Prinomide Tromethamine Pharmacokinetics: Mutually Dependent Saturable and Competitive Protein Binding Between Prinomide and Its Own Metabolite

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Received April 7, 1992; accepted July 20, 1992

Prinomide tromethamine, a nonsteroidal antiinflammatory drug, was orally administered at doses of 250, 500, and 1000 mg every 12 hr for 28 days to healthy male volunteers. The pharmacokinetic behavior of prinomide and its primary plasma metabolite displayed nonlinear characteristics, while those of free prinomide and its metabolite were dose proportional. The nonlinear pharmacokinetic behavior of total prinomide and *p*-hydroxy metabolite was found to be caused by both saturable and mutually dependent competitive Langmuir-type plasma protein binding between prinomide and its *p*-hydroxy metabolite. The extent of the protein interaction displayed at steady state was due to the extensive accumulation of the *p*-hydroxy metabolite. While ligand–protein interactions are known for xenobiotic competitors, the characteristic behavior of prinomide is the first known example to be reported for a competitive protein interaction between a xenobiotic and its own *in vivo* generated metabolite. The findings of this study may have implications regarding the disposition of other extensively bound nonsteroidal antiinflammatory drugs with long-lived metabolites.

KEY WORDS: prinomide tromethamine pharmacokinetics; Langmuir-type protein binding; competitive cobinding; *in vivo* metabolite–protein interaction.

INTRODUCTION

Prinomide tromethamine is the tris(hydroxymethyl)aminomethane salt of a novel pyrrolepropionitrile compound (Fig. 1) with antiarthritic activity. In experimental models and limited clinical trials, prinomide has exhibited properties of nonsteroidal antiinflammatory drugs (1,2).

Prinomide is extensively metabolized by P-450 oxidation in laboratory animals (3) and man (4). In man, the drug is metabolized to at least six oxidative adducts, in which three are known to undergo further conjugative metabolism. Little or no unchanged prinomide is eliminated in the urine. In plasma, however, unchanged prinomide and its phenyl-ring *p*-hydroxy metabolite account for approximately 75% of the total radioactivity after single-dose administration of labeled prinomide. Both prinomide and its *p*-hydroxy metabolite exhibit >97% plasma protein binding.

Initial pharmacokinetic studies assessing the single-dose proportionality were unremarkable and demonstrated a linear relationship between dose and AUC. After chronic administration, prinomide's pharmacokinetic disposition was unexpectedly peculiar, showing no accumulation despite an apparent 16-hr single-dose half-life (4). Although prinomide did not accumulate, its *p*-hydroxy metabolite showed extensive accumulation. Since both prinomide and its *p*-hydroxy metabolite are extensively plasma protein bound, it was anticipated that a protein binding interaction may have contributed to the unusual characteristics displayed by prinomide after chronic administration. Saturable plasma protein binding of prinomide, however, cannot by itself explain the differences observed between single-dose and multiple-dose pharmacokinetics since plasma concentrations resulting from both were virtually identical. Therefore, potential differences in binding fractions could not be attributed to differences in concentration of prinomide.

The objective of this study was to examine the dependence of total and free prinomide disposition on dose, metabolic product formation, and protein interaction at steady state. In addition, the *in vitro* competitive binding of prinomide and its *p*-hydroxy metabolite was assessed.

METHODOLOGY

Clinical Study Design and Procedures

This was a single-center, open-label, steady state, randomized crossover trial of prinomide tromethamine at three dose levels. Doses of 250 mg (one 250-mg formulated tablet), 500 mg (one 500-mg formulated tablet), and 1000 mg (two 500-mg formulated tablets) where taken orally twice a day for 28 consecutive days each. Serial plasma and urine samples were collected following the final dose of each dose-level phase. At the conclusion of each phase, subjects received the first dose of the next assigned dose regimen and continued until the study was completed. No intervening washout period was prescribed.

Subjects were permitted to follow their usual routine regarding activity and eating. Each received a diary in which they recorded the time of their meals, the time they took the drug, and any other important information. Subjects were instructed not to take any other drug, either prescribed or over-the-counter. On each day preceding the final dose of each phase, subjects fasted overnight and remained fasted until 4 hr after receiving the dose. At this time they received a standardized lunch.

The subjects took the drug orally twice daily, at 8 AM and 8 PM. On the day of the final dose of each phase subjects were administered the drug with 200 ml of water.

Subjects

Twelve male subjects participated in this study. One subject was black and the others were Caucasian. Their average age was 45 years (range, 31–54 years) and average weight was 84 kg (range, 63–97 kg). All subjects were in good health on the basis of their medical history, physical examination, clinical laboratory tests, ECG, and chest X-ray.

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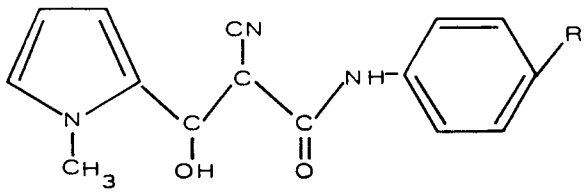

Prinomide Tromethamine R = H , $H_2NC(CH_2OH)_3$

Metabolite R = OH

Fig. 1. Prinomide tromethamine and its primary plasma metabolite, *p*-hydroxyphenylprinomide.

Plasma Sample Collection and Analysis

A control blood and urine sample was collected prior to the active phases of this study. During the active phases, blood samples (10 ml) for drug and metabolite estimation were drawn by venipuncture. Single blood samples were obtained prior to the morning dose on the 2 days preceding the final dose of each phase for the determination of steady state. On the day of the final dose of each phase, samples were collected at the following times after administration of the morning dose: 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hr. Additional samples were obtained following completion of the final phase at 24, 36, 48, 72, 96, 120, 144, 192, 288, and 360 hr after drug administration.

Each blood sample was collected into an evacuated blood collection tube containing 143 USP units of heparin. The heparinized blood was centrifuged and the separated plasma was transferred to a clean polypropylene tube and frozen.

Urine samples for drug and metabolite estimation were collected during the 12-hr study interval on days of the final dose of each phase. The volume was measured, and a 20-ml aliquot retained and frozen.

Plasma and urine samples were analyzed for total prinomide and its *p*-hydroxy metabolite utilizing a previously reported HPLC method (5,6). The minimum level of quantitation was 0.2 μg/ml in plasma and 0.5 μg/ml in urine for both analytes. Prinomide concentrations are reported in terms of the tromethamine salt concentration, whereas the concentration of the *p*-hydroxy metabolite is reported in terms of the free acid concentration. The molecular weight ratio of prinomide free acid to salt is 0.69.

Plasma Protein Binding

The free and bound concentrations of prinomide and its *p*-hydroxy metabolite were estimated in each plasma sample from the free and bound fractions and the total concentration. The following analytical procedure was used.

[^{14}C]Prinomide tromethamine (CGS 10787D, Ciba-Geigy, Ardsley, NY), with a specific activity of 8.78 μCi/mg and chemical purity of >98%, was dissolved in methanol to a final concentration of 10 μg/ml. The metabolite, *p*-[^{14}C]hydroxyphenylprinomide (CGS 12094, Ciba-Geigy), with a specific activity of 9.95 μCi/mg and chemical purity of >98%, was dissolved in methanol to a final concentration of 40 μg/ml. Dialyzing buffer was prepared by dissolving 9 g of sodium chloride in 197 ml of 0.067 *M* aqueous monobasic potassium phosphate in a 1-liter volumetric flask and brought to volume with 0.067 *M* aqueous dibasic sodium phosphate. The pH was adjusted to 7.4 with the addition of a few drops of 10 *N* sodium hydroxide. One hundred fifty-microliter aliquots of [^{14}C]prinomide or 50-μl aliquots of *p*-[^{14}C]hydroxyphenylprinomide stock solution were pipetted into 40-ml centrifuge tubes. Solvent was evaporated with a stream of nitrogen, and 1.5 and 0.5 ml of plasma containing unlabeled prinomide and metabolite (subject plasma samples) were added to the prinomide and metabolite standard, respectively. This provided spiked plasma concentrations of 1.0 μg/ml for prinomide and 4.0 μg/ml for its metabolite.

Protein binding determinations were carried out by equilibrium dialysis in a Dianorm apparatus (Spectrum Medical Industries, Inc., Los Angeles, CA), comprised of 20 pairs of chambers. For prinomide, chambers with a 1.0-ml capacity were used. For the *p*-hydroxy metabolite, chambers with a 0.25-ml capacity were used. A Spectrapor semipermeable membrane, with a molecular weight cutoff of 12,000–14,000 (Spectrum Medical Industries, Inc.) was pretreated by soaking it sequentially in distilled water and 30% isopropanol for 15 and 30 min, respectively. The membrane was thoroughly washed with water, soaked in dialysis buffer for 30 min, and superficially dried by blotting with tissue paper. The membrane was mounted between a pair of chambers, and the assembled unit was positioned with others in a rack. Plasma samples were dialyzed for 1.5 hr against buffer solution with constant rotation at 15 rpm and at 37°C. After dialysis, samples from each side of the dialysis cell were removed, and aliquots were dissolved in Scintisol scintillation fluid (Isolab, Akron, OH). Samples were counted for radioactivity in a Spectral, Liquid Scintillation Counter (LKB Instruments, Inc., Gaithersburg, MD). Counting efficiencies were determined by the external standard method.

The protein bound fraction, at equilibrium, was calculated as follows:

$$\text{fraction bound} = [\text{dpm (plasma)} - \text{dpm (buffer)}]/\text{dpm (plasma)}$$

Volume shifting was not observed in these analysis. Binding of prinomide or its metabolite to the cell membrane or the cell body did not occur to any appreciable extent since the recoveries of radioactivity from the two sides of the dialysis cells were >96% of the spiked radioactivity for all analysis.

Pharmacokinetic Analysis

Total and free steady-state plasma concentration–time curves for prinomide and its *p*-hydroxy metabolite were characterized at each dose level by the maximum concentration (C_{max}), the time to the maximum concentration (t_{max}), and the steady-state dosage interval area under the plasma concentration versus time curve [AUC(0–τ)]. AUC(0–τ) was estimated using the linear trapezoidal method. The oral clearance (CL_o) of prinomide was estimated by CL_o = dose/AUC(0–τ). The renal clearance of the

p-hydroxy metabolite was estimated by CL_r = 12-hr urinary recovery at steady state/AUC(0–τ), metabolite.

Statistical Analysis

Statistical analysis followed traditional methods for investigating dose proportionality. The pharmacokinetic variables total and free AUC(0–τ) and C_{max} were normalized to the 250-mg dose. The resulting dose-normalized values were then tested for equivalence by analysis of variance (ANOVA). Factors evaluated by the ANOVA were sequence, subject within sequence, period, direct effect of dose, and carryover. The equivalence of steady-state concentration minima prior to the final dose for each phase was evaluated in a similar fashion. T_{max} values were compared nonparametrically by the Wilcoxon signed rank test. A P value of 0.05 or less was considered to be statistically significant.

In Vitro Binding Studies

Prinomide and the p-hydroxy metabolite were added to aliquots of freshly obtained human plasma. A single pool of plasma with an assayed plasma total protein concentration of 6.9 g/dl and an albumin concentration of 4.4 g/dl (733 μM) was utilized in these experiments. The aliquots were prepared to give final concentrations of total prinomide and p-hydroxy metabolite as follows: 120, 240, 360, 480, 600, and 720 μM prinomide and 88, 177, 353, 530, 706, and 883 μM metabolite. These concentrations correspond to approximately 50 to 300 μg/ml prinomide tromethamine and 25 to 250 μg/ml p-hydroxy metabolite acid, where the molecular weights of prinomide tromethamine and the metabolite acid are 388.4 and 283.3, respectively. Sets of samples also contained 120–720 μM prinomide plus 88, 177, 353, and 706 μM metabolite and, 88–883 μM metabolite plus 120, 240, and 480 μM prinomide. Once prepared, all samples were dialyzed and assayed for free and bound fractions as above. Dialysis of samples containing either prinomide or the p-hydroxy metabolite alone, and those for each combination were replicated in triplicate.

Binding of prinomide and its p-hydroxy metabolite to isolated plasma constituents was evaluated by dissolving prinomide or the p-hydroxy metabolite in dialysis buffer containing either 4.0% (w/v) human serum albumin (Fraction V, 96–99%, Sigma Chemical Co., St. Louis, MO), 0.1% (w/v) α_1-acid glycoprotein (Sigma Chemical Co.), or 1.0% (w/v) γ-globulin (Sigma Chemical Co.). The concentration of prinomide was 600 μM, and the concentration of the p-hydroxy metabolite was 353 μM. The solution was dialyzed at 37°C and analyzed for free and bound fractions as above. In addition, binding to erythrocytes was evaluated by centrifuging whole blood at 1500g for 10 min and harvesting the erythrocytes. Erythrocytes were then suspended in a volume of dialysis buffer equal to the plasma volume and incubated for 1 hr at 37°C. After centrifugation, an aliquot of buffer was assayed for radioactivity. The erythrocyte concentration of prinomide or p-hydroxy metabolite was estimated by $C_e = [C_t - C_{bu} \cdot (1 - HCT)]/HCT$, where C_e and C_{bu} are the concentrations in the erythrocytes and buffer, C_t is the total concentration, and HCT is the hematocrit. The erythrocyte fraction bound was estimated as $(C_e/C_t) \cdot HCT$.

RESULTS

Pharmacokinetics

The total free plasma concentration–time curves for each dose are illustrated in Figs. 2 to 4. Plasma concentrations at −48, −24, and 0 hr were not statistically differentiable at any of the specified dose levels, indicating that steady state was achieved in the 28-day period allotted in this study. Of qualitative interest, a notable, apparently dose-related metabolite concentration minima was observed at 1 hr following the oral dose. This minima corresponded to the time at which maximum plasma concentrations of prinomide were observed. This phenomenon was not observed in the free concentration–time curves of the p-hydroxy metabolite, although the absence of the minima may have been obscured by the variability in free concentrations at the 1000-mg dose level. Terminal elimination phases, also, did not exhibit the usual dose-related parallel decline in total concentrations usually associated with linear systems. Terminal phase concentrations of free prinomide and its p-hydroxy metabolite were not determined in this study due to the quantitative limitations of the dialysis method with regard to accuracy of free concentrations at the observed total concentrations. The observed relationship between maximal prinomide concentrations and minimal metabolite concentrations, and the nonlinearity of the system, suggests a potential protein binding interaction.

The qualitative observations concerning nonlinearity and a protein binding interaction were confirmed by quantitative pharmacokinetic measures (Table I). Dose-related AUC values based on total prinomide and metabolite were not proportional and exhibited a declining relative AUC with increasing dose. The dose-normalized AUC values for both prinomide and its p-hydroxy metabolite were statistically different such that $P < 0.001$ and $P = 0.005$, respectively. Similar results were observed for C_{max}. The dose-normalized C_{max} values were statistically different such that $P < 0.001$ and $P = 0.009$, respectively. No dose-related

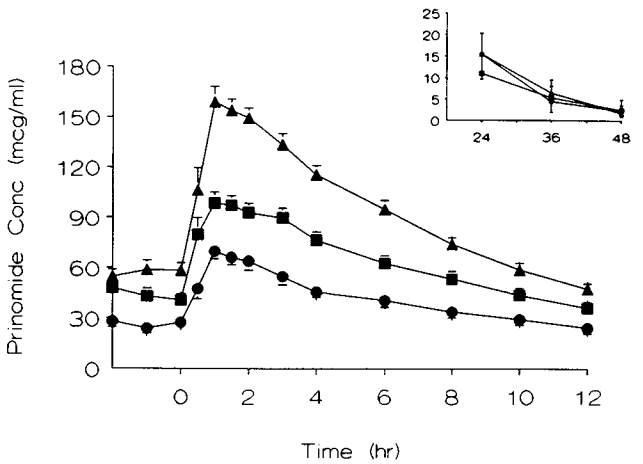

Fig. 2. Mean (SE; n = 12) steady-state total prinomide tromethamine plasma concentrations at −48 and −24 hr and from 0 to τ following administration of 250 (●), 500 (■), and 1000 (▲) mg prinomide tromethamine every 12 hr. Inset: Terminal elimination following final dose; n = 4.

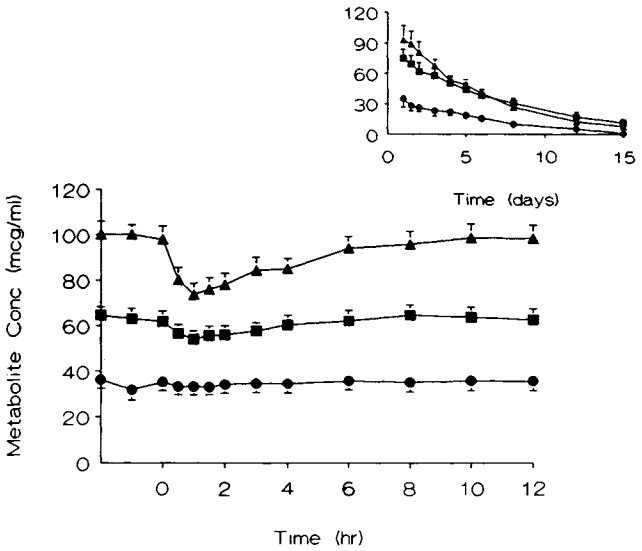

Fig. 3. Mean (SE; n = 12) steady-state total p-hydroxyphenylprinomide plasma concentrations at −48 and −24 hr and from 0 to τ following administration of 250 (●), 500 (■), and 1000 (▲) mg prinomide tromethamine every 12 hr. Inset: Terminal elimination following final dose; n = 4.

difference in t_{max} was found. AUC values based on free prinomide and p-hydroxy metabolite were found to be dose proportional. The dose-normalized AUC values for neither prinomide nor its p-hydroxy metabolite were statistically different. Again, similar results were observed for C_{max}, whereby the dose-normalized C_{max} values for neither prinomide nor its p-hydroxy metabolite were statistically different. These findings confirm a protein interaction at steady state.

Among the other pharmacokinetic parameters evaluated, it was found that the average free fraction (free AUC/total AUC) increased with increasing dose for both prinomide and its p-hydroxy metabolite. These values are reported for prinomide based on 11 subjects at the 250- and 1000-mg dose levels. One subject had a value of 4.5% at the 250-mg dose level, which was about 10-fold greater than the mean, and another subject had a value of 6.7% at the 1000-mg dose level, which was about 6-fold greater than the mean. Both of these values were considered to be unrepresentative extremes. No extreme values were noted for the p-hydroxy metabolite.

As expected, the apparent oral clearance of prinomide was dose related. The free oral clearance, however, was not dose related and resulted in an average value of approximately 1800 ml/min. Only unquantifiable trace levels of prinomide were detected in urine, whereas quantities of up to 170 mg at the 1000-mg dose level of the p-hydroxy metabolite were found in urine in a 12-hr interval collection at steady state. The average free renal clearance of the p-hydroxy metabolite was approximately 275 ml/min.

In order to evaluate further the nature of the protein interaction between prinomide and its p-hydroxy metabolite, the relationship between the free fraction of prinomide (f_d) and its dose-related maximal concentration was assessed. Also, the dependency of f_d on the free fraction of the p-hydroxy metabolite (f_m) at prinomide's maximal concentration was evaluated (Fig. 5). Similarly, relationships were evaluated with regard to the p-hydroxy metabolite at the metabolite's maximal concentration. Both f_d and f_m were found to be related to their respective maximal concentrations. In addition, however, both f_d and f_m were found to be correlated and therefore related at corresponding maximal concentrations of prinomide and its p-hydroxy metabolite. These findings suggest that prinomide and its metabolite exhibit not only saturable protein binding, but also mutually dependent competitive binding.

In Vitro Plasma Protein Binding

Both prinomide and the p-hydroxy metabolite were found to conform to Langmuir-type plasma protein binding (Fig. 6). Protein binding to isolated plasma constituents at physiological relevant concentrations and at prinomide and p-hydroxy metabolite concentrations representative of those observed at steady state in the *in vivo* study showed only limited binding, <7%, to α_1-acid glycoprotein and γ-globulin (Table II). In contrast, the protein binding to human serum albumin was >98%. The low order of affinity of prinomide and its p-hydroxy metabolite for α_1-acid glycoprotein and γ-globulin, and high order of affinity for albumin, suggests that a Langmuir-type isotherm with a single class of binding sites may be sufficient to describe analytically the plasma protein binding of prinomide and its p-hydroxy metabolite.

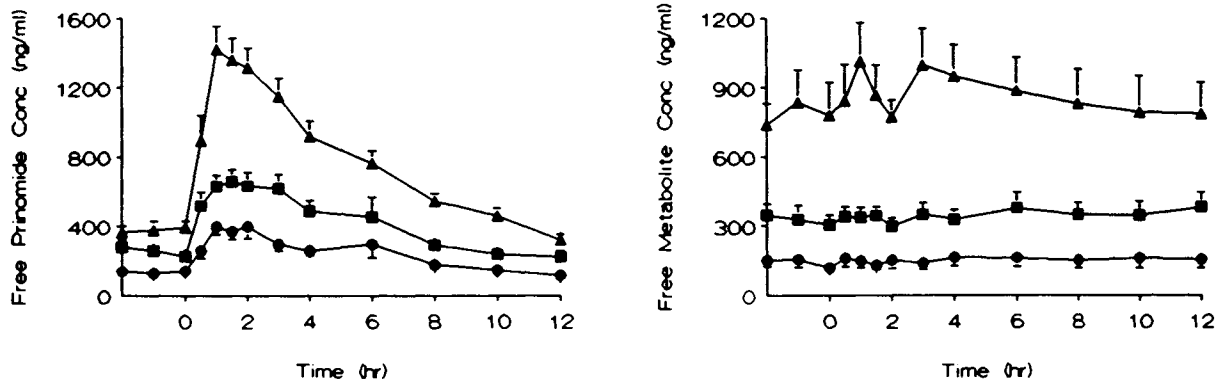

Fig. 4. Mean (SE; n = 12) free concentrations of prinomide tromethamine and p-hydroxyphenylprinomide corresponding to total concentrations in Figs. 2 and 3.

Table I. Steady-State Pharmacokinetic Parameters for Total and Free Prinomide Tromethamine and Its *p*-Hydroxy Metabolite

	Dose level[a]		
	250 mg	500 mg	1000 mg
Prinomide tromethamine			
Total AUC(0–τ), μg · hr/ml	503 (138.4)[b]	778 (181.5)	1147 (172.3)
Free AUC(0–τ), μg · hr/ml	2.9 (1.15)	4.9 (2.02)	9.2 (2.62)
Total C_{max}, μg/ml	74 (17.2)	110 (19.6)	169 (21.4)
Free C_{max}, ng/ml	483 (286.6)	797 (335.3)	1557 (314.0)
t_{max}, hr	1 (0.5–2)[c]	1.3 (0.5–3)	1 (0.5–3)
Free AUC/total AUC, %	0.58 (0.184)[d]	0.63 (0.177)	0.82 (0.180)[d]
Total CL_o, ml/min	8.8 (2.20)	11.3 (2.70)	14.9 (2.38)
Free CL_o, ml/min	1672 (654.6)	1929 (690.0)	1928 (485.4)
Metabolite			
Total AUC(0–τ), μg · hr/ml	421 (160.6)	734 (178.0)	1088 (219.4)
Free AUC(0–τ), μg · hr/ml	2.2 (1.80)	4.1 (2.30)	10.4 (6.22)
Total C_{max}, μg/ml	39 (15.0)	67 (16.5)	105 (19.7)
Free C_{max}, ng/ml	238 (186.9)	463 (219.8)	1070 (599.7)
t_{max}, hr	2.8 (0–12)	8 (0–12)	8 (0–12)
Free AUC/total AUC, %	0.49 (0.295)	0.56 (0.290)	0.91 (0.386)
Total CL_r, ml/min	1.6 (1.31)	1.1 (0.68)	1.3 (0.91)
Free CL_r, ml/min	432 (444.4)	225 (154.5)	165 (138.1)

[a] Prinomide tromethamine administered every 12 hr for 28 days.
[b] Mean (SD); $n = 12$.
[c] Median (range).
[d] $n = 11$.

Accordingly, the relationship between the bound and the free concentrations of prinomide, and those of the *p*-hydroxy metabolite, was evaluated by least-squares regression analysis utilizing a Langmuir binding model with only one class of binding sites, such that

$$C_b = PT \cdot C_f / (K_d + C_f)$$

where C_b is the bound concentration, C_f is the free concentration, PT is the total protein binding site density, and K_d the equilibrium dissociation constant.

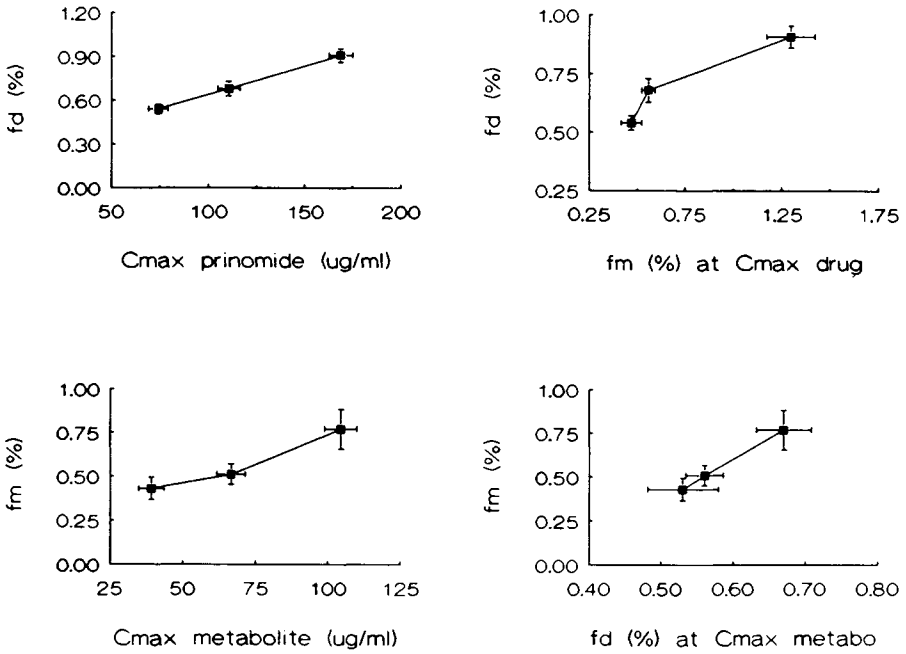

Fig. 5. *In vivo* dependency of prinomide tromethamine's free fraction (f_d) and its metabolite's free fraction (f_m) on their corresponding total C_{max} values after oral dosing, and the mutual association of f_d and f_m at their C_{max} values with f_m and f_d at times corresponding to the C_{max} of the drug and metabolite. Values are represented by the means (SE) corresponding to dose levels of 250, 500, and 1000 mg prinomide tromethamine.

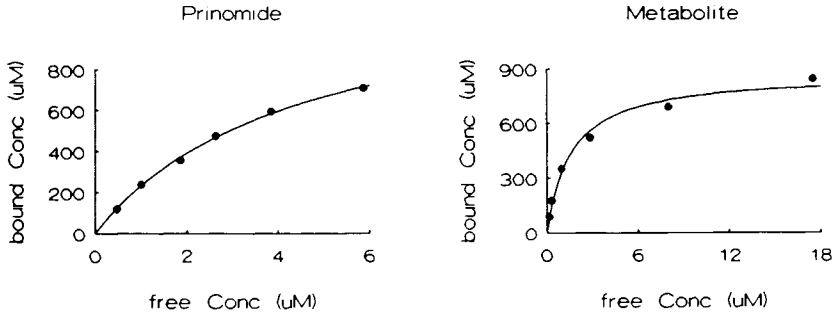

Fig. 6. Isolated prinomide and metabolite plasma protein binding isotherms demonstrating Langmuir-type interactions. Each value is represented by the mean of triplicate determinations. The solid line is the minimized least-squares fit corresponding to a Langmuir-type model with one class of binding sites.

Good agreement with the prescribed model was found for both prinomide and the *p*-hydroxy metabolite. The coefficients of determination were 0.998 and 0.986, respectively. For prinomide, the estimated (SD) value for K_d was 4.42 (0.356) μM and the value for PT was 1253 (56.1) μM, which corresponds to 18.2 μmol prinomide/g protein. For the *p*-hydroxy metabolite, the estimated value for K_d was 1.60 (0.290) μM and the value for PT was 876 (43.4) μM, which corresponds to 12.7 μmol metabolite/g protein.

Bivariate isotherms again displayed characteristics of Langmuir-type plasma protein binding (Fig. 7). In addition, these isotherms displayed characteristics of multiple-ligand cobinding typified by the isotherm displacements. These displacement interactions were related to the coexisting concentration of either prinomide or the *p*-hydroxy metabolite and were mutual for both prinomide and the *p*-hydroxy metabolite. These findings indicate that the nature of the plasma protein binding of prinomide or the *p*-hydroxy metabolite is capacity limited and subject to mutually competitive cobinding.

DISCUSSION

The pharmacokinetic behavior of prinomide is complex and related to several mutually dependent factors. First, and foremost, is the dependency of prinomide's disposition on the disposition of its own metabolic product. Unlike single-dose studies, where the relative total concentration of the *p*-hydroxy metabolite compared to that of prinomide is minimal, following multiple-dose administration the concentration of the metabolite is comparable to that of prinomide. This comparability results from the extensive accumulation of the long-lived metabolite, which is of the order of days. At steady state, mutually dependent competitive protein binding due to the accumulation of the metabolite partially contributes to the nonlinear behavior of total prinomide.The *in vitro* results confirming the competitive nature of the protein binding interaction support the observed *in vivo* dependency of prinomide's free fraction on that of the metabolite.

Another factor contributing to the observed nonlinear behavior of prinomide is capacity-limited protein binding of prinomide. Saturable protein binding of prinomide is, however, not exclusive of coexisting concentrations of metabolite since linearity was indicated in single-dose proportionality studies. This is additionally supported by the magnitude of the bound concentration of isolated prinomide at the capacity maximum in plasma. The estimated maximal value of 1253 μM prinomide corresponds to a concentration of 487 μg/ml prinomide tromethamine. This value is approximately threefold larger than the C_{max} observed for the 1000-mg dose level, indicating that cobinding of the metabolite probably significantly reduces the binding capacity reserve for prinomide.

Although the pharmacokinetics of total prinomide and metabolite are mutually dependent and complicated, the pharmacokinetics of free prinomide and its *p*-hydroxy metabolite are independent and generally unremarkable. Both prinomide and *p*-hydroxy metabolite free AUC values were linearly related to the dose, indicating independent linear elimination processes. The fact that the intrinsic free clearance of prinomide is constant in spite of increasing f_d values with increasing doses additionally indicates that prinomide has a low extraction ratio. Prinomide may thus be regarded as a restrictively cleared drug.

The potential for displacement interactions has been well recognized. While ligand–protein interactions have been confirmed for xenobiotic competitors (7), there has been no reported interaction with an endogenously generated competitor of a xenobiotic. In this study we have confirmed this potential. The lack of recognition of this type of competitive interaction may be due to several factors. First, if a wide disparity between affinities of precursors and metabolites exists, detection of the interaction may be obscured if only one entity is preferentially measured. This is generally the case in pharmacokinetic studies because of the relatively late stage in drug development in which metabolite elucidation and identification occurs. Second, protein bind-

Table II. Protein Binding of Prinomide and Its *p*-Hydroxy Metabolite to Isolated Plasma Constituents

		Fraction bound, %	
	% protein (w/v)	Prinomide (600 μM)	Metabolite (353 μM)
Human serum albumin	4.0	98.5 (0.24)[a]	99.7 (0.02)
α_1-Acid glycoprotein	0.1	4.3 (0.13)	2.3 (0.30)
γ-Globin	1.0	5.9 (0.14)	6.2 (0.31)
Erythrocytes		16.5 (0.24)	6.8 (0.13)

[a] Mean (SD); $n = 3$.

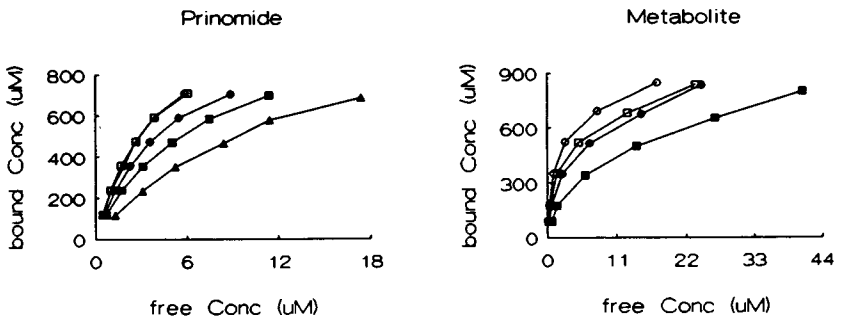

Fig. 7. Langmuir-type cobinding isotherms for prinomide with coexisting metabolite concentrations of 0 (○), 88 (□), 177 (●), 353 (■), and 706 (▲) μM and for the metabolite with coexisting prinomide concentrations of 0 (○), 120 (□), 240 (●), and 480 (■) μM. Isotherm displacements demonstrate mutually dependent competitive protein binding.

ing isotherms are generally evaluated *in vitro* in the absence of potential metabolites. The results of this study suggest that optimal *in vitro* evaluation of protein interactions should consider the competitive effect of metabolites, particularly for highly bound drugs with chemically similar metabolite products.

The findings reported in this study may have implications regarding the disposition of other nonsteroidal antiinflammatory drugs (NSAIDs). For the majority of NSAIDs, hepatic metabolism represents the primary route of elimination and usually accounts for >90% of their elimination (8–10). Most NSAIDs are also plasma protein bound to an extent of >99%. A large number of protein interactions have been reported for the coadministration of aspirin with other NSAIDs, including naproxen, ibuprofen, flurbiprofen, fenbufen, fenoprofen, ketoprofen, pirprofen, indomethacin, tolmetin, diflunisal, diclofenac, meclofenamate, isoxicam, and tenoxicam, all of which resulted in reduced plasma concentrations of the NSAID and an increase in apparent clearance. Considering these interactions, and reported cases of saturable plasma protein binding including salicylate, naproxen, diflunisal, and phenylbutazone, it is reasonable to suggest that metabolic product formation may have influenced the disposition of some of these NSAIDs. Protein interactions involving these NSAIDs usually are attributed to either saturable protein binding of the parent drug alone or competitive binding with a coadministered displacer, with little regard to the effect that coadministered displacers may have on metabolites or the role metabolic products may have on the parent drug. Potential interactions may be particularly noteworthy for NSAIDs with long-lived metabolites which demonstrate extensive accumulation such as phenylbutazone. In this case both phenylbutazone and its *p*-hydroxy metabolite, oxyphenbutazone, are long-lived, with half-lives of 75 and 27–64 hr, respectively. Both are bound to an extent of >98%, and consequently phenylbutazone exhibits both dose- and time-dependent pharmacokinetics, suggesting both saturable and metabolic product-dependent competitive protein binding similar to that demonstrated with prinomide.

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