IN VITRO AND IN VIVO EVALUATIONS OF THE METABOLISM, PHARMACOKINETICS, AND BIOAVAILABILITY OF ESTER PRODRUGS OF L-767,679, A POTENT FIBRINOGEN RECEPTOR ANTAGONIST

An Approach for the Selection of a Prodrug Candidate

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ABSTRACT:

The present study demonstrates the utility of an in vitro–in vivo correlative approach in the selection of an optimum prodrug candidate of L-767,679 (N-(7-(piperazin-1-yl)-3,4-dihydro-1H-isquinolin-2-yl)acetyl(3S)-(ethynyl)-β-alanine), a potent fibrinogen receptor antagonist. As an initial screening step, a comparative in vitro hepatic metabolism study was conducted for L-767,679 and a series of aliphatic and aromatic ester prodrugs in dogs, monkeys, and humans. In all species, the active acid L-767,679, but not the ester prodrugs, was resistant to metabolism. Only the methyl, ethyl, and isopropyl esters were converted exclusively to the active acid in liver microsomal preparations from dogs and humans, and thus were selected for further studies. In the preparations from monkeys, all of the esters investigated were metabolized efficiently to both the active acid and several other products. The absolute formation rates of L-767,679 from the esters followed the rank order: methyl > ethyl > isopropyl in all species, and in humans > dogs for the three esters. The three ester prodrugs did not undergo appreciable hydrolysis in blood or upon incubation with intestinal S9 from any of the studied species.

In vivo evaluation of the previous three aliphatic esters in dogs and monkeys supported the in vitro findings. L-767,679 was metabolically stable in both dogs and monkeys. After intravenous administration of the prodrugs to either species, the extent of acid formation was higher in dogs than in monkeys. In addition, the extent of L-767,679 formed from these prodrugs followed the rank order: methyl > ethyl > isopropyl. Similar results were obtained after oral dosing of the prodrugs, such that the bioavailability of L-767,679 was higher in dogs than in monkeys, and the bioavailability was higher after the ethyl ester than after the isopropyl ester prodrug in both species. In either species, both ethyl and isopropyl ester prodrugs were better absorbed than L-767,679.

Overall, the results suggested that the bioavailability of the active acid after administration of an ester prodrug was dictated primarily by two factors, viz.: 1) the relative rates of ester hydrolysis versus competing metabolic reactions and 2) the absolute rates of ester hydrolysis. In the case of L-767,679 prodrugs, absorption was not a limiting factor. Consequently, the bioavailability of L-767,679 after oral administration of the ester prodrugs would likely be greater in humans than in dogs, and in humans would be higher with the ethyl ester than with the isopropyl ester. On this basis, the ethyl ester was considered as a promising candidate for clinical evaluation as a fibrinogen receptor antagonist prodrug.

In the pharmaceutical industry, a number of in vitro systems are being used increasingly to screen compounds for metabolic and pharmacokinetic behavior in the early phase of drug discovery and development (1–3). To assess the relevance of findings from animal studies to the situation in humans, in vitro experiments with human tissues now are finding widespread application in the screening process (1–4). Provided that in vitro–in vivo correlations can be established, such in vitro approaches, due to their simplicity and rapidity relative to in vivo studies, often can serve as valuable tools in preclinical drug metabolism. The present study demonstrates the utility of an in vitro–in vivo correlative approach in the selection, during our drug discovery, of potential prodrug candidates of a given active drug. Specifically, the approach was used to screen a series of esters of L-767,679 (fig. 1), as potential prodrugs for the efficient delivery of L-767,679 after oral administration to humans.

In common with other highly polar compounds with zwitterionic properties (5–8), L-767,679 was found to exhibit low F1 in animal models, and therefore it was decided to evaluate the feasibility of developing a lipophilic ester prodrug derivative of this agent. In general, the F of an active compound after administration of a prodrug depends on a number of factors, including absorption of the prodrug, metabolism/elimination of the prodrug, and metabolism/elimination of the active compound (9–12). In a recent study (12), we demonstrated that the ethyl ester prodrug of a compound structurally related to L-767,679 failed to improve the bioavailability of its active acid despite the markedly increased lipophilicity of the prodrug. By means of in vitro techniques used in conjunction with in vivo studies, it was demonstrated that the low bioavailability was not due to poor absorp-

1 Abbreviations used are: F, oral bioavailability of the administered compound; iv, intravenous; po, per oral; UDPGA, uridine diphosphate glucuronic acid; CLint, intrinsic clearance; F27h (prodrug iv), oral bioavailability of L-767,679 after intravenous administration of a prodrug; F27h (prodrug po), oral bioavailability of L-767,679 after oral administration of a prodrug; AUC0-inf, area under the plasma concentration-time curve from time 0 to infinity; t1/2, half-life.
2 L. M. Gorham and T. Prueksaritanont, manuscript in preparation.

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were synthesized at Merck Research Laboratories (West Point, PA) as described (5). NADPH and UDPGA were purchased from Sigma Chemical Co. (St. Louis, MO). Solvents used for analysis were of analytical or HPLC grade (Fisher Scientific, Pittsburgh, PA).

In Vitro Studies. Subcellular fractions of liver (N = 4–9) and intestine (N = 3, each) from beagle dogs, rhesus monkeys, and humans were prepared as previously described (19). Studies on the metabolism of L-767,679 and its esters were performed in a 1-ml incubation mixture, using 0.5 mg of liver microsomal protein, 1 μmol NADPH, 1 mmol L-767,679 or a prodrug ester, 10 μmol MgCl₂, and 100 μmol phosphate buffer (pH 7.4). Metabolism in intestinal preparations was conducted similarly, except that 1 mg of intestinal S9 fraction protein and 2 nmol substrate were used. The reaction was terminated after incubation at 37°C for 60 min, by the addition of 5 ml acetone. For kinetic studies, the incubation time was 6 min in the case of monkey and 15 min in the case of dog liver preparations. A preliminary experiment showed that the rate of ester disappearance and the rate of L-767,679 formation were linear during these incubation periods. After centrifugation, the supernatant was evaporated, reconstituted in a mixture of 35% acetonitrile in 10 mM ammonium acetate buffer (pH 3.2), and analyzed by HPLC.

Studies on the hydrolysis of prodrugs I, II, and III in blood were performed using 0.5 ml whole blood obtained on the day of the experiment from dog, monkey, and human (N = 2, each), and various concentrations of the prodrugs (0.05, 0.5, and 20 μM). At various times during incubation at 37°C, the reaction was stopped by the addition of 5 ml acetone. After centrifugation, the supernatant was subjected to solid-phase extraction, as described herein, and the resulting extract was analyzed by HPLC.

In Vivo Studies. In vivo studies were conducted with L-767,679 and its esters I, II, and III, in a cross-over design, in four male beagle dogs (9–12 kg) or four male rhesus monkeys (4–6 kg). L-767,679 was given by iv injection at doses of 0.25 mg/kg (monkeys) or 0.08 mg/kg (dogs), and by po dosing at 1.25 mg/kg (monkeys) or 0.4 mg/kg (dogs). The esters were administered to both species at doses of 0.1 mg/kg by iv bolus (I, II, and III) and 0.5 mg/kg orally (II and III). Dosing solutions were prepared using sterile-buffered saline containing 2% (v/v) of 1 M HCl (for the ester prodrugs) or sterile-buffered saline alone (for L-767,679). All compounds were stable in these dosing solutions at least for 1 day. Blood was drawn at predose and at appropriate time intervals after drug administration. Plasma samples were separated immediately by centrifugation and kept frozen at −20°C until analyzed. Urine samples were collected on dry ice for up to 48 hr after dosing. All samples were analyzed within 2 weeks of the experiment.

Analytical Procedure. HPLC Analysis of Microsomal Incubations. Standard curves for L-767,679 and its esters were prepared by the addition of known quantities of these compounds to boiled microsomal preparations, followed by extraction as previously described. The HPLC system consisted of a Waters 600E multisolvent delivery system, a Waters 717 plus autosampler, and a Jasco 821-FP fluorescence detector. The sample analysis was conducted on a Zorbax C8-Rx column (4.6 × 250 mm, 5 μm), with a step gradient of acetonitrile (A) and 10 mM ammonium acetate buffer, pH 5.2 (B), at a flow rate of 1 ml/min (13–35% A in 6 min, then 35–70% A in 2 min, and held at 70% A for 5 min). The effluent was monitored at λ ex = 259 and λ em of 245 and 440 nm, respectively.

HPLC Analysis of Urine and Plasma Samples. Concentrations of L-767,679 and its ester prodrugs in urine and plasma were determined using HPLC methods developed in our laboratory. In brief, urine (0.2 ml) or plasma (0.5 ml) samples were vortex mixed with an equal volume of 0.1 M potassium phosphate buffer (pH 6) and then applied to preequilibrated C18 solid-phase extraction columns (Bakerbond, 3 ml). The columns were washed with 2 ml buffer, followed by 2 ml water; the compounds were eluted with 1.5 ml of methanol containing 1% (v/v) HCl. Eluates were evaporated and reconstituted in 150 μl of the HPLC mobile phase (52% acetonitrile in 0.04 M phosphate buffer, pH 4.2). An aliquot (60–120 μl) of this solution was analyzed by injection onto a Spherisorb SCX column (4.6 × 250 mm, 5 μm), with the mobile phase delivered isocratically at a flow rate of 1 ml/min. The eluate was monitored by a fluorescence detector (λ ex = 245 nm and λ em = 440 nm). Satisfactory linearity and precision (<15% coefficient of variation) were achieved for both L-767,679 and its prodrugs. The limit of detection of assay was 1 ng/ml for L-767,679 and its esters.

Data Analysis. The extent of formation of the active acid relative to all
Results are means of duplicate determinations using pooled liver microsomes (N = 4 for dogs and monkeys, and N = 9 for humans). All incubations were conducted in duplicate, at 37°C for 60 min, using liver microsomes (0.5 mg protein/ml) and the prodrug (1 μM) with NADPH (1 mM). Values above each bar represent the conversion efficiency.

In vitro metabolism of prodrugs of L-767,679 in the presence of NADPH by liver microsomal preparations from (A) dogs, (B) monkeys, and (C) humans.

Results

In Vitro Studies

Metabolism in Liver. L-767,679 was resistant to metabolism in liver microsomal preparations from all species. The prodrugs did not undergo conjugation in the presence of UDPGA, but were metabolized by liver microsomes in the presence and absence of NADPH. In the absence of NADPH, L-767,679 was the only metabolic product formed (data not shown). In some cases, formation of metabolites other than L-767,679 was observed in the presence of NADPH. This was shown by incomplete recovery of the substrate when only L-767,679 formation was taken into account, i.e. <100% conversion efficiency (figs. 2A, B, and C), and by the presence of unidentified products in HPLC chromatograms (data not shown). In general, the alkyl esters were found to undergo metabolism at a much slower rate than the aromatic esters. Among the alkyl esters, the 1-ethylpropyl derivative (IV) was consumed at the highest rate and was converted mostly to products other than the active acid (figs. 2A, B, and C). Species similarities were observed in the apparently stereoselective metabolism of the methylbenzyl esters; VII was metabolized to products other than L-767,679 more rapidly than its epimer VI (figs. 2A, B, and C).

Qualitatively, the metabolism of the alkyl ester prodrugs was similar in dog and human liver microsomes. In both species, the methyl, ethyl, and isopropyl prodrugs (I, II, and III) were converted exclusively to L-767,679, yielding essentially a 100% conversion efficiency (figs. 2A and C). All other esters were metabolized to several products, with a conversion efficiency ranging from 6% to 25% in dogs, and 8% to 75% in humans (figs. 2A and C). In all cases, the rate of formation of L-767,679 observed in human preparations was notably higher (up to 5-fold) than that in dogs. In monkeys, all esters underwent metabolism to the active acid as well as to other products, with a wide range of conversion efficiencies (5-90%, fig. 2B). For all species, the formation rate of L-767,679 from the isopropyl ester (III) was ~2-fold less than that from the ethyl (II) and methyl (I) esters. Presumably, this was a result of the increased steric bulk of the isopropyl group. Similar observations have been reported for branched alkyl ester prodrugs of compounds in different structural classes (20, 21).

In dogs, the CLint of L-767,679 formation (3 and 1 ml/min/g microsomal protein with II and III, respectively) should essentially be equal to the CLint of ester disappearance because no other metabolites were formed. In monkeys, the CLint for L-767,679 formation (5 and 2.5 ml/min/g microsomal protein with II and III, respectively) accounted for ≤10% of the CLint of the disappearance of esters (~50 ml/min/g microsomal protein for both prodrugs). Thus, on a per milligram protein basis, values for the CLint of ester disappearance obtained with either ester in dogs were ~15-fold lower than those in monkeys. In both species, the CLint of formation of L-767,679 from II was ~2- to 4-fold higher than that from III.

Metabolism in Intestine. In all species, prodrugs I, II, and III were metabolized only minimally by intestinal S9 preparations (fig. 3A–C). Under similar conditions, these subcellular fractions were catalytically active, with activities against various known enzyme markers comparable with those previously described (19). In all cases, L-767,679 was the only metabolic product formed. Similar to the observations with liver microsomal preparations, III was converted to L-767,679 at a slower rate than I and II.

Hydrolysis in Blood. The hydrolysis of prodrugs I, II, and III in whole blood from dogs, monkeys, and humans was <5% over a 60-min incubation, in agreement with our previous observation that alkyl ester prodrugs of compounds in this structural class undergo minimal hydrolysis in blood from these three species (12). The results suggested that neither blood nor intestine would likely play a significant role in the metabolism of these prodrugs in dogs, monkeys, or humans.

In Vivo Studies. Dog Study. Metabolism and excretion were investigated after iv administration to dogs of L-767,679 and the three selected esters. It was found that L-767,679 was eliminated mainly by renal excretion and not by metabolism, because >80% of the dose was recovered as the unchanged drug after iv administration (table 1). In contrast, the esters were eliminated primarily by nonrenal routes.
Results are means of duplicate determinations using pooled intestinal S9 fractions (N = 3 for each species). All incubations were conducted in duplicate, at 37°C for 60 min, using intestinal S9 fractions (1 mg protein/ml) and prodrugs I, II, and III (2 µM) with NADPH (1 mM).

TABLE 1
Urinary recovery and pharmacokinetic parameters for L-767,679 and prodrugs I, II, and III in dogs

<table>
<thead>
<tr>
<th>Compound Administered</th>
<th>Dose mg/kg</th>
<th>Urinary Recovery % dose</th>
<th>F_{679} (prodrug iv) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-767,679</td>
<td>0.08</td>
<td>82 ± 10</td>
<td>100</td>
</tr>
<tr>
<td>I</td>
<td>0.1</td>
<td>59 ± 10</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>II</td>
<td>0.1</td>
<td>60 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>III</td>
<td>0.1</td>
<td>33 ± 2</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>

Upon oral dosing of L-767,679 to dogs, the bioavailability of L-767,679 was estimated to be ~18% (table 2). Because the iv pharmacokinetics of I and II were comparable (table 1), only II and III were studied further after po administration. Based on the values for total urinary recovery (L-767,679 and the administered ester) (table 2), it may be concluded that at least 30% of the dose of the prodrugs was absorbed orally. An increase in the values for F_{679} (prodrug po) (1.5- to 2.3-fold; table 2) was obtained after administration of either prodrug, compared with that after administration of L-767,679 itself. The F_{679} (prodrug po) values were higher after administration of II (41%) than after III (27%). However, the reverse was observed with respect to values for F of the prodrugs (38% and 21% after III and II, respectively; table 2).

Pharmacokinetic parameters of L-767,679 and the ethyl ester prodrug (II) also were obtained from plasma data. Plasma levels of L-767,679 after iv dosing with L-767,679 exhibited a biexponential decay (fig. 4A), with a terminal t_{1/2} of ~4 hr and a clearance of 3.6 ± 0.3 ml/min/kg. After iv administration of ester II, plasma concentrations of L-767,679 increased slowly and then declined relatively slower than those of the ester (fig. 4A). The extent of conversion, F_{679} (prodrug iv), was calculated to be 60 ± 9%, comparable with the value estimated from the urine data (72%; table 1). Upon oral dosing of L-767,679 (fig. 4B), the bioavailability of L-767,679 was estimated to be 13 ± 2%. After po administration of II to the same dogs, plasma concentration-time profiles of L-767,679 and its ester (fig. 4B) were similar to those obtained after iv dosing (fig. 4A). The mean value of F_{679} (prodrug po) was 36 ± 9%. These results were in good agreement with those obtained on the basis of urinary recovery data (table 2).

**Monkey Study.** As was the case in dogs, L-767,679 was eliminated in monkeys primarily by renal excretion, with ~90% of the iv dose recovered as the unchanged drug in urine. After iv administration of the prodrugs, the urinary recoveries were ~18–32% for the active acid and ~20% for the unchanged prodrugs (table 3). The extent of conversion of esters to L-767,679, as reflected by values for F_{679} (prodrug iv) was 20–37% (table 3), corresponding to about one-half of those observed with the corresponding esters in dogs. However, the finding of higher F_{679} (prodrug iv) values after I or II than after III (table 3) was in line with the results obtained in dogs (table 1).

L-767,679 seemed to be less absorbed (F ~7%) in monkeys than in dogs (table 4). After po administration of the prodrugs, values for both F and F_{679} (prodrug po) were <15%, which were much lower than those observed in dogs (table 4). An increase (~2-fold) in F of L-767,679 was obtained after po dosing with II, but not after III. As was the case in dogs, the values for F_{679} (prodrug po) were higher after administration of II than after dosing with III.

**Discussion**

In the present study, an in vitro–in vivo correlative approach was used to identify an optimal prodrug candidate of L-767,679. Because it was demonstrated previously (12) that an ester prodrug of a structurally related acid served as a poor delivery form of the active acid due to appreciable metabolism by nonhydrolytic pathways, the study focused initially on the extent to which prodrugs were converted to the active acid, relative to their overall metabolism, in liver microsomal preparations. To examine potential metabolic pathways, the in vitro hepatic metabolism study was conducted at a prodrug concentration of 1 µM. This low concentration was considered to be appropriate based on the expected plasma concentrations of these esters (≤1 µM) and
the relatively high-affinity characteristics of enzymes that catalyzed the formation of NADPH-dependent metabolites ($K_M \approx 2 \mu M$). The in vitro studies were conducted in three different tissues (liver, intestine, and blood) known to contain xenobiotic-metabolizing enzymes, including esterases. The in vitro metabolism results suggested that the liver likely was the major metabolic organ of the three prodrugs in all of the species investigated.

Assuming that a prodrug is metabolized by a single organ in vivo, the conversion efficiency obtained from an in vitro study, theoretically, could only be higher than or at least equal to, the extent of formation of the active moiety in vivo after administration of its prodrug. This is because the in vitro condition is static (i.e., metabolism is the only elimination pathway for the prodrug). In addition, in this case, the active acid L-767,679 was not subject to further metabolism in vitro. In vivo, the extent of formation of the active moiety could be lower due to the potential contribution of other competing pathways, such as biliary and/or urinary excretion for both the prodrug and the active compound. Assuming also that the extent of the excretion pathways are comparable, the prodrugs with the highest conversion efficiency should yield the optimum conversion in vivo and vice versa. On this basis, the methyl, ethyl, and isopropyl esters, which exhibited a 100% conversion efficiency in human and dog liver preparations, but possessed low conversion efficiency in monkey liver microsomes, were selected for further studies in dogs and monkeys. This strategy allowed confirmation of in vitro results to be made in animal models, for compounds with both good (dog) and poor (monkey) conversion efficiency. It should be noted that this selection criterion would exclude only the prodrugs of potentially poor performance, but it is very possible that prodrugs with an incomplete but reasonable conversion efficiency may exhibit a high yield of conversion in vivo. Evaluation of such compounds in vivo may also be needed.

For the purpose of validation of these in vitro results, it was necessary to first demonstrate that hepatic metabolism also played an important role in the in vivo elimination of the prodrugs. In the present study, these goals were achieved by monitoring the urinary recovery of both the active acid and intact prodrugs after administration of the esters, as well as determining the plasma concentrations of the parent acid after administration of the acid itself. In dogs, the values of $F_{679}$ (prodrug iv) of 40–70%, and of the small unchanged fraction in urine of $\approx 10\%$, implied that metabolism, apparently in the liver, played an important role in the elimination of the three prodrugs. The values of $F_{679}$ (prodrug iv) obtained from plasma data (60%) were slightly lower than those estimated from the urinary recovery (72%), thus suggesting that the ester-to-acid conversion in kidney was relatively minor. This conclusion was consistent with an earlier finding that, in dogs, the kidney contains esterases at a level much lower than Table 3

**Urinary recovery and pharmacokinetic parameters for L-767,679 and prodrugs I, II, and III in monkeys**

Results (means ± SD, $N = 4$) were obtained after iv administration.

<table>
<thead>
<tr>
<th>Compound Administered</th>
<th>Dose (mg/kg)</th>
<th>Urinary Recovery</th>
<th>$F_{679}$ (prodrug iv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-767,679</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>% of dose</td>
<td>% of dose</td>
<td>% of dose</td>
</tr>
<tr>
<td>L-767,679</td>
<td>0.25</td>
<td>89 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>I</td>
<td>0.1</td>
<td>29 ± 14</td>
<td>21 ± 15</td>
</tr>
<tr>
<td>II</td>
<td>0.1</td>
<td>32 ± 5</td>
<td>22 ± 9</td>
</tr>
<tr>
<td>III</td>
<td>0.1</td>
<td>18 ± 3</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 4

**Urinary recovery and pharmacokinetic parameters for L-767,679 and prodrugs II and III in monkeys**

Results (means ± SD, $N = 4$) were obtained after po administration.

<table>
<thead>
<tr>
<th>Compound Administered</th>
<th>Dose (mg/kg)</th>
<th>Urinary Recovery</th>
<th>$F_{679}$ (prodrug po)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-767,679</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>% of dose</td>
<td>% of dose</td>
<td>% of dose</td>
</tr>
<tr>
<td>L-767,679</td>
<td>1.25</td>
<td>6 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>II</td>
<td>0.5</td>
<td>12 ± 2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>III</td>
<td>0.5</td>
<td>9 ± 9</td>
<td>—</td>
</tr>
</tbody>
</table>
IN VITRO AND IN VIVO EVALUATIONS OF ESTER PRODRUGS

the liver (22). These results justified the use of urine data for evaluation of the prodrugs in vivo and suggested that, when renal excretion is the major elimination pathway of the active acid, the in vivo data may be based solely on the urinary recovery of the acid and its intact CL. The in vivo data for the prodrugs in dogs than in monkeys (tables 1 and 3), consistent with the in vitro observations that the values for conversion efficiency were higher in dog than monkey liver preparations. Specifically, in the case of I and II, values of F(679) (prodrug) were 72% in dogs and 34–37% in monkeys, agreeing with the in vitro conversion efficiencies of 100% and 40–50% in dog and monkey livers, respectively. The finding that the values of F(679) (prodrug) from III were ~2-fold lower than those from I or II in both species also was in line with the in vitro observations that the CL_total of formation of L-767,679 from III was ~2- to 4-fold lower than that from II. Interestingly, this in vivo/in vitro agreement implied that, in a given species, the clearances of the prodrugs by all elimination pathways (metabolism + excretion) were similar. The results suggested that, in addition to the conversion efficiency, the absolute rate of formation of the active acid also would be an important criterion for the selection of a prodrug of L-767,679.

Considering that the clearance of L-767,679 was low and mostly renal, the low F of L-767,679 in both animal species could not be attributed to extensive first-pass elimination by hepatic metabolism or biliary excretion, but, as expected, to low intestinal absorption. Consistent with the increased lipophilicity of the prodrugs (>100-fold), as compared with L-767,679 (fig. 1), the absorption of esters II and III in both species seemed to be much higher than their active acid and may be comparable. These conclusions were based on the following analyses. In dogs, the sum of F and F(679) (prodrug) values after oral administration of either prodrug was >50% (table 2). Also, there was a similar rank order between F(679) (prodrug) and F(679) (prodrug) and an inverse relationship between F and F(679) (prodrug) for both prodrugs (table 2). In monkeys, both prodrugs were expected to be well absorbed, based on similar rank orders between F(679) (prodrug) and F(679) (prodrug). The finding that values for F(679) (prodrug) were higher in dogs than in monkeys (tables 2 and 4) was in agreement with the in vitro observations that the CL_total of ester disappearance (and thus the hepatic first-pass metabolism) was less in dogs than in monkeys. Thus, overall results suggested that, for compounds in this structural series, structural differences of the ester moieties affected greatly the metabolism of the prodrugs and, consequently, the bioavailability of the active acid, but affected minimally the absorption of the prodrugs.

The apparent in vitro-in vivo correlations observed in the present study, not only for the two species investigated, but also among the compounds examined, provide bases for extrapolation of in vitro human studies. Because much more rapid metabolism of the three esters was observed with human than with dog liver microsomes, it may be anticipated that, in humans, in vivo hepatic metabolism also would play an important role. Based on the in vitro metabolic pattern (fig. 2), the dog seemed to be a better animal model for humans than the monkey, and it is likely that, in humans, F(679) (prodrug) follows the rank order: I ~ II > III. In addition, assuming that absorption of the prodrugs also was not a limiting factor in humans, an increase in F(679) (prodrug) values after administration of these esters in humans, as compared with that after administration of the active acid, would be expected in the rank order of II > III. Considering that formation of L-767,679 from these esters occurred at a much higher rate in humans than in dogs, and assuming that there are species similarities in the pharmacokinetics of L-767,679, it is possible that, for the corresponding ester prodrug, F(679) (prodrug) values would be higher in humans than in dogs. On this basis, the ethyl ester seemed to be a promising candidate for clinical evaluation as a fibrinogen receptor antagonist prodrug.

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References


