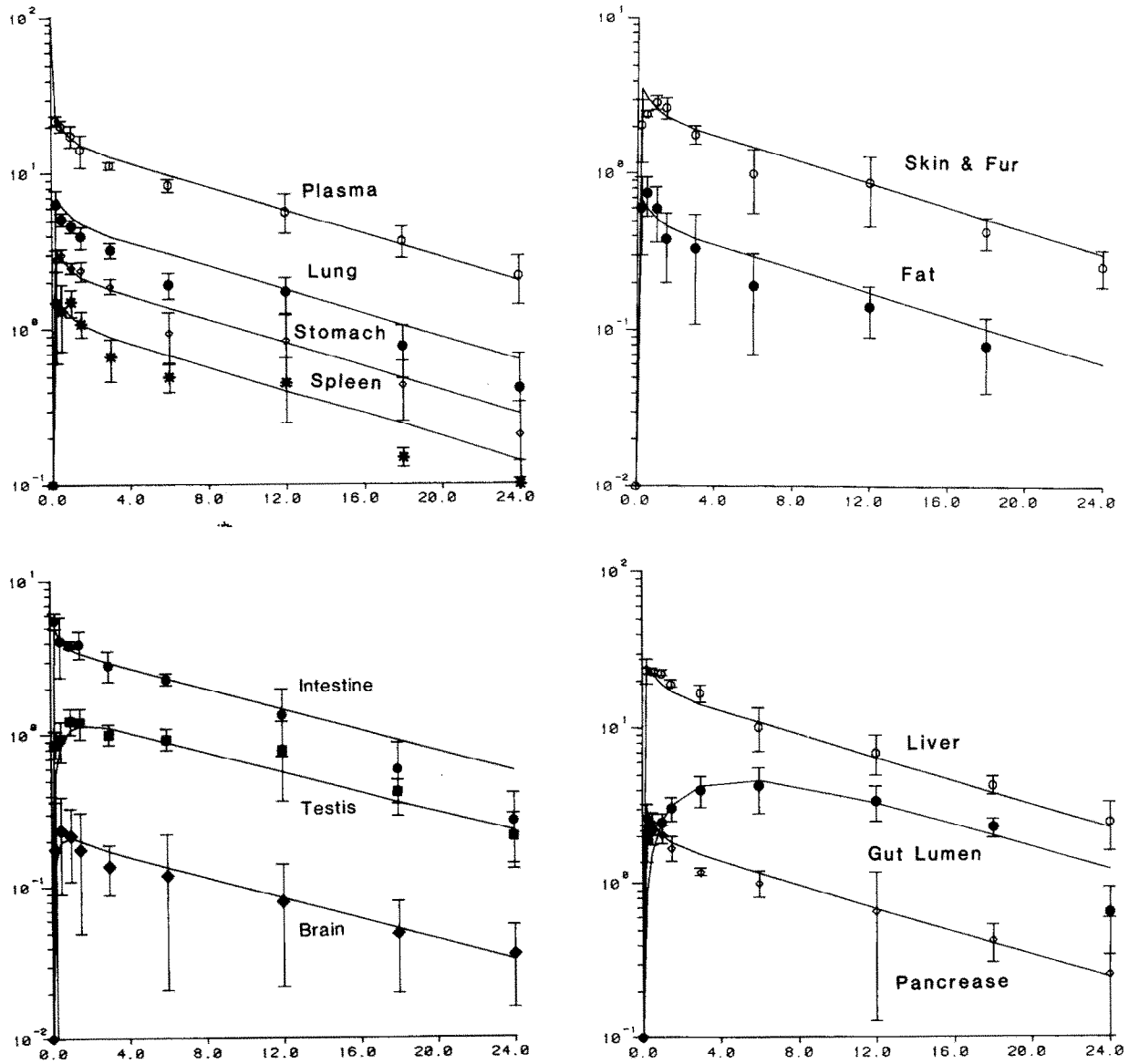
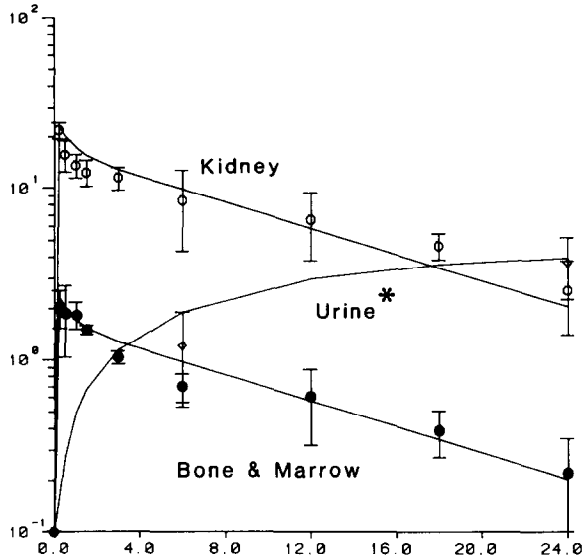


Fig. 3. Model predicted (lines) vs observed (points) tenoxicam concentrations in plasma and tissues after a 4.5 mg/kg i.v. bolus dose to rats. Each point and bar represents a mean of 5 experimental data and S.D., respectively. * Cumulative amount (μg) of tenoxicam excreted in the urine. X-axes, time (h); Y-axes, tenoxicam concentration (μg/ml).



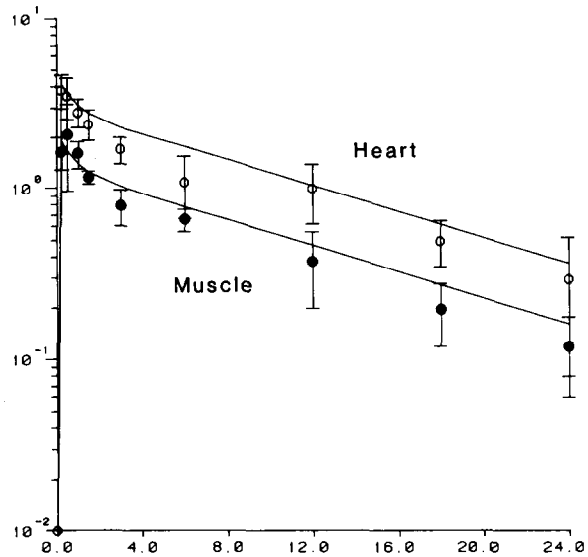


Fig. 3 continued.

98.8% over the plasma tenoxicam concentration range of 5.7 to 21.9 μg/ml. The solid line represents the curve calculated by fitting the total and free tenoxicam concentrations to Eqn. 1 (Bevill et al., 1982) using the digital computer program NONLIN modified to operate on a mini-computer (Bourne and Wright, 1981).

$$\text{Free} = \frac{(KF \times Total - 1 - KF \times Pt)}{2KF} + \frac{\sqrt{(KF \times Total - 1 - KF \times Pt)^2 + 4KF \times Total}}{2KF} \quad (1)$$

The parameters KF and Pt are the association constant and the total concentration of protein binding sites in plasma, respectively. The parameter values of KF and Pt obtained in this study were 5.09×10^5 M^{-1} and 1.56×10^{-4} M respectively. The plasma protein binding term for tenoxicam in the model was omitted as the agreement between predicted and experimental data was sufficient without it.

The physiological pharmacokinetic model presented here contributes towards a more complete understanding of tenoxicam disposition. The model should be readily applicable to estimate the kinetics of the drug in other species including

TABLE 2

Pharmacokinetic parameter values for tenoxicam [a] in the rat

Organ	AUC (μg.h/ml)	Half-life [b] (h)	Organ	AUC (μg.h/ml)	Half-life [b] (h)
Plasma	190.2	8.8	Liver	232.2	7.9
Brain	2.7	10.6	Kidney	210.0	10.5
Heart	28.3	8.2	Muscle	13.0	7.2
Lung	46.1	7.4	Fat	4.7	7.6
Stomach	24.4	7.0	Testis	20.0	9.9
Spleen	10.6	6.7	Skin and fur	25.5	7.3
Pancreas	22.2	9.0	Bone and marrow	18.6	9.2
Intestine	40.5	6.1	Gut lumen	79.3	10.5

[a] Tenoxicam 4.5 mg/kg i.v. injection.
[b] Terminal half-life.

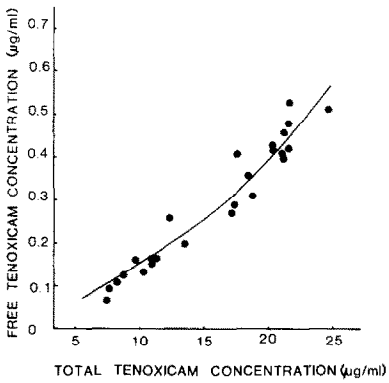


Fig. 4. In vivo protein binding of tenoxicam in rat plasma. The line represents the curve calculated by NONLIN using Eqn. 1 (see text).

man, and should also prove useful as a predictive tool when the drug is administered to patients with altered physiological status (e.g. liver or renal failure, etc). Although the parameters are different between various species, scale-up is possible since parameters can be adjusted on the basis of animal size (Ichimura et al., 1984).

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