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Enantioselective Disposition of 2-Arylpropionic Acid Nonsteroidal Anti-Inflammatory Drugs. III. Fenoprofen Disposition[1]

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ABSTRACT

The disposition of fenoprofen enantiomers has been studied in nine healthy rabbits. A mean (S.E.M.) of 0.73 (0.07) of *R*-fenoprofen was inverted to *S*-fenoprofen and the distribution volumes for bound plus unbound *R*-fenoprofen and *S*-fenoprofen were 50.3 (4.5) and 98.5 (5.9) ml/kg, respectively. A model was developed which predicted the area under the *S*-fenoprofen plasma concentration-time curve after bolus administration of racemic fenoprofen. The mean (S.E.M.) predicted area, 2.1 (0.2) mg·min/ml/kg, was within 94% of the observed area 2.2 (0.2) mg·min/ml/kg. The effect of phenobarbital on the disposition of fenoprofen enantiomers was examined in an additional eight rabbits. During the control study the glucuronidation of *R*-fenoprofen exceeded the corresponding clearance term for the *S*-enantiomer by 2.1-fold. The clearances of individual enantiomers to their respective glucuronides increased after phenobarbital pretreatment by a mean 1.6-fold for *R*- and 2.3-fold for *S*-fenoprofen. The clearance of *S*-fenoprofen by processes other than glucuronidation and elimination of unchanged drug in urine was increased by a mean of 2.1-fold after phenobarbital pretreatment but the fractional inversion and the inversion clearance of *R*- to *S*-fenoprofen were not affected. These data indicate that on racemic fenoprofen administration the area under the curve for the pharmacologically active *S*-enantiomer would be reduced by phenobarbital pretreatment.

Fenoprofen, in common with the other 2-arylpropionic acids, exists in two enantiomeric forms. As an inhibitor of human platelet cyclooxygenases, *S*-fenoprofen is approximately 35 times as potent as the *R*-enantiomer (Rubin *et al.*, 1985); however, the drug is marketed as a racemic mixture. Other 2-arylpropionic nonsteroidal anti-inflammatory drugs have been shown to undergo enantiospecific chiral inversion from the less active *R*- to the active *S*-enantiomer (Hutt and Caldwell, 1983), but until recently there were no published data on fenoprofen inversion. Rubin *et al.* (1985) reported that after the administration of the racemic fenoprofen to humans approximately 80% of the racemic dose was recovered in urine as *S*-fenoprofen or *S*-fenoprofen metabolites, suggesting a high degree of *R*- to *S*-inversion. However, the fraction of the *R*-dose inverted could not be quantitatively determined from this study with racemic drug.

The principle routes of metabolism of racemic fenoprofen in humans are aromatic hydroxylation and acylglucuronide conjugation (Rubin *et al.*, 1972a,b), and these metabolites are also reported to be the major products of racemic fenoprofen elimination in rabbits (Culp, 1971). Thus, rabbits may be a good model to examine the enantioselectivity of fenoprofen disposition. The ability to administer separate enantiomers of fenoprofen to rabbits enables the quantitation of inversion and the enantioselectivity of other metabolic processes which is not possible after the administration of racemic drug.

Phenobarbital has been reported to reduce the area under the plasma fenoprofen concentration-time curve after the administration of racemic drug to humans (Helleberg *et al.*, 1974). Phenobarbital is well known to induce the hydroxylation of a wide variety of drugs. In addition recent reports have also focused on the ability of phenobarbital to induce the glucuronidation of a number of alcoholic and phenolic substrates; this effect being enantioselective (Yost and Finley, 1984; Okulicz-Kozaryn *et al.*, 1981). The formation of the acylglucuronides of clofibric acid (Odum and Orton, 1983) and diflunisal (Faed *et al.*, 1984) has also been reported to be induced by phenobarbital. Based on the above observations we have examined the effect of phenobarbital on fenoprofen disposition and its enantiomeric consequences.

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ABBREVIATIONS: $Cl_{R\to S}$, clearance of *R*-fenoprofen by inversion to the *S*-enantiomer; Cl_{RnonS}, clearance of the *R*-enantiomer by mechanisms other than inversion; Cl_S, clearance of *S*-fenoprofen; Cl_R, clearance of *R*-fenoprofen; $F_{R\to S}$, fraction of *R*-dose undergoing inversion of *S*-fenoprofen; V_R, steady-state distribution volume of *R*-fenoprofen; V_s, steady-state distribution volume of *S*-fenoprofen; $AUC_{S,pred}$, predicted area under the *S*-fenoprofen plasma concentration-time curve; $AUC_{S,obs}$, observed area under the *S*-fenoprofen plasma concentration-time curve; Cl_{Rren}, renal clearance of *R*-fenoprofen; Cl_{Rgluc}, clearance by glucuronidation of *R*-fenoprofen; Cl_{Roth}, clearance of *R*-fenoprofen by unknown processes; Cl_{Sren}, renal clearance of *S*-fenoprofen; Cl_{Sgluc}, clearance by glucuronidation of *S*-fenoprofen; Cl_{Soth}, clearance of *S*-fenoprofen by unknown processes.

Methods

Resolution of racemic fenoprofen. The resolution of racemic fenoprofen was accomplished by fractional recrystallization of the (+)-1-methybenzylamine (Sigma Chemicals, St. Louis, MO) salt of fenoprofen from ethylacetate. After six recrystallizations the salt contained 99.1% *S*-fenoprofen as measured by an enantiospecific assay (Sallustio *et al.*, 1986). To obtain *R*-fenoprofen the mother liquors retained after the previous six recrystallizations were combined, fenoprofen-free acid was extracted into dichloromethane and the solvent was removed under reduced pressure at 40°C. An equimolar quantity of (−)-1-methylbenzylamine was added and six recrystallizations were undertaken from ethylacetate to yield *R*-fenoprofen (98.9%). Analysis of these compounds by a reversed phase high-performance liquid chromatographic method (Sallustio *et al.*, 1986) showed only peaks corresponding to authentic fenoprofen. Proof of absolute enantiomer identity was based on which fenoprofen enantiomer preferentially crystallized from ethylacetate as the particular (+)- and (−)-1-methylbenzylamine diastereoisomeric salt as described by Marshall (1971).

Disposition Studies

Model definition. Male New Zealand White rabbits ranging in weight from 3.05 to 4.20 kg (mean 3.45 kg) were used in these studies. Nine rabbits were administered i.v. bolus doses of *S*-fenoprofen (5 mg/kg), *R*-fenoprofen (5 mg/kg) and racemic fenoprofen (5 mg/kg) as solutions (4 mg/ml) in distilled water adjusted to pH 8.0 with sodium hydroxide and hydrochloric acid. Racemic fenoprofen was used as received (Eli Lilly, New South Wales, Australia). The doses were administered in random order on consecutive days *via* an i.v. cannula and blood samples were obtained from an arterial cannula as described previously (Meffin *et al.*, 1983). Blood samples (1 ml) were collected immediately before and at approximately 0.25, 1, 3, 5, 7, 10, 15, 20, 25 and 30 min and at 15-min intervals until 120 min after the *R*-fenoprofen and racemic fenoprofen doses and before and at 2, 4, 6, 8, 10, 15, 20, 25 and 30 min and at 15-min intervals until 120 min after the *S*-fenoprofen dose. Plasma was obtained by centrifugation and was stored at −20°C until analyzed.

Effect of phenobarbital. Eight additional rabbits each received i.v. bolus doses of *S*-fenoprofen (9.18 mg/kg) and *R*-fenoprofen (9.92 mg/kg) in random order on consecutive days constituting a control study, followed by phenobarbital pretreatment for the next 3 days (50 mg/kg/day i.p.). *S*- and *R*-fenoprofen boluses were administered to each animal as described for the control study commencing a day after the final phenobarbital dose, and constituted the phenobarbital treatment study. Plasma sample collections were performed immediately before and at intervals after each fenoprofen enantiomer dose as described above for the model definition study. Twenty-four hour rabbit urine collections were made after each fenoprofen dose in both the control and phenobarbital studies using the technique described by Meffin *et al.* (1983). The volume of each urine collection, including normal saline urinary bladder flushes, was measured and five 2-ml aliquots were diluted immediately with equal volumes of cold 0.5 M glycine buffer (pH 2.5) and stored at −20°C until analyzed.

Analysis of *R*- and *S*-fenoprofen in plasma and urine. A high-pressure liquid chromatographic analysis which enables the separate measurement of either enantiomer of fenoprofen was used in these studies (Sallustio *et al.*, 1986). In outline the method involved solvent extraction of fenoprofen and an internal standard and quantitation of the sum of both enantiomers by reversed-phase chromatography. The single peak corresponding to *R*- and *S*-fenoprofen was collected and reacted with *R*-1-phenylethylamine to form the corresponding *RR*- and *RS*-amides of fenoprofen which were then separated and quantitated on normal phase chromatography in order to obtain the fraction of each enantiomer in the sample. The assay was calibrated from plasma in the range of 2.5 to 150 mg/l. For the 12 calibration curves run concurrently with samples during the present study, the mean coefficient of variation over this range was 5.7% for the reversed-phase analysis. Regression of the known enantiomer fraction against the observed peak height fraction yielded a (mean S.D.) r^2 of 0.999, 0.001 ($n = 12$).

The determination of urinary unchanged fenoprofen was a modification of the method described above for plasma fenoprofen analysis. To overcome the broad initial interference peak associated with rabbit urine samples, the retention times of the internal standard and fenoprofen during reversed-phase chromatography were increased to 5.0 and 7.25 min, respectively, by reducing the methanol concentration of the mobile phase to 40% in pH 7.0, 0.05 M phosphate buffer. Normal phase chromatographic conditions were identical to those used with plasma samples. Calibration of the reversed-phase chromatography was carried out using urine standards containing known masses of fenoprofen from 0.5 to 20.0 μg. For calibration curves run concurrently with samples during the present study, the mean coefficient of variation over this range was 7.9% for the reversed-phase analysis. Regression of the known enantiomer fraction against the observed peak height fraction yielded a (mean, S.D.) r^2 of 0.998, 0.0005 ($n = 4$).

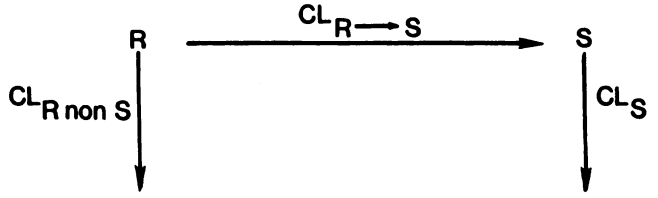

Fig. 1. A model of fenoprofen disposition in which the *R*-enantiomer is cleared by inversion to *S*-fenoprofen ($CL_{R\rightarrow S}$) and by noninversion processes (CL_{RnonS}). *S*-fenoprofen is also cleared (CL_S) but does not undergo inversion to *R*-fenoprofen.

Chromatographic conditions for the determination of hydrolyzed fenoprofen were identical to those described above for unchanged fenoprofen in urine. Hydrolysis of fenoprofen glucuronides was achieved by incubating the 0.2-ml urine sample and internal standard with 0.3 ml of 1 M NaOH for 30 min in a water-bath at 37°C. Samples were then cooled to room temperature and carried through the high-performance liquid chromatography procedure described above for plasma samples. Calibration of the reversed-phase chromatography was carried out using urine standards of known masses of fenoprofen from 1 to 20 μg. A mean coefficient of variation of 8.24% was obtained for four calibration curves run concurrently with unknown samples during the reversed-phase chromatography. Regression of the known enantiomer fraction against the observed peak height fraction yielded a (mean, S.D.) r^2 of 0.998, 0.0009 ($n = 4$). The difference between the analysis of NaOH-treated urine and that of untreated urine was taken as a measure of fenoprofen glucuronides.

Calculation of model parameters. For the purpose of the model definition study, a simplified dispositional model (fig. 1) has been proposed. Thus, the clearance of *R*-fenoprofen by inversion to the *S*-enantiomer is given by $CL_{R\rightarrow S}$. The clearance of the *R*-enantiomer by mechanisms other than inversion has been expressed as a single clearance term (CL_{RnonS}). Similarly, the clearance of the *S*-enantiomer by all pathways including arylhydroxylation, glucuronide conjugation and elimination of unchanged drug have been summed to yield a single term, CL_S.

After single i.v. doses of *R*- or *S*-fenoprofen the clearance of *R*- and *S*-fenoprofen, (CL_R, CL_S) the fraction of the *R*-dose undergoing inversion to *S*-fenoprofen ($F_{R\rightarrow S}$), the clearance of *R*-fenoprofen by inversion ($CL_{R\rightarrow S}$), the clearance of *R*-fenoprofen by processes other than inversion (CL_{RnonS}) and the respective steady-state distribution volumes of *R*- and *S*-fenoprofen (V_R, V_S) were calculated as described previously (Meffin *et al.*, 1986).

The predicted area under the *S*-fenoprofen plasma concentration-time curve after administration of an i.v. bolus of racemic fenoprofen ($AUC_{S,pred}$) was determined as:

$$AUC_{S,pred} = \frac{0.5 \text{ Racemic Dose}\left(1 + \frac{CL_{R\rightarrow S}}{CL_R}\right)}{CL_S} \quad (1)$$

For the purpose of the calculation of the model parameters in the eight rabbits receiving fenoprofen enantiomer administrations before and after phenobarbital treatment, the model shown in figure 1 was extended to encompass partial clearance terms (fig. 2). Thus, the clearance of *R*-fenoprofen (CL_R) is given by the sum of its partial clearances:

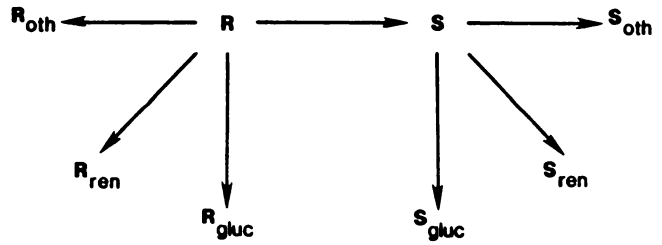

Fig. 2. A model of the disposition of fenoprofen enantiomers showing the enantiospecific *R*- to *S*-inversion and the clearance of each enantiomer renally (R_{ren}, S_{ren}), by glucuronidation (R_{gluc}, S_{gluc}), and by unknown processes (R_{oth}, S_{oth}). The relationship of these partial clearances to other clearance processes are given by equations 2 and 3 (see "Methods").

$$CL_R = CL_{R\to S} + CL_{Rren} + CL_{Rgluc} + CL_{Roth} \quad (2)$$

where $CL_{R\to S}$ is the clearance of *R*-fenoprofen by inversion, CL_{Rren} its renal clearance, CL_{Rgluc} its clearance by glucuronidation and CL_{Roth} its clearance by known processes. By analogy with the clearance of *R*-fenoprofen the clearance of the *S*-enantiomer (CL_S) is given by:

$$CL_S = CL_{Sren} + CL_{Sgluc} + CL_{Soth} \quad (3)$$

The partial clearances in equation 2 were calculated as:

$$CL_{Rren} = CL_R \cdot F_{Rren} \quad (4)$$

where F_{Rren} is the fraction of the dose of *R*-fenoprofen recovered unchanged in urine. Similarly CL_{Rgluc} is given by:

$$CL_{Rgluc} = CL_R \cdot F_{Rgluc} \quad (5)$$

where F_{Rgluc} is the fraction of the dose of *R*-fenoprofen recovered as *R*-fenoprofen glucuronide. CL_{Roth} is given by a rearrangement of equation 2.

$$CL_{Roth} = CL_R - CL_{R\to S} - CL_{Rren} - CL_{Rgluc} \quad (6)$$

The partial clearances for *S*-fenoprofen in equation 3 are analogous to those shown for the corresponding partial clearances of *R*-fenoprofen (equations 4 and 5), where F_{Sren}, F_{Sgluc} are the respective fractions recovered as unchanged *S*-fenoprofen and its glucuronide after administration of *S*-fenoprofen.
Similarly

$$CL_{Soth} = CL_S - CL_{Sren} - CL_{Sgluc} \quad (7)$$

All of the clearance and distribution volumes are calculated for total (bound + unbound) fenoprofen.

The statistical significance of mean differences between treatments or in model parameters within a single treatment was assessed using the Wilcoxon matched-pairs test (Siegel, 1956).

Results

Model definition. Log-linear plasma concentration-time plots of each fenoprofen enantiomer after *R*-fenoprofen and racemic fenoprofen administration and of *S*-fenoprofen after *S*-fenoprofen administration are illustrated for a single animal in figure 3. Mean model parameters are presented for nine animals in table 1. The duration of plasma sampling was of sufficient length to adequately define the terminal slope of each enantiomer, the total plasma sampling time (120 min) corresponded to approximately four half-lives for the *S*-enantiomer which has the longer half-life (table 1). Frequent plasma sampling was needed in the first 30 min to define the distribution phase for *S*-fenoprofen and to determine the terminal elimination phase for more rapidly eliminated *R*-fenoprofen.

Metabolic chiral inversion was unidirectional (*R* to *S*) as no *R*-fenoprofen was found in plasma samples taken from animals administered *S*-fenoprofen. On average 73% of the dose of *R*-fenoprofen was inverted to *S*-fenoprofen. Large interanimal variation in $F_{R\to S}$ was found (range from 0.304–1.00) (table 1).

Model evaluation. The nine rabbits received an i.v. bolus of racemic fenoprofen in order to test the predictive ability of the model to estimate the area under the curve of the pharmacologically active *S*-enantiomer (AUC_S). These data are presented in table 2.

The mean predicted AUC_S upon racemic fenoprofen administration is 94.2% of the observed area. The mean V_R after racemic fenoprofen administration was 112% of the mean V_R attained after *R*-fenoprofen dosage. The mean CL_R after racemic fenoprofen was 140% of the mean CL_R after *R*-fenoprofen administration. Neither of these mean differences reached statistical significance (table 2).

Enantioselective disposition. The steady-state distribution volumes for each enantiomer, after administration of individual enantiomers were different. The mean V_S being 196% of the mean V_R (table 1). Plasma clearances were different, the mean CL_R being 318% of the mean CL_S implying marked stereoselectivity in the bound + unbound plasma clearances of fenoprofen enantiomers. The model in figure 1 indicates that the clearance of the *R*-enantiomer is the sum of its clearance to the *S*-enantiomer ($CL_{R\to S}$) and its clearance by other processes (CL_{RnonS}). Thus, CL_{RnonS} is equivalent to CL_S for the *S*-enantiomer. These two clearance terms were not significantly different (table 1).

Log-linear plasma concentration-time plots of each fenoprofen enantiomer after administration of *R*-fenoprofen and *S*-fenoprofen during both the control and the phenobarbital studies are illustrated for a single animal (fig. 4). Model parameters are presented for animals before and after phenobarbital pretreatment in table 3. Inasmuch as complete urine collections were obtained for six of the eight animals, partial clearance terms are expressed for six of these animals. All other dispositional parameters are expressed for eight animals.

In the control study the mean plasma clearance of *R*-fenoprofen to its acyl glucuronide exceeded the corresponding clearance of *S*-fenoprofen by 2.12-fold (table 3). The mean CL_{Roth} exceeded CL_{Soth} by 2.83-fold in the control study but this was not statistically significant (table 3). The renal clearances of fenoprofen enantiomers were not different.

Effect of phenobarbital. The mean plasma clearances of both *R*- and *S*-fenoprofen to their respective glucuronides increased after phenobarbital pretreatment by 1.61- and 2.29-fold, respectively (table 3). The mean CL_{Soth} increased by 2.11-fold after phenobarbital pretreatment but the 1.25-fold increase in the mean CL_{Roth} after phenobarbital pretreatment did not reach statistical significance (table 3). The renal clearances of *R*- and *S*-fenoprofen (CL_{Rren} and CL_{Sren}) were not affected by phenobarbital pretreatment. The clearance of *R-fenoprofen by metabolic chiral inversion to S-fenoprofen* ($CL_{R\to S}$) was unaffected by phenobarbital pretreatment.

There were no alterations to steady-state distribution volumes of either enantiomer after phenobarbital pretreatment (table 3), suggesting that the plasma-protein binding of fenoprofen enantiomers was unaffected by phenobarbital.

Discussion

Limitations of bound plus unbound fenoprofen concentration data. Jones *et al.* (1986) report stereoselective binding

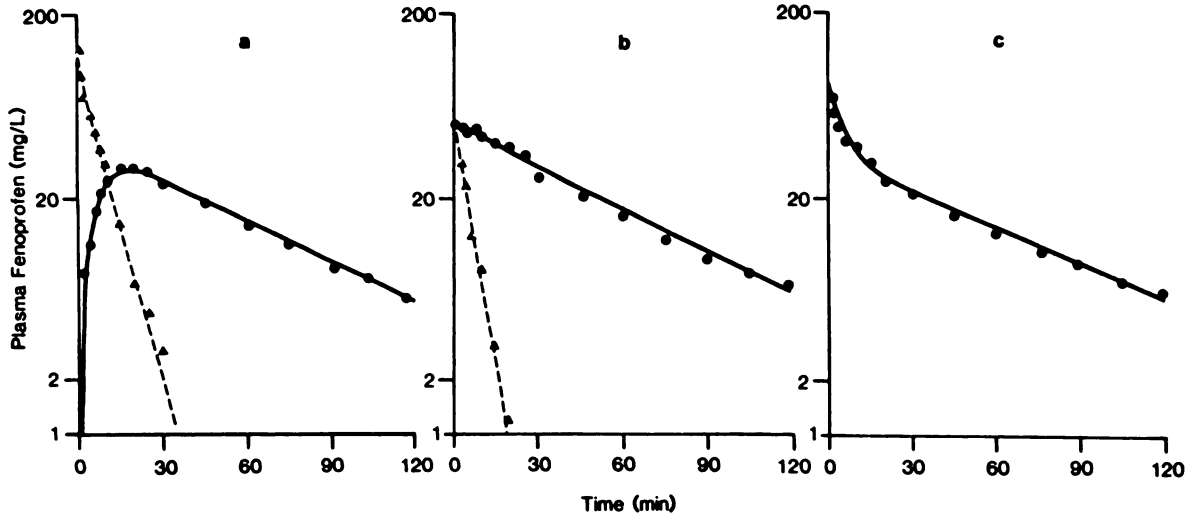

Fig. 3. Log-linear plasma concentration-time plots of fenoprofen enantiomers after administration of 5.0 mg/kg of *R*-fenoprofen (a) and racemic fenoprofen (b). c, log-linear plasma concentration *vs.* time plot for *S*-fenoprofen after *S*-fenoprofen administration. ▲, *R*-fenoprofen; ●, *S*-fenoprofen.

TABLE 1

Mean (S.E.M.) parameters of fenoprofen disposition in nine healthy rabbits administered 5 mg/kg of *R*, *S* and racemic fenoprofen i.v.

Parameter	Mean (s.e.m.)
CL_S (ml/min/kg)	2.27*,‡ (0.26)
CL_R (ml/min/kg)	7.21‡ (0.50)
$CL_{R\rightarrow S}$ (ml/min/kg)	5.46 (0.76)
CL_{RnonS} (ml/min/kg)	1.75* (0.43)
$F_{R\rightarrow S}$	0.731 (0.074)
V_S (ml/kg)	98.5** (5.9)
V_R (ml/kg)	50.3** (4.5)
Half life of *S*-fenoprofen (min)	37.7† (3.5)
Half life of *R*-fenoprofen (min)	6.14† (1.17)

* CL_S *vs.* CL_{RnonS}, $P > .05$; ** V_S *vs.* V_R, $P < .01$; † half-life of *S*-fenoprofen *vs.* half-life of *R*-fenoprofen, $P < .01$; ‡ CL_S *vs.* CL_R, $P < .01$.

TABLE 2

Mean (S.E.M.) predicted and observed areas under the curve of *S*-fenoprofen after racemic administration, and model parameters after *R*-fenoprofen and racemic fenoprofen doses in nine healthy rabbits

Parameter	Mean (s.e.m.)
$AUC_{S,predicted}$ (mg·min/ml/kg)	2.10* (0.21)
$AUC_{S,observed}$ (mg·min/ml/kg)	2.23* (0.22)
$CL_R{}^a$ (ml/min/kg)	7.21† (0.50)
$CL_R{}^b$ (ml/min/kg)	10.1† (1.7)
$V_R{}^a$ (ml/kg)	50.3‡ (4.5)
$V_R{}^b$ (ml/kg)	56.5‡ (5.4)

[a] Model parameter obtained after R-fenoprofen administration.
[b] Model parameter obtained after racemic fenoprofen administration.
* Predicted *vs.* observed AUC_S, $P > .05$; † $CL_R{}^a$ *vs.* $CL_R{}^b$, $P > .05$; ‡ $V_R{}^a$ *vs.* $V_R{}^b$, $P > .05$.

of 2-phenylpropionic acid to rabbit plasma and ketoprofen, a related 2-arylpropionate exhibits stereoselective binding to human serum (Rendic *et al.*, 1980). Fenoprofen has been reported not to exhibit stereoselective binding to human serum albumin as assessed by induced circular dichroic measurements at molar fenoprofen to albumin ratios of 14 to 1 (Perrin, 1973). However, the relevance of these measurements to the fenoprofen concentrations used in this study in which fenoprofen albumin ratios were approximately 1 or less and to rabbit plasma is uncertain. On balance it is likely that binding of fenoprofen enantiomers to rabbit plasma is stereoselective. If such stereoselective binding occurs, then estimates of clearance and distribution processes of the individual enantiomers and the influences of concomitant drug administration upon them will be biased as we have shown previously for 2-phenylpropionic acid (Meffin *et al.*, 1986; Jones *et al.*, 1986). For these reasons it would have been desirable to express model parameters in terms of unbound drug; however, the assay methodology available for this study is not sufficiently sensitive to measure unbound fenoprofen enantiomer concentrations. The free fraction of fenoprofen in rabbit plasma is likely to be low given that the free fraction in human plasma is less than 0.01 (Brogden *et al.*, 1977).

Extent of metabolic chiral inversion. No other study has quantitated fenoprofen chiral inversion. Rubin *et al.* (1985) administered racemic fenoprofen p.o. to humans and determined the stereochemistry of fenoprofen metabolites in urine. Urinary metabolite enrichment with respect to the *S*-enantiomer could be due to inversion or the selective loss of *R*-fenoprofen as a result of enantioselective metabolism to unknown products. Thus, unless the entire dose can be accounted for the extent of chiral inversion cannot be determined from excretion data after racemic drug administration and can only be determined after administration of separate enantiomers. The 73% inversion of *R*-fenoprofen is high with respect to its close structural analog ketoprofen which is 9% inverted in rabbit (Abas and Meffin, 1987). The high coefficient of variation of $F_{R\rightarrow S}$ (30%) is a result of the composite nature of this parameter. $F_{R\rightarrow S}$ is a function of the chiral inversion clearance of *R*-fenoprofen and the individual clearance processes involved in the total plasma clearance of the *R*-enantiomer (equation 2)

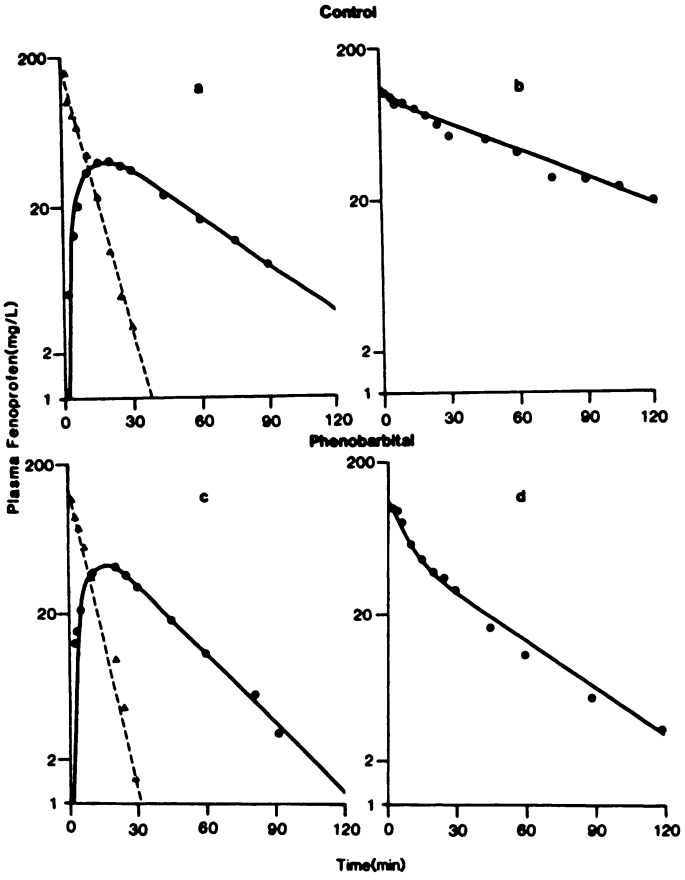

Fig. 4. Log-linear plasma concentration-time plots of fenoprofen enantiomers after administration of 9.92 mg/kg of *R*-fenoprofen in the control phase (a) and of *S*-fenoprofen after administration of 9.18 mg/kg in the control phase (b). c and d, log-linear plasma concentration time plots of fenoprofen enantiomers after phenobarbitone pretreatment for *R*-fenoprofen (9.92 mg/kg) and *S*-fenoprofen (9.18 mg/kg), respectively. ▲, *R*-fenoprofen; ●, *S*-fenoprofen.

and as such does not measure the efficiency of the inversion process (Abas and Meffin, 1987).

Model evaluation. The proposed model (fig. 1) was based on the assumption that the clearance processes were linear for bound plus unbound drug. AUC_S upon racemic fenoprofen administration is a function of all three model clearances processes (equation 1). The close agreement found between $AUC_{S,pred}$ and $AUC_{S,obs}$ after racemic fenoprofen administration (table 2) implies that the three model clearance processes acting together at the 2.5- and 5.0-mg/kg doses are able to predict the concentrations of the active enantiomer in plasma.

Stereoselective fenoprofen disposition. The investigation of stereoselective fenoprofen disposition, apart from chiral inversion in the model definition study, was largely preliminary as the clearance terms CL_S and CL_{RnonS} were measured as composite terms. Based on the studies of racemic fenoprofen metabolism in the rabbit (Culp, 1971), CL_S and CL_{RnonS} were assumed to chiefly compose 4-arylhydroxylation and acylglucuronidation. Although no difference was found between these clearances (table 3), it was possible that stereoselectivity occurred in individual clearance processes (glucuronidation and hydroxylation) in a manner whereby they masked each other when summed together. This was examined in greater detail in the subsequent study in which partial clearances were determined.

TABLE 3

Mean (S.E.M.) parameters of fenoprofen disposition in rabbits before (control) and after phenobarbital pretreatment (phenobarbital)

Parameter	Control	Phenobarbital	P Value[a]
CL_S (ml/min/kg)	1.53 (0.12)	3.13 (0.31)	<.016
CL_R (ml/min/kg)	6.94 (0.70)	8.64 (0.75)	>.05
$CL_{R\rightarrow S}$ (ml/min/kg)	3.88 (0.68)	4.59 (0.62)	>.05
CL_{RnonS} (ml/min/kg)	3.06 (0.81)	4.36 (0.85)	>.05
V_S (ml/kg)	103 (13)	89.1 (9.4)	>.05
V_R (ml/kg)	82.7 (9.9)	69.0 (6.2)	>.05
CL_{Sgluc} (ml/min/kg)	0.375[b] (0.053)	0.860 (0.104)	<.016
CL_{Rgluc} (ml/min/kg)	0.794[b] (0.072)	1.28 (0.097)	<.016
CL_{Soth} (ml/min/kg)	0.974* (0.070)	2.06 (0.26)	<.016
CL_{Roth} (ml/min/kg)	2.76* (0.81)	3.45 (0.97)	>.05
CL_{Sren} (ml/min/kg)	0.164† (0.024)	0.157 (0.017)	>.05
CL_{Rren} (ml/min/kg)	0.162† (0.058)	0.181 (0.019)	>.05
$F_{R\rightarrow S}$	0.575 (0.107)	0.548 (0.065)	>.05

[a] Control *vs.* phenobarbital treatment.
[b] CL_{Sgluc} (control) *vs.* CL_{Rgluc} (control), $P < .016$.
* CL_{Soth} (control) *vs.* CL_{Roth} (control), $P > .05$; † CL_{Sren} (control) *vs.* CL_{Rren} (control), $P > .05$; Determined from plasma and urine measurements in six of eight animals.

Stereoselctive glucuronidation. The clearance terms CL_{Rgluc} and CL_{Sgluc} of the model (fig. 2) are estimates of the net ability of the animals to conjugate *R*- and *S*-fenoprofen with glucuronic acid. The greater than 2-fold mean value for the *R*-enantiomer over the *S*-enantiomer is dependent on glucuronide conjugation, hydrolytic clearance and the renal clearance of glucuronide (Meffin *et al.*, 1983). Thus, any or all of these processes which contribute to the acyl glucuronide futile cycle (Meffin *et al.*, 1983; Rowe and Meffin, 1984) are potential sources for the observed stereoselective glucuronidation of fenoprofen in rabbits. A recent study (Lee *et al.*, 1985) showed the *in vivo* glucuronidation of *S*-ibuprofen to exceed the clearance of its antipode to the acylglucuronide by approximately 9-fold. Our data show clearly that whether stereoselectivity resides in glucuronyltransferase or esterase enzymes, renal elimination of glucuronides or plasma binding of the enantiomers, the net balance of all these processes is more strongly in favor of glucuronidation of *R*-fenoprofen.

Effect of phenobarbital. Based on a portal blood flow in rabbits of 33 ml/min/kg reported by White *et al.* (1967) and assuming a hematocrit of 0.5, equal partition of fenoprofen between blood and plasma, and exclusive hepatic clearance for CL_{Rgluc}, CL_{Sgluc}, CL_{Roth} and CL_{Soth}; the mean hepatic extraction ratios for these clearance terms are all less than 0.1. Thus, any observed differences to total (bound plus unbound) plasma clearances after phenobarbital pretreatment can be interpreted primarily in terms of potential displacement of fenoprofen enantiomers from plasma binding sites by phenobarbital or changes in intrinsic clearance rather than being due to increases in hepatic blood flow (Rowland *et al.*, 1973). Because there was no significant difference in V_R and V_S between treatments it was unlikely that such binding changes occurred. To effect

plasma binding changes while maintaining constant distribution volumes, tissue binding would have to change in a similar direction. Based on the reports of phenobarbital-induced acylglucuronide formation of clofibric acid (Odum and Orton, 1983) and diflunisal (Faed *et al.*, 1984), it seemed likely that the increased clearances of *R*- and *S*-fenoprofen to their respective acylglucuronides (table 3) was due to direct glucuronyltransferase induction by phenobarbital.

The clearance of *S*-fenoprofen, by processes other than glucuronidation and elimination of unchanged drug in urine (CL_{Soth}), was induced by phenobarbital (table 3). Inasmuch as this process is thought to chiefly compose aromatic hydroxylation in rabbits and humans (Culp *et al.*, 1971; Rubin *et al.*, 1972b), it appears likely that phenobarbital has induced the oxidative metabolism of *S*-fenoprofen. This finding is consistent with the report that in both rats and humans, phenobarbital decreases the area under the plasma fenoprofen concentration-time curve after racemic fenoprofen administration (Helleberg *et al.*, 1974). That such an effect was shown with CL_{Soth} but not CL_{Roth} (table 3) is of relevance to the recent report that after the administration of racemic fenoprofen to humans, the 4-hydroxy metabolite was almost exclusively of the *S*-configuration (Rubin *et al.*, 1985). Taken together these findings support the concept that 4-hydroxylation in rabbit may have similar enantioselectivity to that observed in humans and that this metabolic pathway may be similarly inducible by phenobarbital in both species.

Racemic fenoprofen has been reported to be able to substitute for endogenous fatty acids in triglyceride formation in rats (Fears *et al.*, 1978). In a preliminary communication we reported recently that in rat hepatocytes this process was enantiospecific for *R*-fenoprofen (Meffin and Sallustio, 1986). Triglyceride formation and chiral inversion are both thought to proceed *via* an initial stereospecific activation of the *R*-enantiomer (Hutt and Caldwell, 1983; Caldwell and Marsh, 1983). As it is considered unlikely that phenobarbital would have any effect on the activity of the acylcoenzyme A synthetase(s), the lack of an increase in CL_{Roth} and $CL_{R\rightarrow S}$ in response to phenobarbital (table 3) is consistent with the hypothesis that CL_{Roth} may have a substantial contribution from triglyceride incorporation.

Implications for fenoprofen use in humans. Using the mean values of CL_S and $F_{R\rightarrow S}$ (table 3) before and after phenobarbital pretreatment we predict that upon administration of racemic fenoprofen to phenobarbital-pretreated animals the AUC_S for bound plus unbound drug will be reduced by approximately 50% (equation 1). As it appeared unlikely, based on the argument expressed above, that phenobarbital altered the plasma binding of *S*-fenoprofen, we would predict the area under the curve for the true pharmacologically active species (unbound *S*-fenoprofen) would be halved after enzyme induction. It appears likely on the basis of available data that in humans, rats and rabbits, fenoprofen metabolic pathways are quantitatively similar (Rubin *et al.*, 1972a,b; Culp, 1971) show similar stereoselectivity and degree of inversion (Rubin *et al.*, 1985) and are similarly induced by phenobarbital treatment (Helleberg *et al.*, 1974). To the extent that the above approximations are true, they suggest that the reduction in the area under the fenoprofen plasma concentration-time curve observed in humans by Helleberg *et al.* (1974) after phenobarbital treatment can be ascribed primarily to an effect on the *S*-enantiomer which is the pharmacologically active species.

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