Relationship Between Enoxacin and Ciprofloxacin Plasma Concentrations and Theophylline Disposition

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Received February 3, 1994; accepted May 5, 1994

Certain fluoroquinolone antibiotics affect theophylline (THEO) disposition by inhibition of its metabolism, yet no studies to date have investigated the relationship between fluoroquinolone plasma concentration and THEO pharmacokinetics. The effects of two fluoroquinolones, enoxacin (ENOX) and ciprofloxacin (CIPRO), have been studied in male Sprague-Dawley rats (n = 33–46) at steady state plasma concentrations of 0–33 mg · l^{-1}, achieved by supplementing an intravenous bolus dose with a constant rate infusion. The effects of steady state ENOX and CIPRO plasma concentrations on the clearance of THEO determined after an intravenous bolus dose of 6 mg · kg^{-1} were described using a competitive inhibition model. The model consisted of two components, one describing a residual component of THEO clearance, which was unaffected by fluoroquinolone, the other describing the non-linear reduction of THEO clearance by fluoroquinolone. The residual clearance estimated from the model was comparable to renal clearance for THEO in the rat. The potency of each fluoroquinolone was characterised by a Ki value, the concentration reducing THEO clearance by 50% of the maximum change. These values were 4.7 μM and 16.3 μM for ENOX and CIPRO, respectively. Thus, in this study, ENOX was found to be a more potent inhibitor of THEO clearance than CIPRO. The method allowed direct *in vivo* comparison of potency between different fluoroquinolones, as pharmacokinetic differences, such as clearance, volume of distribution and bioavailability, were 'designed out.'

KEY WORDS: enoxacin; ciprofloxacin; theophylline; pharmacokinetics; drug-drug interactions.

INTRODUCTION

Ciprofloxacin (CIPRO) and enoxacin (ENOX) are both fluoroquinolone antibiotics that have been used for a number of years in the oral treatment of Gram negative bacterial infections. A number of authors have reported the interaction of CIPRO and ENOX with concurrently administered theophylline (THEO) (1,2). These reports have indicated that this interaction is due to a reduction in the total plasma clearance of THEO. It has been shown that the interaction of ENOX with THEO occurs *via* inhibition of its metabolism (3,4). Further *in vitro* studies (5) have shown that this is a competitive type interaction.

Other authors (1,2,6,7) have studied a range of fluoroquinolones and have shown that the effects of individual fluoroquinolones on THEO are markedly different. However, there have been no studies reported that address the relationships between fluoroquinolone plasma concentration and THEO clearance. A valid comparison of the ability of the various fluoroquinolones to inhibit THEO metabolism *in vivo* can only be achieved by assessing the plasma concentration-effect relationship for each fluoroquinolone.

The proposed studies were designed to compare the effect of two fluoroquinolones, CIPRO and ENOX, on THEO disposition in the rat, as these two fluoroquinolones would be expected to have the most pronounced effects. A constant intravenous infusion of fluoroquinolone was used, as conventional multiple oral dosing only achieves a pseudo-steady state—minimum plasma concentrations are constant from dose to dose, but the concentration of fluoroquinolone in plasma changes throughout the dosing interval. To adequately describe potency, the effect of specific plasma concentrations on THEO clearance should be established. In addition, the use of intravenous constant infusions enable pharmacokinetic differences (e.g. bioavailability, clearance and volume of distribution) to be designed out, by using regimes that result in constant plasma concentrations of fluoroquinolone.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (250–300 g) were obtained from the University of Manchester Biological Services Unit. The animals were housed 2–3 per cage on a bedding of sawdust and fed with CRN rat diet. They had access to water *ad libitum*. The holding room was kept in a constant range of 20–21°C and alternated between 12 hours of light and darkness.

Polyethylene cannulae were inserted, under halothane anaesthesia, into the (right) jugular vein and carotid artery (8). All cannulations were performed during the afternoon of the day before the experiment, the animals therefore having about 18 hours recovery from the surgery. Once the animals had been cannulated, they were housed individually.

Preliminary Bolus Experiments

To be able to predict steady state plasma concentrations from infusion experiments, the basic pharmacokinetic parameters of CIPRO and ENOX were assessed in a small number of preliminary experiments. After cannulation and recovery, animals received either 10 mg or 25 mg CIPRO by rapid intravenous bolus. The resulting plasma concentration-time curve was used to estimate clearance (CL) and volume of distribution at steady state (Vss). A simple exponential model [1] was used to describe the data:

$$C = \sum_{i=1}^{n} C_i \cdot e^{-\lambda_i t} \qquad [1]$$

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0724-8741/94/1000-1424$07.00/0

Thus

$$CL = \frac{Dose}{\sum_{i=1}^{n} \frac{C_i}{\lambda_i}}, \quad MRT = \frac{\sum_{i=1}^{n} \frac{C_i}{\lambda_i^2}}{\sum_{i=1}^{n} \frac{C_i}{\lambda_i}}, \quad \text{Vss} = \text{CL} \cdot \text{MRT}.$$

For ENOX, a short intravenous infusion of 4 mg or 10 mg over 30 minutes was given. This enabled a better estimate of area under the plasma concentration-time curve (AUC). Convolution of a simple exponential disposition function with a zero order input function yields the description of the plasma concentration-time data [2]:

$$C = \frac{Dose}{\tau} \sum_{i=1}^{n} \left(\frac{1 - e^{+\theta\lambda_i}}{-\lambda_i} \right) C_i' e^{-\lambda_i t} \qquad [2]$$

Where τ is the infusion duration, $C_i' = C_i/Dose$ and $\theta = t$ for $t \leqslant \tau$, $\theta = \tau$ for $t \geqslant \tau$. In both experiments ten samples were taken over an eight hour period. The models were fitted to the data by weighted least squares regression (weighting schemes of 1/y(calc) gave the best fits), using the Siphar version 3.3 software (Simed, Créteil, France).

Design of Steady State Studies

Mean values of clearance (CL) and volume of distribution (Vss) calculated from the preliminary bolus experiments were used in the steady state experiments. To achieve the desired steady state plasma concentration (Css), a bolus of fluoroquinolone, calculated using the relationship

$$Dose = V_{ss} \cdot C_{ss}$$

was given *via* the venous cannula. This was followed immediately by an infusion of fluoroquinolone (R_0), which was calculated for the desired steady state concentration using the relationship

$$R_0 = CL \cdot C_{ss}$$

CIPRO was dissolved in distilled water up to a concentration of 30 mg · ml^{-1}. ENOX was dissolved up to a concentration of 20 mg · ml^{-1} in 0.1 M NaOH to obtain sufficiently high solubility. A Sage infusion pump model 355 (A. H. Horwell Ltd., London, England) was used to deliver the infusion into the jugular vein. The flow rate was 0.15–1.1 ml · h^{-1}.

A bolus of 1.5 mg THEO was given *via* the venous cannula immediately before the bolus of fluoroquinolone. A range of fluoroquinolone concentrations were studied (0.75–33.8 mg · l^{-1} for CIPRO and 0.45–24.6 mg · l^{-1} for ENOX) and the clearance of THEO assessed at each different steady state concentration of fluoroquinolone (n = 33 for CIPRO and n = 46 for ENOX). Each experimental group also contained a control infusion, of the solvent containing no fluoroquinolone. The cannulae were flushed with heparinised saline (100 units ml^{-1}) between each drug, and after each blood sample, to maintain their patency. Serial blood samples were taken into Eppendorf tubes, containing one or two drops of heparinised saline (5000 units ml^{-1}), over a 6–7 hour period, from the carotid artery. Plasma was separated and stored at −20°C until analysis, usually on the next day.

HPLC Analysis

Plasma samples (100 µl) were diluted in phosphate buffer pH 7.4 (CIPRO) or saturated sodium bicarbonate solution (ENOX) and extracted with 5 ml 95:5 chloroform:isopropanol (CIPRO) or 5 ml 9:1 dichloromethane:isopropanol (ENOX), after the addition of 50 µl internal standard (20 µgml^{-1} β-hydroxypropyl theophylline for CIPRO analysis or 20 µg · ml^{-1} CIPRO for ENOX analysis). After reconstitution, the samples were analysed using reversed phase HPLC with a detector wavelength of 280 nm and a mobile phase consisting of 15% acetonitrile in a phosphate buffer containing 4.4 mM tetrabutyl ammonium hydrogen sulphate at pH 3.0, flowing at 1.3 ml · min^{-1}. Limits of detection were 0.5–1.0 µg · ml^{-1} and intra-day variability was less than 15%. The assay has been described elsewhere in more detail (9).

Data Analysis

From conventional Michealis-Menten assumptions for competitive inhibition of metabolism it can be shown that the rate of drug metabolism is given by

$$RATE = \frac{S \cdot V_{MAX}}{S + K_M\left(1 + \frac{i}{K_i}\right)} \qquad [3]$$

where S is the concentration of substrate and i the concentration of inhibitor. The effect of adding a competitive inhibitor is therefore to increase the effective K_M without affecting V_{MAX}. Clearance is defined as the rate of removal of drug per unit concentration and thus metabolic clearance (CL_M) for a pathway sensitive to inhibition can be defined for the situation where $S \ll K_M$, as

$$CL_M = \frac{CL_1}{1 + \frac{i}{K_i}} \qquad [4]$$

Table I. Estimates of Pharmacokinetic Parameters for ENOX Using Short Intravenous Infusions, mean (±CV%) of 3 Animals

Dose mg/30 min	C_1 mg · l^{-1}	λ_1 h^{-1}	C_2 mg · l^{-1}	λ_2 h^{-1}	$t_{1/2}$ h	MRT h	CL l · h^{-1}	Vss l
4	9.8 ± 17	3.7 ± 30	1.9 ± 16	0.34 ± 15	2.1 ± 14	2.3 ± 9	0.47 ± 2	1.0 ± 10
10	38.2 ± 56	6.6 ± 70	4.9 ± 57	0.48 ± 37	1.6 ± 31	1.6 ± 31	0.49 ± 10	0.7 ± 17
Mean ± CV%					1.81 ± 25	1.95 ± 25	0.48 ± 6	0.86 ± 23

Table II. Estimates of Pharmacokinetic Parameters for CIPRO Using Rapid Intravenous Bolus Dosing, mean (±CV%) of 3 Animals

Dose mg	C_1 mg · l^{-1}	λ_1 h^{-1}	C_2 mg · l^{-1}	λ_2 h^{-1}	$t_{1/2}$ h	MRT h	CL l · h^{-1}	Vss l
10	15 ± 47	2.3 ± 52	4.1 ± 15	0.40 ± 17	1.8 ± 22	1.8 ± 22	0.57 ± 19	0.98 ± 3
25	48 ± 10	2.4 ± 12	9.4 ± 35	0.37 ± 14	1.9 ± 11	1.6 ± 0.2	0.55 ± 20	0.95 ± 23
Mean ± CV%					1.82 ± 13	1.66 ± 13	0.56 ± 18	0.96 ± 17

where CL_1 is the formation clearance of the drug by the sensitive pathway in the absence of inhibitor. Total clearance is simply the sum of metabolic clearance plus clearance *via* other routes (CL_2), which are not sensitive to inhibition.

$$CL_{TOT} = \frac{CL_1}{1 + \frac{i}{K_i}} + CL_2 \quad [5]$$

CL_{TOT} was calculated for THEO from Dose/AUC.

RESULTS

Fluoroquinolone Disposition

The parameters describing the disposition of ENOX and CIPRO after bolus intravenous administration are shown in Tables I and II, respectively. No dose dependency was seen over the limited dose range studied for either fluoroquinolone. A biexponential model was required to describe the data in both cases. The CL and Vss for ENOX were found to be 2.0 ± 0.1 l · h^{-1} · kg^{-1} and 3.6 ± 0.8 l · kg^{-1}. Similar estimates were observed for CIPRO: 2.4 ± 0.4 l · h^{-1} · kg^{-1} and 4.1 ± 0.7 l · kg^{-1}, respectively.

There was a linear relationship between infusion rate and steady state plasma concentration of ENOX, over the concentration range 2–20 mg · l^{-1}. The average CL estimate in these experiments was 3.1 l · h^{-1} · kg^{-1}. However, the relationship for CIPRO (Figure 1) was found to be nonlinear and was best described using equation [6]:

$$C_{SS} = \frac{R_0 \cdot K_M}{V_{MAX} - R_0} \quad [6]$$

Equation [6] was fitted to the data using ordinary least squares regression and estimates of V_{MAX} and K_M of 111 ± 6.7 mg · h^{-1} · kg^{-1} and 24 ± 2.8 mg · l^{-1}, respectively were obtained. At low concentrations (<5 mg · l^{-1}), CL was calculated to be 3.7 l · h^{-1} · kg^{-1}, by linear regression.

Effect of Fluoroquinolone Concentration on Theophylline Clearance

Typical fluoroquinolone concentration-time profiles are shown in Figure 2 together with corresponding THEO concentration-time profiles.

A monoexponential model was required to describe THEO disposition. In all studies, the volume of distribution of THEO was not affected by either CIPRO or ENOX over the concentration ranges studied (Vss = 0.60 l · kg^{-1}). The area under the curve for THEO progressively increased as fluoroquinolone steady state plasma concentrations increased.

The maximum effect of CIPRO was a threefold increase in THEO AUC, occurring above 30 mg · l^{-1}. The maximum effect of ENOX was a fourfold increase in AUC at concentrations above 20 mg · l^{-1}. These changes corresponded to falls in total plasma THEO clearance of 67% and 79% respectively.

DISCUSSION

The results of the disposition experiments agree with those of Siefert *et al.* (10) for CIPRO. There are no similar reports in the literature for ENOX. It is interesting that the disposition parameters for CIPRO and ENOX were so similar. The use of short infusion experiments to determine the disposition characteristics of ENOX allowed better estimations (due to a lower % extrapolation in AUC) than the rapid bolus dose method.

From equation [5], the effect of a competitive inhibitor on THEO metabolism would be to reduce metabolic clearance in a hyperbolic fashion. This assumes however that the concentration of THEO is much less than the K_M for its metabolism. In the interaction experiments, the dose of THEO used was such that the maximum concentration was

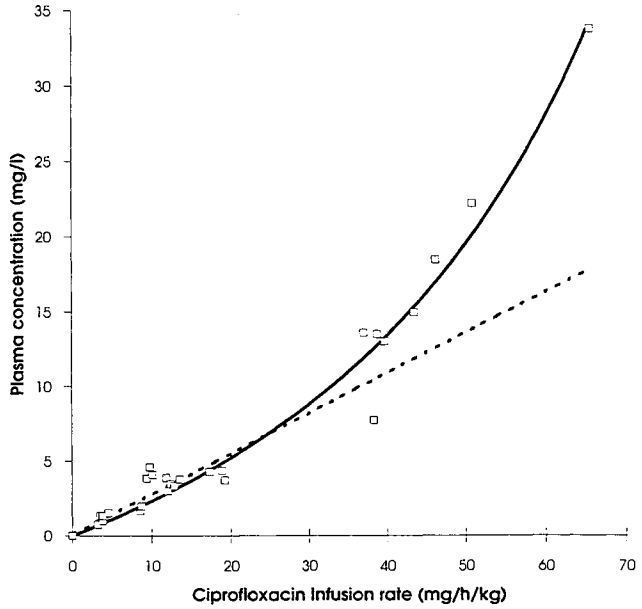

Figure 1 Effect of infusion rate on CIPRO plasma concentration. Data from 33 animals are shown. The dashed line indicates a linear clearance estimate of 3.7 l · h^{-1} · kg^{-1} (applicable to concentrations < 20 mg · l^{-1}) and the solid line the nonlinear clearance (V_{MAX} 111 mg · h · kg^{-1} and K_M 24 mg · l^{-1}) over the full concentration range.

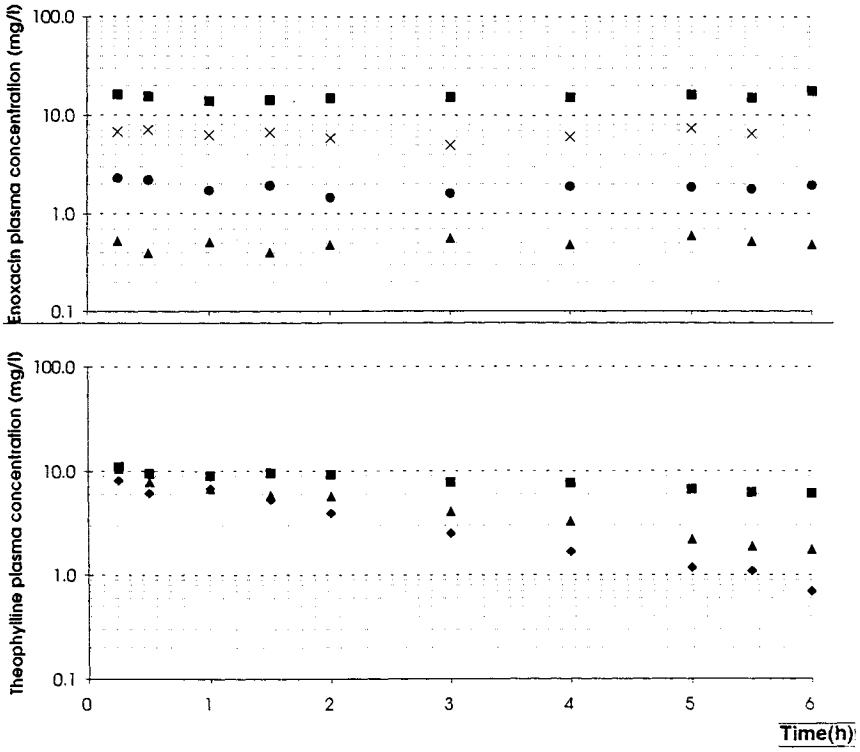

Figure 2 Typical ENOX and corresponding THEO concentration-time data. ◆— 0 mg · l^{-1}, Δ—0.5 mg · l^{-1}, ●—1.9 mg · l^{-1}, ×—6.4 mg · l^{-1}, ■—15 mg · l^{-1} ENOX.

around 55 μM (10 mg · l^{-1}). It has been found in rat hepatic microsomes that the K_M for the major metabolite of THEO, 1,3 dimethyl uric acid, is greater than 1000 μM (J. D. Davis, L. Aarons & J. B. Houston, *in preparation*), consequently equation [5] is appropriate. Figures 3 and 4 show the relationship between THEO clearance and steady state plasma concentration of ENOX and CIPRO, respectively. It can be seen that equation [5] describes the data well. The parame-

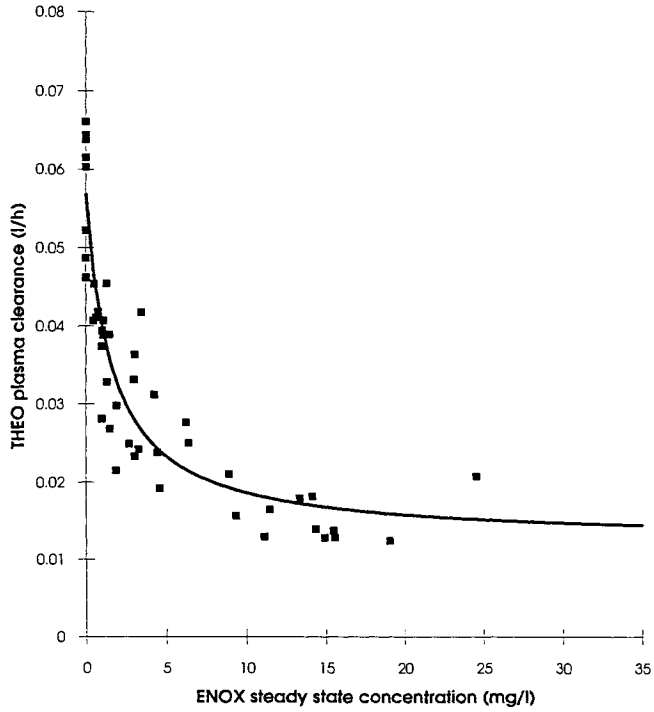

Figure 3 Effect of ENOX concentration on THEO clearance. Data from 46 animals which can be described by equation [5], with a Ki estimate of 1.62 mg · l^{-1}.

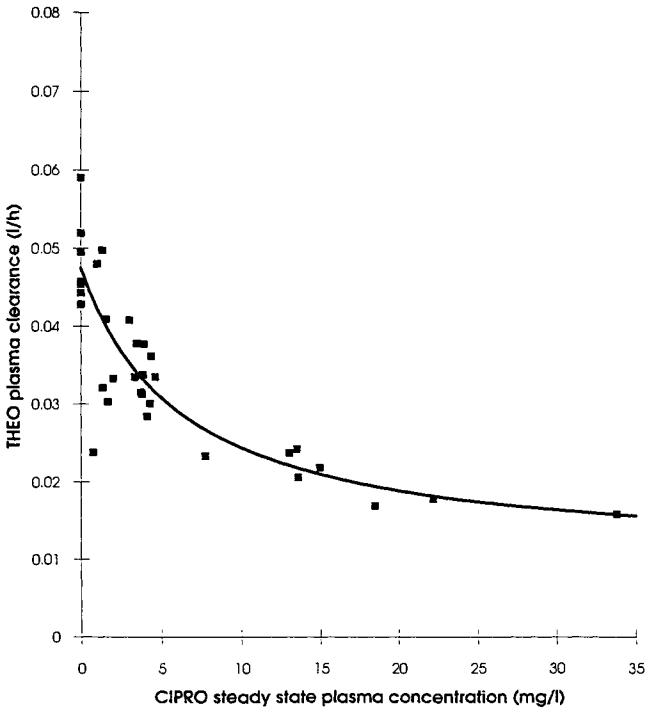

Figure 4 Effect of CIPRO concentration on THEO clearance. Data from 33 animals which can be described by equation [5], with a Ki estimate of 6.28 mg · l^{-1}.

ters estimated by the Siphar software for the fitted model are summarised in Table III.

The sum of CL_1 and CL_2 is equal to THEO total plasma clearance (CL_{TOT} = 0.227 and 0.190 $l \cdot h^{-1} \cdot kg^{-1}$ for the ENOX and CIPRO groups, respectively). These values compare well with previously published results from our laboratory (11). In both experiments, the clearance of THEO falls to a residual value (CL_2), when fluoroquinolone concentrations are increased, suggesting that there are elimination pathways unaffected by fluoroquinolone. Renal excretion of THEO at a dose of 6 $mg \cdot kg^{-1}$ accounts for about 20% of the dose (12; J. D. Davis, unpublished results). The predicted residual clearances (CL_2) correspond to 22% and 21% of total clearance for ENOX and CIPRO, respectively, suggesting that CL_2 may represent clearance due to the renal elimination pathway.

CONCLUSIONS

The effect of steady state ENOX and CIPRO plasma concentrations on the clearance of THEO was described using a competitive inhibition model. This model consisted of two components, one describing a residual component of THEO clearance, which was unaffected by fluoroquinolone, the other describing the non-linear reduction of THEO clearance by fluoroquinolone. The residual clearance estimated from the model is approximately equal to renal clearance for THEO in the rat.

The potency of each fluoroquinolone has been characterised by a Ki value, the concentration reducing THEO clearance by 50% of the maximum change. These values were 4.7 μM and 16.3 μM for ENOX and CIPRO, respectively. Thus ENOX is more potent in reducing THEO clearance than CIPRO. The finding that ENOX has a more potent effect on THEO confirm clinical reports of greater changes in THEO disposition during concurrent ENOX therapy when compared with CIPRO comedication. The issue of potency has only been examined by Rogge *et al.* (4), who found a dose dependent reduction in THEO clearance for ENOX in healthy human volunteers. No comparison of potency between different fluoroquinolones in terms of a concentration-effect relationship has been previously described.

Table III. Parameter Estimates (±CV%) from Equation [5] for Interaction of ENOX or CIPRO with THEO

	CL_1 $ml \cdot h^{-1}$	CL_2 $ml \cdot h^{-1}$	K_i $mg \cdot l^{-1}$	K_i μM
ENOX (n = 46)	44.4 (1.8)	12.4 (3.2)	1.62 (6.2)	4.7
CIPRO (n = 33)	37.5 (2.3)	9.9 (9.1)	6.28 (9.0)	16.3

ACKNOWLEDGMENTS

JDD is grateful to Mrs B Brennan for expert technical assistance and the Science and Engineering Research Council for financial support.

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