Pharmacokinetics and Interactions of a Novel Antagonist of Chemokine Receptor 5 (CCR5) with Ritonavir in Rats and Monkeys: Role of CYP3A and P-Glycoprotein

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ABSTRACT

The mechanisms of pharmacokinetic interactions of a novel anti-human immunodeficiency virus (anti-HIV-1) antagonist of chemokine receptor 5 (CCR5) [2-(R)-(N-methyl-N-1-(R)-(3-(4-(3-benzyl-1-ethyl-(1H)-pyrazol-5-yl)piperidin-1-yl)methyl)-4-(S)-(3-fluorophenyl)cyclopent-1-yl)-3-methylbutanoic acid (MRK-1)] with ritonavir were evaluated in rats and monkeys. MRK-1 was a good substrate for the human (MDR1) and mouse (Mdr1a) multidrug resistance protein transporters and was metabolized by CYP3A isozymes in rat, monkey, and human liver microsomes. Both the in vitro MDR1-mediated transport and oxidative metabolism of MRK-1 were inhibited by ritonavir. Although the systemic pharmacokinetics of MRK-1 in rats and monkeys were linear, the oral bioavailability increased with an increase in dose from 2 to 10 mg/kg. The area under the plasma concentration-time curve (AUC) of MRK-1 was increased 4- to 6-fold when a 2 or 10 mg/kg dose was orally coadministered with 10 mg/kg ritonavir. Further pharmacokinetic studies in rats indicated that P-glycoprotein (P-gp) inhibition by ritonavir increased the intestinal absorption of 2 mg/kg MRK-1 maximally by ~30 to 40%, and a major component of the interaction likely resulted from its reduced systemic clearance via the inhibition of CYP3A isozymes. Oral coadministration of quinidine (10 and 30 mg/kg) increased both the extent and the first-order rate of absorption of MRK-1 (2 mg/kg) by ~40 to 50% and ~100 to 300%, respectively, in rats, thus further substantiating the role of P-gp in modulating the intestinal absorption of MRK-1 in this species. At the 10 mg/kg MRK-1 dose, however, the entire increase in its AUC upon coadministration with ritonavir or quinidine could be attributed to a reduced systemic clearance, and no effects on intestinal absorption were apparent. In contrast to rats, the effects of P-gp in determining the intestinal absorption of MRK-1 appeared less significant in rhesus monkeys at either dose.

The CCR5 chemokine receptor is expressed on both monocytes and T-lymphocytes and is believed to play a pivotal role in the pathogenesis of the human immunodeficiency virus (HIV-1) infection. It has been suggested that the entry of HIV-1 into the host cell is facilitated by the interaction of the viral envelope glycoproteins gp120 and gp41 with the host cell CD4, and then either the chemokine receptor CCR5 or CXCR4 and appear later in persistent infection concomitant with a catastrophic decline in CD4+ T cell numbers and the development of clinical acquired immunodeficiency syndrome (Connor et al., 1997). Human genetic evidence supports CCR5 as a potentially attractive antiviral target. A 32-base pair deletion in the CCR5 coding region (CCR5Δ32) generates a nonfunctional receptor, and homozygosity for CCR5Δ32 confers resistance to HIV-1 infection in populations at high risk for exposure but does not manifest any adverse health effect (Liu et al., 1996). Studies of infected humans heterozygous for CCR5Δ32 have shown that the genotype is associated with delayed pro-

ABBREVIATIONS: CCR5, chemokine receptor 5; HIV-1, human immunodeficiency virus; AUC, area under the plasma concentration-time curve; MDR, multidrug resistance; PEG, polyethylene glycol; ETOH, ethanol; MRK-1, 2-(R)-(N-methyl-N-1-(R)-(3-(4-(3-benzyl-1-ethyl-(1H)-pyrazol-5-yl)piperidin-1-yl)methyl)-4-(S)-(3-fluorophenyl)cyclopent-1-yl)-3-methylbutanoic acid; CLblood, systemic blood clearance; Clint, intrinsic clearance; Cmax, maximal plasma concentration; CLint, systemic plasma clearance; P450, cytochrome P450; K1o, first-order absorption rate constant; Koo, first-order elimination rate constant; MS, mass-spectrometry; LC-MS/MS, liquid chromatography tandem mass spectrometry; P-gp, P-glycoprotein; Tmax, time to reach maximal plasma concentration.
ggression to clinical acquired immunodeficiency syndrome (Balfe et al., 1998). A number of CCR5 receptor antagonists with antiviral activity have been identified and are in various stages of clinical development (Moore and Stevenson, 2000; Eckert and Kim, 2001; Finke et al., 2002).

Over the past several years, multidrug therapy has shown a considerable advantage over the use of a single drug in the management of HIV infection (Torres and Barr, 1997; Palella et al., 1998). This has been propelled by the need to delay the development of resistance and avoid dose-limiting adverse effects with a single agent. Currently, a triple or a quadruple therapy with two nucleoside analogs, plus one or two protease inhibitors, is considered essential for optimal efficacy and to avoid rapid development of viral resistance (Barry et al., 1997, 1999). Although the availability of a CCR5 antagonist may offer another powerful pharmacological intervention for the management of HIV infection, it is almost certain that a combination therapy would be required to achieve reasonable reductions in disease progression and to circumvent rapid development of resistance.

Ritonavir is one of several HIV-protease inhibitors (ritonavir, indinavir, saquinavir, nelfinavir, amprenavir, and lopinavir) approved for the management of HIV infection in the United States. Protease inhibitors, especially ritonavir, have potent inhibitory effects on drug-metabolizing enzymes such as CYP3A4, CYP2C9, CYP2C19, and CYP2D6 (Eagling et al., 1997; von Moltké et al., 1998). Ritonavir, when given in combination with other protease inhibitors, serves to enhance their pharmacokinetics by providing an increased plasma concentration and prolonged drug residence in the circulation (Hsu et al., 1998; Barry et al., 1999). Thus, potent antiviral effects can be achieved with lower doses of each protease inhibitor and with a less frequent dosing regimen. This has become an important therapeutic strategy for the pharmacotherapy of HIV. In addition to their inhibitory effects on cytochrome P450 (P450) enzymes, protease inhibitors including ritonavir are also good substrates for the human multidrug resistance proteins MDR1 or P-gp (Alsenz et al., 1998; Kim et al., 2001; Finke et al., 2002). Ritonavir was obtained as the commercially available Norvir solution from Abbott Laboratories (Abbott Park, IL). Polyclonal rabbit antiserum against rat cytochrome P450 CYP3A2, CYP2C11, and the corresponding control antiserum (non-immune serum) were purchased from Sigma-Aldrich (St. Louis, MO).

Identification of Cytochrome P450 Isozymes Responsible for MRK-1 Metabolism. [3H]MRK-1 was incubated at 37°C with microsomes prepared from baculovirus-infected cells expressing individually expressed P450 isozymes and cytochrome P450 reductase. Each incubation contained 10 μM [3H]MRK-1; 500 pmol/ml P450 protein; an NADPH-regenerating system consisting of 10 mM glucose 6-phosphate, 2 mM NADP+, and 2.8 units/ml glucose-6-phosphate dehydrogenase; and 10 mM magnesium chloride in 100 mM potassium phosphate buffer (pH 7.4). Incubations were carried out for 60 min, after which the reaction was halted by the addition of an equal volume of acetonitrile. After centrifugation, the supernatant was analyzed by HPLC with an on-line radioactivity detector.

Further confirmation of the P450 isof orm(s) responsible for the in vitro metabolism of 10 μM [3H]MRK-1 in human liver microsomes was obtained by incubating the compound in the presence of monoclonal antibodies against CYP2C9, CYP2D6, and CYP3A4, and also with the cytochrome P450 isof orm-specific inhibitors including sulfaphenazole (CYP2C9), tranylcypromine (CYP2C19), quinidine (CYP2D6), and ketoconazole and troleandomycin (CYP3A4). The effect of the above-mentioned monoclonal antibodies on MRK-1 metabolism was also examined in male rhesus monkey liver microsomes. In addition, male rat liver microsomes were incubated with polyclonal antibodies against CYP2C11 and CYP3A2 to examine their role in MRK-1 metabolism in the rat. Each incubation contained 1 mg/ml microsomal protein and 25 μl/ml of the antibody preparation along with the above-described buffer and NADPH-regenerating system. The disappearance of MRK-1 from the incuba-

![Chemical structure of MRK-1](image)
The potential of ritonavir to inhibit the metabolism of MRK-1 was also examined in rat, monkey, and human liver microsomes. MRK-1 (10 μM) was incubated with the above, with liver microsomes from the three species in the absence and presence of varying concentrations of ritonavir for 15 min. Preliminary studies indicated that metabolism was linear for the duration of the incubation. At the end of the incubation, the reaction was stopped by the addition of an equal volume of acetonitrile. Samples were spun in a centrifuge and the supernatant was analyzed by LC-MS/MS. The rates of MRK-1 metabolism in the presence of ritonavir were calculated relative to the control and the data were fit to the following equation: % Activity Remaining = 100 · IC50/[IC50 + I] to determine the ritonavir concentration required for 50% inhibition of MRK-1 metabolism (IC50) under these conditions; I represents the inhibitor concentration.

Transethelial Transport of MRK-1 across Monolayers of Cell Lines Transfected with Human MDR1 and Mouse Mdr1a

Transporter and the Effect of Ritonavir. Human MDR1 transfectants (L-MDR1), Mouse Mdr1a transfectants (L-Mdr1a), and parental pig kidney epithelial cell line (LLC-PK1) were kindly provided by Dr. Alfred H. Schinkel (The Netherlands Cancer Institute, Amsterdam) and used under a license agreement. Cells were cultured in Medium 199 (Invitrogen, Carlsbad, CA) supplemented with 2 mM L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, and 10% (v/v) fetal calf serum (Invitrogen) (Schinkel et al., 1995). For L-MDR1 and L-Mdr1a, cells were maintained in the continuous presence of 640 nM vincristine (Schinkel et al., 1995). Confluent monolayers were subcultured every 3 to 4 days by treatment with 0.25% trypsin and 1 mM EDTA in Ca2+- and Mg2+-free Hank’s balanced salt solution. All cultures were incubated at 37°C in a humidified atmosphere of 5% CO2/95% air.

Transethelial transport studies were carried out as described by Schinkel et al. (1995) with minor modifications. L-MDR1, L-Mdr1a, and LLC-PK1 cells were plated at a density of 4–12×104 cells/12-mm well on porous (3-μm) polycarbonate membrane filters (Transwell; Costar Corp., Cambridge, MA). Cells were supplemented with fresh media every 2 days and used in the transport studies on the fourth day after plating. Transethelial resistance was measured in each well using a Millicell ohmmeter (model ERS; Millipore Corp., Bedford, MA); wells registering a resistance of ≥300 Ω or greater, after correcting for the resistance obtained in control blank wells, were used in the transport experiments.

About 1 to 2 h before the start of the transport experiments, the medium in each compartment was replaced with fresh transport medium. The transport experiment was then initiated (t = 0) by replacing the medium in each compartment with 700 μl transport medium with (donor compartment) and without (receiver compartment) the radiolabeled substrate ([14C]MRK-1, 10 μM, 0.5 μCi/ml). Directional transport of vinblastine ([3H]vinblastine, 10 μM, 0.5 μCi/ml) was examined in parallel as a positive control for P-gp activity. After 0.5, 1, and 2 h 50-μl aliquots were taken from the receiver (L-MDR1) and replaced with fresh transport medium. Samples were placed in scintillation vials containing 5 ml scintillation cocktail (Ultima-Flo M, PerkinElmer Life Sciences, Boston, MA), and total radioactivity was measured by liquid scintillation counting. The data were calculated either as the fraction of the total added radioactivity that appeared in the receiver compartment or as the total amount transported as a function of time.

To examine the effect of ritonavir on MRK-1 transport in L-MDR1 cell monolayers, varying amounts of ritonavir (in ethanol at a final concentration of 2%) were added to both the donor and receiver compartments to provide final concentrations of 0, 10, 25, 50, and 75 μM. The effect of cyclosporin A (10 μM) on MRK-1 and vinblastine transport was examined also as a positive marker for P-gp inhibition. Directional transport was measured in three individual cell cultures on three separate days and in triplicate during each experiment. The data are presented as the mean ± S.D.

Pharmacokinetics in Rats and Monkeys. All animal procedures were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Rats and monkeys were housed in temperature- and humidity-controlled rooms with a 12-h light/dark cycle.

Cannulas were implanted in the femoral artery and vein of male Sprague-Dawley rats (250–300 g, n = 3 or 4/group) and animals were allowed to recover from surgery for at least 1 day before experimentation. Similarly, 5- to 7-year-old male adult rhesus monkeys (Macaca mulatta, n = 4/group) were surgically prepared by placing catheters either into the saphenous vein via percutaneous venipuncture or by surgically placing indwelling catheters into the femoral vein and connecting them to a subcutaneous vascular access port. Monkeys were transferred to restraint chairs on the day of experiment for dosing and blood collection. Rats and monkeys were fasted.
overnight before drug administration, whereas access to water was provided ad libitum. Food was restored after the collection of 4-h blood samples.

Intravenous dosing solutions of MRK-1 were prepared in a PEG400/EtOH/H2O (2:2:6, v/v) vehicle. The compound was administered as an i.v. bolus via the femoral vein (or saphenous vein in the case of monkeys) at 0.5 and 2 mg/kg doses at a dose volume of 1 (rats) or 0.2 (monkeys) ml/kg. Oral dosing solutions of MRK-1 were prepared as a suspension in 0.9% NaCl, and the doses were 2 and 10 mg/kg in a dosing volume of 1 (monkeys) or 5 (rats) ml/kg. Different groups of rats were used for oral and i.v. administration experiments. However, a randomized two-way crossover design was used for the oral and i.v. administration experiments in monkeys.

**Effect of Ritonavir Oral Coadministration on the Pharmacokinetics of MRK-1 in Rats and Monkeys.** Separate groups of rats were surgically prepared as above to examine the effect of ritonavir oral coadministration on the pharmacokinetics of MRK-1. However, the same set of rhesus monkeys that was used in the previous pharmacokinetic experiments (vide supra) was used for these studies. Appropriate doses of ritonavir were administered as the commercially available Norvir solution. The oral doses and dosing volumes of MRK-1 were the same as described above for the pharmacokinetic studies. Animals were administered the ritonavir dose via oral gavage followed immediately by the MRK-1 suspension via the same route.

**Effect of Quinidine Oral Coadministration on the Oral Pharmacokinetics of MRK-1 in Rats.** Rats (n = 3/group) were surgically prepared as above. Formulations were prepared by dissolving appropriate amounts of MRK-1 and quinidine (quinidine gluconate; Sigma-Aldrich) in an EtOH/PEG400/H2O (2:2:6, v/v) vehicle. The dosing volume was 5 ml/kg in each case. MRK-1 was administered either alone (at 2 and 10 mg/kg doses) or in combination with 10 and 30 mg/kg quinidine.

**Effect of Oral Ritonavir and Quinidine on the Systemic Pharmacokinetics of MRK-1 in Rats.** Quinidine formulations were prepared at appropriate concentrations, as described above, in EtOH/PEG400/H2O (2:2:6, v/v). The Norvir solution was used for ritonavir doses. A 0.5 mg/ml solution of MRK-1 was prepared in the EtOH/PEG400/H2O (2:2:6, v/v) vehicle. Rats (n = 3/group) were administered vehicle, ritonavir (10 mg/kg), or quinidine (30 mg/kg) doses via oral gavage 30 min before the administration of a 0.5 mg/kg i.v. bolus dose of MRK-1; the 30-min time-point corresponds to plasma concentrations of ritonavir that are near-maximal (Cmax) with the dosing regimen (data not shown).

In all pharmacokinetic and interaction studies, blood samples (250 µl for rats and 1 ml for monkeys) were collected at predetermined time points up to 24 h after drug administration. Plasma was obtained by centrifugation of the blood and stored at −20°C until LC-MS/MS analysis.

**LC-MS/MS Analysis.** Plasma samples were extracted by a solid phase extraction procedure that utilized Waters Oasis 96-well extraction plates. Briefly, the 96-well plates were equilibrated, successively, in two steps with 1 ml each methanol and water. An aliquot of 1 M phosphoric acid (0.5 ml) was added to each sample well. Appropriate volumes of calibration curve standard solutions, quality control samples (prepared in control rat or monkey plasma), and plasma samples (0.1 ml) were pipetted into the predetermined sample wells. Control plasma (0.1 ml) was included in each of the calibration curve samples. The internal standard (a close analog of MRK-1, 50 ng) was added to all wells and the contents of each well were thoroughly mixed. The plate was eluted slowly under vacuum until the wells were dry and each sample well was then washed with 0.5 ml distilled water. The sample wells were eluted with 300 µl acetonitrile/distilled water mixture (90:10, v/v) into a 96-well collection plate and analyzed using LC-MS/MS. Chromatography was performed on an ABZ+ column (100 mm × 2.1 mm, 5 µm; Supelco, Bellefonte, PA) and an HPLC system consisting of PerkinElmer Series 200 Micro Pumps and autosampler using a gradient mobile phase of acetonitrile, methanol, and 1 mM ammonium acetate. The HPLC flow rate was 0.35 ml/min. Detection of the analyte and internal standard was performed using a Sciex API 3000 mass spectrometer in the positive ion mode using the Turbo-Ion Spray source at 400°C. Mass transitions (m/z) monitored were 575 → 444 for MRK-1 and 547 → 282 for the internal standard. Triplicate calibration curves were constructed by plotting peak area ratio of the analyte to internal standard against the analyte concentration. The concentrations of MRK-1 in plasma samples were determined by comparing the analyte to internal standard peak area ratios against the calibration curve. Calibration curves for MRK-1 were constructed at a concentration range of 1−1000 ng/ml and the data were fitted to a power model of the form y = ax^z. The variability and bias of the LC-MS/MS assay for MRK-1 at all quality control (QC) levels was <15%.

**Pharmacokinetic Analyses.** Pharmacokinetic parameters of MRK-1 were calculated by standard pharmacokinetic approaches (Gibaldi and Perrier, 1982). The AUC up to the last sampling point was calculated by the linear trapezoidal rule. Extrapolation to infinity was performed by the factor C∞/λz, where C∞ is the plasma concentration at the last sampling time and λz is the terminal elimination rate constant. For determination of the first-order absorption and elimination rate constant in MRK-1-quinidine interaction studies, the plasma concentration-time data were fitted to a one-compartment model with first-order absorption and elimination.

The intrinsic clearance (CLint) of MRK-1 was calculated from the i.v. bolus clearance data, assuming a well stirred model of hepatic clearance and using the following equations.

\[
\begin{align*}
\text{CL}_{\text{blood}} &= \frac{Q_H \cdot \text{CL}_{\text{out}}}{Q_H + \text{CL}_{\text{out}}} \\
\text{CL}_{\text{int}} &= \frac{Q_H \cdot \text{CL}_{\text{blood}}}{Q_H - \text{CL}_{\text{blood}}}
\end{align*}
\]

(1)

where \(Q_H\) and \(\text{CL}_{\text{blood}}\) refer to hepatic blood flow and systemic blood clearance, respectively.

Also, after oral administration

\[
\text{CL}_{\text{int}} = \frac{F_e \cdot \text{Dose}_{\text{oral}}}{\text{AUC}_{\text{oral}}} \quad \text{or} \quad \frac{\text{Dose}_{\text{oral}}}{\text{AUC}_{\text{oral}}} = \frac{\text{CL}_{\text{int}}}{F_e}
\]

(2)

where \(F_e\), \(\text{Dose}_{\text{oral}}\), and \(\text{AUC}_{\text{oral}}\) refer to the fraction of orally administered dose absorbed into the circulation, total administered oral dose, and systemic blood AUC of MRK-1 following an oral dose, respectively.

Assuming linear systemic pharmacokinetics and constant plasma protein binding, the fraction of the orally administered dose that was absorbed after administration of different doses was calculated using the pharmacokinetic data from the i.v. bolus and oral administration experiments and the above two equations. As described under Results, the assumptions of linear systemic pharmacokinetics and constant plasma protein binding were largely true at the plasma concentrations encountered in our studies.

**Results**

**MRK-1 is a Substrate for the CYP3A Isozymes in Rat, Monkey, and Human Liver Microsomes.** The relative rates of metabolism in liver microsomes from different species followed the rank order monkey > human > rat. Approximately 90, 100, and 60% of the compound was metabolized at the end of a 60-min incubation period when MRK-1 (10 µM) was incubated with human, rat, and monkey liver microsomes (1 mg/ml microsomal protein), respectively. The oxidative metabolism of MRK-1 in human and monkey liver microsomal incubations was completely inhibited when microsomes were preincubated with monoclonal antibodies against the CYP3A4 isozyme. In contrast, anti-CYP2C8/9 and anti-CYP2D6 antibodies had no significant inhibitory
effect on MRK-1 metabolism in either human or monkey liver microsomes. Similarly, anti-rat CYP3A2 antibody inhibited MRK-1 metabolism in rat liver microsomes by ~70%. Consistent with results from antibody studies, incubation of [3H]MRK-1 with microsomes containing individually expressed recombinant human P450 isoforms showed that MRK-1 was metabolized by only CYP3A4; no metabolism was detectable in microsomes containing any of the other human P450 isoforms. In addition, the use of specific chemical inhibitors of human P450 isoforms indicated that the phase I metabolism of MRK-1 in human liver microsomes could be inhibited completely by ketoconazole (a potent reversible inhibitor of CYP3A4) and by >70% by tronloamycin (a mechanism-based inhibitor of CYP3A4). In contrast, the inhibitors of other P450 isoforms such as sulfaphenazole (CYP2C9), quinidine (CYP2D6), and tranalyepromine (CYP2C19) exhibited only minor inhibitory effects (<20% inhibition) on the metabolism of MRK-1 in human liver microsomes up to a high concentration of 50 μM. Thus, data from experiments with human in vitro systems and from the effect of anti-CYP3A antibodies on MRK-1 metabolism in liver microsomes suggest that the compound was metabolized primarily by the CYP3A isoforms in the three species. Ritonavir was a potent inhibitor of MRK-1 metabolism in rat, monkey, and human liver microsomal incubations, with IC50 values of 0.29, 0.53, and 0.23 μM, respectively.

**MRK-1 Is a Substrate for P-Glycoprotein.** MRK-1 showed a substantially greater B-to-A than A-to-B transport in monolayers of human MDR1 or mouse Mdr1a-transfected cell lines, while the transport in the two directions was roughly equal in the parental LLC-PK1 cells (Table 1). These data suggest that MRK-1 is a good substrate for human MDR1 and mouse Mdr1a transporters. However, the B-to-A/A-to-B transport ratio of MRK-1 was consistently lower than that of the prototypical P-gp substrate vinblastine, suggesting that the latter may be a better P-gp substrate than MRK-1. The preferential B-to-A efflux transport of MRK-1 and vinblastine in L-MDR1 cells was significantly inhibited by 10 μM cyclosporin A (a known P-gp inhibitor). The P-gp mediated efflux transport of MRK-1 in L-MDR1 cell monolayers was inhibited effectively by ritonavir, with an IC50 value of ~15 μM (Fig. 2). However, cyclosporin A appeared to be somewhat more potent as an inhibitor of human MDR1 relative to ritonavir (Fig. 2).

**Plasma Protein Binding and Blood-to-Plasma Partitioning of MRK-1.** MRK-1 was bound extensively to plasma proteins in all species, with the average plasma protein binding in rat, monkey, and human plasma being 99.6, 99.3, and 99.5%, respectively. The average blood-to-plasma partition ratio of [3H]MRK-1 radioactivity was 0.62, 0.62, and 0.58 in the rat, monkey, and human, respectively. Both MRK-1 plasma protein binding and blood-to-plasma ratio were independent of concentration between 0.01 and 10 μg/ml.

**Rat Intestinal Loop Studies.** Figure 3 shows the net amount of MRK-1 absorbed from a jejunal segment of the rat intestine during 10-min sampling intervals for up to 1 h after the administration of a 0.1 mg dose. Coadministration of verapamil or ritonavir (0.1 mg) with MRK-1 increased the net absorption of the latter compound by ~2–3- and >25-fold,

### TABLE 1

Bidirectional transport of MRK-1 and vinblastine in cell lines transfected with the human MDR1 and mouse Mdr1a transporters and the effect of cyclosporine A (CsA)

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Transport Ratio (B→A/A→B)</th>
<th>MRK-1 (10 μM)</th>
<th>MRK-1 (10 μM CsA)</th>
<th>Vinblastine (10 μM)</th>
<th>Vinblastine (10 μM CsA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC-PK1</td>
<td>1.8 ± 0.23</td>
<td>N.D.</td>
<td>1.4 ± 0.15</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>L-MDR1</td>
<td>10 ± 0.91</td>
<td>3.0 ± 0.2</td>
<td>23 ± 2.4</td>
<td>11 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>L-Mdr1a</td>
<td>4.3 ± 2.0</td>
<td>N.D.</td>
<td>8.2 ± 3.3</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

* Each ratio is an average from three individual experiments conducted on separate days, each with a triplicate determination.

* N.D. Not determined.
respectively, at each sampling time during the 60-min experimental period.

**Pharmacokinetics of MRK-1 in Rats and Monkeys.** Pharmacokinetic parameters of MRK-1 in rats and monkeys after i.v. and oral dosing are presented in Table 2. Plasma concentrations of MRK-1 followed a typical biexponential decline after i.v. bolus dosing at 0.5 and 2 mg/kg in both rats and monkeys. The pharmacokinetics of MRK-1 after i.v. bolus administration were linear as the dose was increased from 0.5 to 2 mg/kg in both rats and adult monkeys. The compound exhibited a low-to-moderate plasma clearance in both species. The terminal elimination half-life of the compound was short (1–3 h) in both species. Because of the very high plasma protein binding of MRK-1, the steady-state volume of distribution was also in the low-to-moderate range (1–2 l/kg). In both rats and adult monkeys, the bioavailability increased nonlinearly with an increase in oral dose from 2 to 10 mg/kg; however, the increase in bioavailability in monkeys was statistically nonsignificant (paired t test, p ≥ 0.05).

The average fraction of administered oral dose of MRK-1 that was absorbed from the intestine into the circulation in rats and monkeys was estimated using the well-stirred model of hepatic elimination as described under Materials and Methods. The average hepatic blood flow values for rats and adult monkeys were assumed to be 45 and 65 ml/min/kg, respectively, for the purpose of this calculation (Davies and Morris, 1993). The data presented in Table 3 suggest that the fraction absorbed increased with an increase in oral dose from 2 to 10 mg/kg in both species. The compound appears to be well absorbed, with the fraction absorbed approaching 80–100% at the 10 mg/kg oral dose in rats and monkeys.

**Effect of Oral Coadministration of Ritonavir on the Pharmacokinetics of MRK-1 in Rats and Monkeys.** As described above, MRK-1 is a P-gp substrate and is metabolized mainly by the CYP3A isozymes. In vitro studies described herein suggest that ritonavir is a potent inhibitor of MRK-1 metabolism and its P-gp mediated efflux transport. Thus, MRK-1 pharmacokinetics can be influenced by ritonavir via inhibition of both of these proteins. The data on the effect of oral coadministration of ritonavir on the pharmacokinetics of MRK-1 are presented in Fig. 4 and Table 4. Thus, when a 2- or 10-mg/kg dose of MRK-1 was orally coadministered with a 10 mg/kg dose of ritonavir to rats, the systemic plasma AUC of MRK-1 was increased between 4- and 6-fold as compared with control. The increases in MRK-1 AUC after coadministration with ritonavir were also accompanied by increases of similar magnitude in maximal plasma concentrations (C_max) (Table 4). Interestingly, however, there ap-

### Table 2

Pharmacokinetic parameters of MRK-1 following i.v. and oral administration in male Sprague-Dawley rats (n = 3/group) and rhesus monkeys (n = 4/group) All values are mean ± S.D.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.5 mg/kg</th>
<th>2 mg/kg</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v. Dose</td>
<td>Oral Dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_int (ng h/ml)</td>
<td>699 ± 147</td>
<td>3462 ± 1318</td>
<td>978 ± 727</td>
<td>12,250 ± 5,115</td>
</tr>
<tr>
<td>CL_int (ml/min/kg)</td>
<td>12.3 ± 2.9</td>
<td>10.9 ± 5.3</td>
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<tr>
<td>CL_h (ml/min/kg)</td>
<td>19.8 ± 4.7</td>
<td>17.6 ± 8.5</td>
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</tr>
<tr>
<td>Vdss (l/kg)</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td></td>
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</tr>
<tr>
<td>T_{1/2} (h)</td>
<td>1.5 ± 0.7</td>
<td>2.9 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.9 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fa (%)</td>
<td></td>
<td></td>
<td>35</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Rhesus Monkeys</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_int (ng h/ml)</td>
<td>691 ± 171</td>
<td>3322 ± 284</td>
<td>884 ± 312</td>
<td>7865 ± 3842</td>
</tr>
<tr>
<td>CL_int (ml/min/kg)</td>
<td>12.1 ± 2.5</td>
<td>10.1 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL_h (ml/min/kg)</td>
<td>19.6 ± 4.0</td>
<td>16.3 ± 1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vdss (l/kg)</td>
<td>1.8 ± 0.6</td>
<td>1.2 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_{1/2} (h)</td>
<td>2.1 ± 0.3</td>
<td>1.6 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>2.4 ± 0.4</td>
<td>2.0 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fa (%)</td>
<td></td>
<td></td>
<td>34 ± 19</td>
<td>49 ± 20</td>
</tr>
</tbody>
</table>

* Calculated by dividing plasma clearance in each animal with the average in vitro blood-to-plasma ratio.

* Bioavailability in rats was determined using average AUC data from i.v. bolus and oral dosing studies in separate groups of animals.

* Bioavailability in monkeys was determined using AUC data from i.v. bolus and oral administration experiments conducted with a randomized two-way crossover design in each monkey.

### Table 3

Estimates of fraction of MRK-1 oral dose absorbed in rats and adult rhesus monkeys

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>A: MRK-1 (0.5 mg/kg i.v.)</td>
<td>Fa = 0.79</td>
</tr>
<tr>
<td></td>
<td>B: MRK-1 (2 mg/kg p.o.)</td>
<td>Fa = 0.79</td>
</tr>
<tr>
<td></td>
<td>Fraction Absorbed (Fa)</td>
<td>= 0.52</td>
</tr>
<tr>
<td></td>
<td>C: MRK-1 (2 mg/kg i.v.)</td>
<td>Fa = 0.79</td>
</tr>
<tr>
<td></td>
<td>D: MRK-1 (10 mg/kg p.o.)</td>
<td>Fa = 0.79</td>
</tr>
<tr>
<td>Rhesus Monkeys</td>
<td>E: MRK-1 (0.5 mg/kg i.v.)</td>
<td>Fa = 0.79</td>
</tr>
<tr>
<td></td>
<td>F: MRK-1 (2 mg/kg p.o.)</td>
<td>Fa = 0.79</td>
</tr>
<tr>
<td></td>
<td>Fraction Absorbed (Fa)</td>
<td>= 0.57</td>
</tr>
<tr>
<td></td>
<td>G: MRK-1 (2 mg/kg i.v.)</td>
<td>Fa = 0.79</td>
</tr>
<tr>
<td></td>
<td>H: MRK-1 (10 mg/kg p.o.)</td>
<td>Fa = 0.79</td>
</tr>
</tbody>
</table>

* Calculation of intrinsic clearances and fraction absorbed was performed according to the well-stirred model of organ clearance. Details are described under Materials and Methods in the Pharmacokinetic Analyses section.
AUC of MRK-1 was also increased during the terminal elimination phase (Fig. 4). Similar to rats, the systemic plasma concentration versus time profile during the terminal elimination appeared to be little change in the slope of MRK-1 plasma concentrations in (A) rats and (B) monkeys.

Effect of ritonavir oral coadministration on the pharmacokinetics of MRK-1 in rhesus monkeys were maintained at or near clearance relative to controls (Table 4). Also, in contrast to rats, the plasma concentrations of MRK-1 in rhesus monkeys was little changed relative to controls (Table 4). Also, in contrast to rats, the plasma concentrations of MRK-1 in rhesus monkeys was little changed relative to controls (Table 4). Also, in contrast to rats, the plasma concentrations of MRK-1 in rhesus monkeys was little changed relative to controls (Table 4).

Effect of Oral Coadministration of Quinidine on the Pharmacokinetics of MRK-1 in Rats. A nonlinear increase in the oral bioavailability of MRK-1 in rats and monkeys raises the possibility of involvement of P-gp-mediated efflux in the absorptive processes of MRK-1 at the intestinal mucosal surface such that at higher doses the saturation of this efflux transport may lead to increased absorption and bioavailability. To confirm this possibility, studies were conducted to examine the oral pharmacokinetics of MRK-1 when administered in combination with quinidine, a known inhibitor of P-gp. Quinidine was selected because of its relatively low inhibitory potential toward CYP3A isozymes (Achira et al., 1999) that are predominantly responsible for MRK-1 metabolism (see above). As shown in Fig. 5 and Table 5, oral coadministration of quinidine at 10 and 30 mg/kg increased the plasma AUC of MRK-1 after a 2 mg/kg oral dose by 3-fold, along with an 3.5- to 5-fold increase in plasma $C_{\text{max}}$ values. There was a dose-dependent increase in the first-order absorption rate constant and a decrease in model-estimated $T_{\text{max}}$ when MRK-1 was administered in combination with quinidine (Fig. 5 and Table 5), suggesting a more rapid MRK-1 absorption in the presence of quinidine. In contrast, the first-order elimination rate constant of MRK-1 was not affected upon coadministration with quinidine. In contrast to these data, at the 10 mg/kg MRK-1 dose coadministration with quinidine (30 mg/kg) increased the plasma AUC of MRK-1 by a somewhat smaller magnitude (~2-fold) relative to control, and there was little change in $C_{\text{max}}$, $K_{\text{a}}$, $K_{\text{at}}$, or $T_{\text{max}}$ values of MRK-1 (Table 5).

Role of Increased Absorption and Reduced Systemic Elimination of MRK-1 in its Pharmacokinetic Interactions with Ritonavir. A profound increase in the plasma AUC of MRK-1 upon oral coadministration with ritonavir in both rats and monkeys raises the question of the relative significance of increased absorption (resulting from inhibition of P-gp at the intestinal mucosal surface) and reduced systemic clearance of MRK-1 (because of inhibition of CYP3A-mediated metabolism) in this interaction. We chose to address this issue by resolving the systemic clearance component of this interaction from the overall interaction. This was achieved by examining the effect of oral ritonavir administration on the systemic clearance of MRK-1, as shown in Table 6. The data presented in Fig. 6 and Table 6 illustrate that a 10 mg/kg oral dose of ritonavir resulted in an ~3-fold reduction in systemic clearance of MRK-1. Considering the magnitude of MRK-1 clearance in rats and assuming a rat hepatic blood flow of 65 ml/min/kg, this amounts to, on average, a 4.5-fold reduction in the intrinsic clearance of MRK-1, based on the well stirred model of hepatic clearance (Table 6). Since, according to the well stirred model, AUCoral = $F_o$ · Dose/CLint, a 4.5-fold reduction in CLint of MRK-1 upon coadministration with ritonavir would result in an increase in its systemic AUC of approximately the same magnitude, due solely to its reduced systemic elimination. Since, at a 2 mg/kg MRK-1 dose, the total change in systemic plasma AUC of MRK-1 was ~6-fold, this amounts to a maximal ~1.3- to 1.4-fold (30–40%) increase in MRK-1 intestinal absorption when MRK-1 was administered to rats in combination with 10 mg/kg ritonavir. Interestingly, at the 10 mg/kg MRK-1 dose, the increase in its AUC upon coadministration with 10 mg/kg ritonavir was 4.3-fold (Table 4); thus, it would appear that all of the increase in AUC of MRK-1 can be accounted for by changes in its systemic $CL_{\text{int}}$ value upon coadministration with ritonavir.

We also examined the possibility of quinidine affecting the systemic clearance of MRK-1 via either inhibition of its metabolism and/or biliary, urinary, and intestinal secretion or transport. Similar to ritonavir, oral administration of quinidine at 30 mg/kg was found to impair the systemic clearance...
of MRK-1, albeit to a lesser extent (−33%). This corresponds to, on average, an ~2-fold reduction in intrinsic hepatic clearance of MRK-1 based on the well stirred model of liver (Table 6). Thus, quinidine at a 30 mg/kg oral dose would be expected to increase the AUC of orally coadministered MRK-1 by ~2-fold, based solely on its interaction with the systemic clearance. This indicates that quinidine may maximally increase the extent of MRK-1 absorption (at a 2 mg/kg dose) by ~40 to 50% (Table 6). Interestingly, at the higher MRK-1 dose (10 mg/kg), there was only a 2-fold increase in the plasma AUC of MRK-1 upon coadministration with quinidine, which can be attributed to the reductions in its systemic clearance (Table 5). Thus, it appears that there is little change in the extent of MRK-1 absorption when the compound is administered at a 10 mg/kg dose in combination with quinidine (30 mg/kg). Overall, the data from the rat indicate that the major component of the MRK-1-ritonavir and MRK-1-quinidine interactions arose from inhibition of the systemic clearance of MRK-1. In addition, there may have been an ~30 to 50% increase in the extent of MRK-1 intestinal absorption when it was administered at a 2 mg/kg dose in combination with either ritonavir (10 mg/kg) or quinidine (10 or 30 mg/kg). Conversely, however, the apparent negligible changes in the intestinal absorption of MRK-1 at the 10 mg/kg dose upon coadministration with ritonavir or quinidine are consistent with nearly complete absorption of the compound at this dose level, likely because of saturation of any P-gp effects (Table 3).

**Discussion**

Combination drug therapy, with intervention at a number of key stages of the viral replication cycle, will likely remain the mainstay of HIV therapy for many years. However, HIV protease inhibitors in general, and ritonavir in particular, have the potential to exhibit significant drug-drug interactions either via the inhibition of P450 isozymes or the efflux transporter P-gp when given in combination with other compounds. It is clear that management of HIV combination therapy in the clinic requires a thorough understanding of the underlying mechanisms and the biochemical bases of these interactions. Hence, we investigated the role of P-gp and CYP3A in the interaction of an investigational CCR5 receptor antagonist, MRK-1, with ritonavir.

Our in vitro studies demonstrated that MRK-1 was metabolized exclusively by the CYP3A isozymes in liver microsomes from the rat, monkey, and human. Ritonavir proved to be a potent inhibitor of the metabolism of MRK-1 in liver microsomes from all species, with an IC_{50} value of ~0.6 μM. MRK-1 was also a good substrate for the human MDR1 and mouse Mdr1a transporters. The inhibitory effect of ritonavir on P-gp was confirmed by potent inhibition of MRK-1 efflux transport in L-MDR1 cell monolayers, with an IC_{50} value of ~15 μM. Furthermore, the data from the intestinal loop studies in the rat suggested that P-gp inhibitors, verapamil, and especially ritonavir, may profoundly influence the absorption of MRK-1. Incidentally, these data also indicate that MRK-1 is a substrate for efflux transporters at the rat intestinal mucosal surface, and at least in the intestinal loop model, the absorption of MRK-1 was significantly limited by this efflux transport. From these data it appears that ritonavir may exhibit drug-drug interactions with MRK-1 either via the inhibition of its CYP3A-mediated hepatic metabolism in the liver or P-gp-mediated transport at the intestinal mucosal surface, resulting in reduced systemic clearance and/or its increased absorption, respectively.

Although it is relatively easy to identify the substrates and inhibitors of P-gp using cell lines overexpressing this transporter or the isolated intestinal loop preparations, the in vivo
significance of these findings is somewhat difficult to ascertain. For example, it is difficult to predict whether the oral absorption of a particular P-gp substrate, as identified from in vitro studies, will be markedly influenced by efflux transport at the intestinal mucosal surface. Similarly, it cannot easily be determined whether a P-gp inhibitor such as ritonavir, which inhibits efflux transport in vitro, would exhibit in vivo drug-drug interactions via this mechanism. The majority of the data in support of the significance of P-gp in determining the disposition and pharmacokinetics of drugs comes from comparative studies in wild-type (Mdr1a+/+) and Mdr1a-deficient (Mdr1a−/−) mice (Schinkel et al., 1994–1997; van Asperen et al., 1996; Sparreboom et al., 1997; Kim et al., 1998; Iyer et al., 2002). However, there are possible species differences in the substrate specificities of these transporters (Yamazaki et al., 2001). Thus, in vivo studies in other species are needed to truly understand the significance of P-gp transport in determining the disposition of a particular drug candidate during its discovery and development. These studies are, however, difficult to conduct because of a large overlap in the affinities of various substrates and inhibitors of P-gp and CYP3A isozymes, so that it becomes difficult to identify whether observed in vivo interaction is a result of CYP3A or P-gp inhibition, or both (Wacher et al., 1995; Kim et al., 1999). Although a few recent reports show that some compounds do indeed exhibit varying degrees of selectivity toward either CYP3A or P-gp (Achira et al., 1999; Dantzig et al., 1999; Wandel et al., 1999; Cummins et al., 2002), the magnitude of these selectivities is likely not sufficient to inhibit one protein without affecting the other in vivo. This is especially true when these compounds are administered orally and high local concentrations are achieved at the intestinal mucosal surface and in the portal venous circulation. Hence, with the exception of anticancer drugs that are metabolized by enzymes other than CYP3A, there are few data in the literature that directly address the role of P-gp in drug disposition in species other than the mouse.

In the present studies, MRK-1 exhibited profound pharmacokinetic interactions after oral coadministration with ritonavir in rats and monkeys. From separate studies on MRK-1 disposition in rats and monkeys we have determined that the systemic clearance of MRK-1 is mediated primarily via hepatic oxidative and conjugative metabolism, with only small contributions from biliary and urinary excretion of the parent compound. Thus, in our studies to investigate the Table 6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>With 10 mg/kg Oral Ritonavir</th>
<th>With 30 mg/kg Oral Ritonavir</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0−∞ (ng·h/ml)</td>
<td>500 ± 150</td>
<td>1370 ± 248</td>
<td>714 ± 104</td>
</tr>
<tr>
<td>CLa (ml/min/kg)</td>
<td>17.6 ± 4.9</td>
<td>6.3 ± 1.3</td>
<td>11.8 ± 1.7</td>
</tr>
<tr>
<td>CLblood (ml/min/kg)</td>
<td>28.5 ± 7.9</td>
<td>10.1 ± 2.0</td>
<td>19.0 ± 2.8</td>
</tr>
<tr>
<td>CLint (ml/min/kg)</td>
<td>54.3 ± 25.6</td>
<td>12.0 ± 2.9</td>
<td>27.0 ± 5.5</td>
</tr>
<tr>
<td>Vdss (l/kg)</td>
<td>0.78 ± 0.14</td>
<td>0.57 ± 0.07</td>
<td>0.61 ± 0.05</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.75 ± 0.09</td>
<td>1.6 ± 0.19</td>
<td>0.85 ± 0.05</td>
</tr>
</tbody>
</table>

* Calculated by dividing plasma clearance in each animal with the average in vitro blood-to-plasma ratio.

* Calculated using the CLblood values and an assumed rat hepatic blood flow of 65 ml/min/kg. A well stirred model of hepatic clearance was assumed for this calculation.

Fig. 6. Effect of ritonavir (10 mg/kg) and quinidine (30 mg/kg) oral pretreatment on the systemic pharmacokinetics of MRK-1 in rats.
relative significance of P-gp and CYP3A in MRK-1/ritonavir interactions, an approach was chosen where the intestinal and systemic components of these interactions were pharmacokinetically resolved. Inhibition of hepatic CYP3A isoforms likely represents the predominant component of the interaction occurring at the systemic clearance level. Similarly, the increase in MRK-1 absorption via P-gp inhibition is likely a primary factor responsible for the intestinal component of this interaction. Resolution of systemic and intestinal interactions was possible because MRK-1 exhibited a low-to-moderate clearance that was markedly lower than the estimates of hepatic blood flow in both rats and monkeys. This approach assumes that there is negligible intestinal metabolism of the compound; this appeared to be largely true because little metabolism of MRK-1 was detected upon incubation with intestinal microsomes from the rat and the monkey. By using this approach we have demonstrated that the reduction of MRK-1 systemic clearance, probably by way of inhibition of hepatic CYP3A isoforms, on average accounted for ~4.5-fold of the observed total 6-fold increase in the plasma AUC of MRK-1 after oral coadministration of a 2 mg/kg dose with 10 mg/kg ritonavir in rats. The remainder of the increase in plasma MRK-1 AUC (~30–40%) likely arose from its increased absorption via inhibition of P-gp. From the present pharmacokinetic studies it appeared that the fraction of the administered MRK-1 dose absorbed after oral dosing at 10 mg/kg approached unity. In agreement with this, when a 10 mg/kg dose of MRK-1 was orally administered in combination with 10 mg/kg ritonavir, almost the entire observed increase in MRK-1 plasma AUC could be accounted for by the reductions in systemic clearance, and the contribution of the intestinal interaction was minimal. The role of P-gp in the absorption of MRK-1 in rats was confirmed further by studies in combination with quinidine when an increased and more rapid absorption of MRK-1 was observed. Interestingly, however, a significant component of the quinidine/MRK-1 interactions also appeared to occur via inhibition of systemic clearance of MRK-1. Although the exact mechanism(s) of this interaction remains to be investigated, it could occur via a combination of inhibition of metabolism and/or biliary and urinary excretion components of MRK-1 clearance. Quinidine, at 10- and 30-mg/kg doses, appeared to result in similar increases in the extent of MRK-1 absorption (40–50%). However, quinidine increased the rate of MRK-1 absorption in a dose-dependent and in a relatively more profound manner (100–300% increase in the first-order absorption rate constant, Table 5). These data seem to suggest that P-gp has a greater role in determining the rate rather than the extent of MRK-1 absorption in rats in vivo. This may be related to the fact that the net absorption of MRK-1 was slowed by P-gp-mediated efflux transport, the transit time of the drug through the gut was still sufficiently protracted to ensure absorption of the majority of the dose.

In comparison to rats, the nonlinearity in both the bioavailability and estimated oral absorption was somewhat less profound in monkeys and was statistically nonsignificant. Furthermore, the shape of the plasma concentration versus time profile of MRK-1 after oral administration suggests a more rapid absorption of the compound in monkeys compared with rats, i.e., the monkeys appear to exhibit a sharper plasma concentration peak as opposed to a more “flat” profile in rats (Fig. 4). A good degree of oral absorption, a less profound nonlinearity in bioavailability with increasing dose, and an apparently more rapid absorption profile suggest a limited role of intestinal P-gp in determining the oral absorption of MRK-1 and its interactions with ritonavir in monkeys. The magnitude of interference between MRK-1 and ritonavir in monkeys as measured by the overall increase in plasma AUC was similar to that in rats. In contrast to rats, however, there was little change in plasma C\textsubscript{max} values of MRK-1 in monkeys when given in combination with ritonavir. Also, when given in combination with ritonavir, the plasma concentrations of MRK-1 were maintained near C\textsubscript{max} for a 6 to 8 h period in monkeys, which may possibly be related to a more potent and prolonged inhibition of CYP3A-mediated MRK-1 metabolism in this species. It appears that the majority of the increase in AUC of MRK-1 in the above ritonavir coadministration experiments arose from its prolonged half-life or reduced elimination during the 6 to 8 h period after dosing and there were minimal, if any, changes in the rate and extent of MRK-1 absorption. Unfortunately, however, the latter could not be confirmed by using quinidine as a P-gp inhibitor (as in the rat) because of a possible stimulatory effect of this compound on CYP3A isoforms in the monkey (Tang et al., 1999).

### Summary and Conclusion

In conclusion, we have demonstrated that ritonavir is a potent inhibitor of the CYP3A-mediated oxidative metabolism of MRK-1 and can also inhibit its P-gp-mediated transport. MRK-1 exhibits significant pharmacokinetic interactions upon coadministration with ritonavir, with the plasma AUC increased 4- to 6-fold in both rats and monkeys. A major mechanism of these interactions is likely the inhibition of hepatic CYP3A-mediated systemic elimination of MRK-1 by ritonavir. At the lower doses of MRK-1 (2 mg/kg), P-gp does appear to play a role in modulating its intestinal absorption and to contribute to its interactions with ritonavir in rats. This is substantiated by the fact that quinidine, a P-gp inhibitor, increases the rate and extent of intestinal absorption of MRK-1 in rats at this dose level. At the higher doses (10 mg/kg), however, the role of P-gp appears to become less significant, possibly because of saturation of P-gp-mediated transport by high concentrations of the compound at the intestinal mucosal surface. In monkeys, the role of P-gp in determining MRK-1 absorption appears less significant and the increase in MRK-1 absorption by ritonavir via inhibition of intestinal P-gp likely accounts for only a small fraction, if any, of the overall increase in systemic MRK-1 exposure.

### Acknowledgments

We thank Drs. Thomas H. Rushmore and Magang Shou of the Department of Drug Metabolism, Merck Research Laboratories, West Point, PA for providing monoclonal antibodies against human P450 isoforms and insect cell microsomes containing individual recombinant human P450 isoforms.

### References


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