Renal Interaction Between Itraconazole and Cimetidine

Chetan S. Karyekar, MD, PhD, Natalie D. Eddington, PhD, Andrew Briglia, DO, Paul O. Gubbins, PharmD, FCCP, and Thomas C. Dowling, PharmD, PhD

Renal drug interactions can result from competitive inhibition between drugs that undergo extensive renal tubular secretion by transporters such as P-glycoprotein (P-gp). The purpose of this study was to evaluate the effect of itraconazole, a known P-gp inhibitor, on the renal tubular secretion of cimetidine in healthy volunteers who received intravenous cimetidine alone and following 3 days of oral itraconazole (400 mg/day) administration. Glomerular filtration rate (GFR) was measured continuously during each study visit using iothalamate clearance. Iothalamate, cimetidine, and itraconazole concentrations in plasma and urine were determined using high-performance liquid chromatography/ultraviolet (HPLC/UV) methods. Renal tubular secretion (CL_{sec}) of cimetidine was calculated as the difference between renal clearance (CL_r) and GFR (CL_{ioth}) on days 1 and 5. Cimetidine pharmacokinetic estimates were obtained for total clearance (CL_T), volume of distribution (Vd), elimination rate constant (K_{el}), area under the plasma concentration-time curve ($AUC_{0\text{-}240\ min}$), and average plasma concentration (Cp_{ave}) before and after itraconazole administration. Plasma itraconazole concentrations following oral dosing ranged from 0.41 to 0.92 μg/mL. The cimetidine $AUC_{0\text{-}240\ min}$ increased by 25% ($p < 0.01$) following itraconazole administration. The GFR and Vd remained unchanged, but significant reductions in CL_T (655 vs. 486 mL/min, $p < 0.001$) and CL_{sec} (410 vs. 311 mL/min, $p = 0.001$) were observed. The increased systemic exposure of cimetidine during coadministration with itraconazole was likely due to inhibition of P-gp-mediated renal tubular secretion. Further evaluation of renal P-gp-modulating drugs such as itraconazole that may alter the renal excretion of coadministered drugs is warranted.

Keywords: *Renal tubular secretion; itraconazole; cimetidine; renal drug interactions*

Journal of Clinical Pharmacology, 2004;44:919-927

The kidney plays a critical role in the removal of a wide range of endogenous substances, drugs, and metabolites from the body. Understanding the role of renal clearance mechanisms as a source of intersubject variability in pharmacokinetics is also important. It is becoming increasingly evident that carrier-mediated drug-efflux transporters in the intestine, liver, and kidney contribute to drug elimination in these organs. For example, primary active transport mechanisms are responsible for biliary excretion of anticancer drugs, endogenous bile acids, and organic anions, including glutathione and glucuronic acid conjugates.[1] Transporters in the kidney are shown to be vital in the body's ability to eliminate organic drugs.[2]

Within the human kidney, several drug transport proteins have been identified, including the basolateral organic anionic transporters (OATs), apical organic cationic transporters (OCTs), apical multidrug resistance protein (MRP2), and apical P-glycoprotein (P-gp).[3] P-gp, a 170-kDa plasma membrane-bound, adenosine triphosphate (ATP)–dependent efflux protein, is likely responsible for transporting a wide variety of drugs and toxins to the extracellular environment. Drug transport has also been shown to be mediated by P-gp in other important areas of drug absorption, distribution, and elimination such as the in-

From the Renal Clinical Pharmacology Laboratory, School of Pharmacy, and the Department of Medicine, Division of Nephrology, School of Medicine, University of Maryland, Baltimore, Maryland, and the College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Arkansas. This work was supported by the University of Maryland General Clinical Research Center, National Institutes of Health Grant M01 RR16500, General Clinical Research Centers Program, National Center for Research Resources. This work was presented in part at the 2003 ACCP Spring Practice and Research Forum. Submitted for publication March 12, 2004; revised version accepted April 25, 2004. Address for reprints: Dr. Thomas C. Dowling, Pharmacokinetics/Biopharmaceutics Laboratory, School of Pharmacy, University of Maryland, 100 N. Penn Street, AHB 540-D, Baltimore, MD 21201.

DOI: 10.1177/0091270004266783

testinal epithelium, blood-brain barrier (BBB), hepatocyte, biliary tract, and kidney. P-gp is expressed in high concentrations in early segments of the nephron, including the glomerular mesangium and proximal tubule.[4,5] Within proximal tubular epithelial cells, P-gp is primarily located along the apical membrane, likely cohabitating with OCT, which also transports drugs by peritubular secretion into the urine. Studies with digoxin,[6] vinblastine,[7,8] and doxorubicin[9] have demonstrated that P-gp is involved in the renal tubular handling of these agents in vitro, and several other cationic drugs, such as quinidine, procainamide, and morphine, are known substrates or inhibitors of P-gp. Therefore, it is likely that P-gp plays an important role in the renal excretion of a wide range of drugs, including organic cations.

Cimetidine is a histamine (H_2)-receptor antagonist that was widely used to treat and prevent gastrointestinal ulcers and is now available without prescription in the United States.[10,11] This organic cation is predominantly excreted renally (approximately 75%-80%), with renal clearance values three- to fourfold greater than the glomerular filtration rate (GFR), indicating extensive tubular secretion.[12] Data obtained from drug transport studies in MDR1-MDCK cells, a P-gp-transfected renal tubular epithelial cell line, and P-gp-enriched renal brush-border membrane vesicles support the hypothesis that cimetidine undergoes P-gp-mediated renal tubular secretion.[13-15]

Itraconazole is an azole antifungal agent that has been shown to inhibit P-gp in experimental models. Itraconazole reversed P-gp-mediated drug resistance to daunorubicin, adriamycin, and etoposide in P388/ADR and K562/ADR cells.[16,17] In humans, itraconazole reduced the renal clearance of digoxin and quinidine, both P-gp substrates, by 20% to 50%.[18,19] Thus, inhibition of P-gp activity in the kidney by itraconazole may lead to altered renal handling of drugs. Therefore, the objective of the present study was to investigate the effect of itraconazole on the P-gp-mediated renal clearance of cimetidine in healthy volunteers.

MATERIALS AND METHODS

Subjects

The protocol for the study was approved by the University of Maryland Institutional Review Board and General Clinical Research Center (GCRC) Advisory Committee. Each subject signed an informed consent prior to enrollment, and all study procedures were conducted in the GCRC at the University of Maryland Medical Center (UMMC).

This was a sequential-design, open-label, two-treatment, single-center study. Eight normal healthy volunteers between the ages of 18 and 40 years were eligible for the study. Subjects underwent a screening physical examination, including serum biochemistries, liver and renal function tests, complete blood cell count, hemoglobin, urinalysis, and review of past medical history. Subjects were excluded from the study if any of following conditions existed: history of acute or chronic kidney disease; evidence of renal insufficiency (calculated screening creatinine clearance < 80 mL/min or serum creatinine > 1.4 mg/dL); allergy to diagnostic iodine compounds (including iothalamate) and drugs such as cimetidine, ranitidine, famotidine, itraconazole, voriconazole, fluconazole, or ketoconazole; history of alcohol or illicit drug abuse; alcohol consumption within 48 hours prior to study; females with a positive pregnancy test, taking oral contraceptives, or lactating; smokers; and use of any concurrent medications within 2 days prior to study day 1.

Materials

Cimetidine (Tagamet®, GlaxoSmithKline), itraconazole (Sporanox® capsules, Janssen Pharmaceutica), and iothalamate (Conray®, Mallinckrodt) were acquired by the UMMC Investigational Drug Pharmacy (IDP). All infusions were prepared by the IDP in 0.9% normal saline on the morning of the study visit. The final infusate concentrations of cimetidine and iothalamate were 1.0 and 2.4 mg/mL, respectively. All analytical-grade high-performance liquid chromatography (HPLC) solvents and reagents were obtained from Fisher Chemical (Fairlawn, NJ) or Sigma-Aldrich (St. Louis, MO).

Study Procedures

In eligible subjects, the study was conducted within 4 weeks after the screening evaluation. Subjects were instructed to avoid intake of grapefruit juice throughout the study period due to its potential effects on P-gp activity.[15] Each subject visited the GCRC on two occasions (day 1 and day 5) after an overnight fast. Vital signs, including heart rate, blood pressure, respiratory rate, and electrocardiogram (ECG), were assessed at arrival and then half-hourly throughout the study visit using continuous ECG monitoring. Intravenous catheters were inserted into forearm veins of each arm of the subjects for blood collection and for intravenous infusion of cimetidine and iothalamate. From 30 minutes before cimetidine administration until the end of each evaluation period, subjects remained in a semireclined

position, except during urine collections. Subjects received 250 mL of water at 30 minutes and 15 minutes prior to the start of the cimetidine infusion. This was followed with a combination of oral or IV fluids (5% dextrose) to maintain fluid intake equal to urine output of the subsequent urine collection throughout the remainder of the study day.

On day 1, subjects received a loading dose of iothalamate (456 mg) over 1 minute followed by a constant-rate infusion of iothalamate (1 mg/min) over 4 hours for continuous measurement of GFR. Subjects also received cimetidine as a loading dose (0.2 mg/kg) over 1 minute followed by a maintenance infusion of 36 mg/h (0.6 mg/min) over 4 hours to achieve an average plasma concentration of approximately 0.7 μg/mL. Blood and urine samples were collected at baseline and every half hour throughout the 4-hour infusion period. Following discontinuation of the infusions, subjects received the first oral dose of itraconazole (200 mg) with a standardized meal. Subjects were then instructed to take 200 mg of itraconazole orally twice daily for 4 days (days 2-5). Subjects were asked to consume the itraconazole capsules with meals and to avoid intake of grapefruit juice. This itraconazole dosing regimen was designed to achieve maximum plasma concentrations of approximately 0.5 to 1.0 μg/mL (0.7-1.4 μM), which have been reported to inhibit P-gp in prior in vitro studies.[16,17]

On day 5, subjects reported to the GCRC after an overnight fast and received the last dose of itraconazole (200 mg). Immediately following the itraconazole dose, iothalamate and cimetidine infusions were initiated as described above. Replacement of fluids was accomplished in a similar manner with IV fluids and oral therapy as described above. Blood samples were taken every 15 minutes for 2 hours (to characterize itraconazole absorption) and then every 30 minutes for the remainder of the study. Urine was collected at baseline and in 30-minute intervals throughout each study visit.

Sample Analysis

All blood samples (7 mL) were collected in heparinized glass test tubes (green top), placed on ice immediately, centrifuged within 15 minutes, split into two plasma aliquots, and frozen at –20°C until analyzed. Urine was placed on ice immediately after collection, volume was accurately recorded, and three 8-mL aliquots were frozen at –20°C until analyzed.

Iothalamate concentrations in plasma and urine were determined by HPLC.[20] Briefly, plasma samples (100 μL) were processed by protein precipitation with 300 μL acetonitrile containing the internal standard beta-hydroxyethyltheophylline (BHET, 12.5 μg/mL), followed by evaporation to dryness under nitrogen and reconstitution in mobile phase (100 μL). The mobile phase consisted of 12% methanol, 3% acetonitrile, and 0.65 mM tetrabutyl ammonium in a 20-mM sodium acetate buffer and was delivered at 1.0 mL/min. Urine samples were diluted in 300 μL of mobile phase prior to injection. The mobile phase used for the urine analysis consisted of 6.5% methanol, 2% acetonitrile, 0.2% tetrahydrofuran, and 3.0 mM tetrabutyl ammonium in 70 mM sodium acetate delivered at 1.4 mL/min. Injection volumes were 20 μL and 10 μL for plasma and urine, respectively. The HPLC system consisted of a Waters 2690 separation module with a Waters dual-mode UV detector set at 254 nm. Separation was achieved using a C_{18} (25 cm × 4.6 mm, 5 μm) column. The assay was linear over the concentration range of 1.0 to 75.0 μg/mL and 25.0 to 1500.0 μg/mL for plasma and urine, respectively. The intra- and interday coefficients of variation for this assay were less than 10%.

Cimetidine concentrations in urine and plasma concentrations were determined by HPLC.[21] Here, a liquid-liquid extraction procedure was employed using 250 μL of plasma, 30 μL of 2 M sodium hydroxide, and 30 μL of internal standard (famotidine 500 μg/mL) with 3 mL of water-saturated ethyl acetate followed by vigorous shaking at 85 cycles/min for 10 minutes. The upper organic phase was separated and evaporated to dryness under a gentle stream of nitrogen with reconstitution in mobile phase. The mobile phase consisted of acetonitrile and heptanesulfonic acid 2.5 g/L in a 20-mM sodium acetate buffer (23:77, v:w:v) adjusted to pH 4.7 and delivered at 1.0 mL/min. Urine processing involved dilution of 100 μL in mobile phase. The injection volume was 50 μL onto a C_{18} (25 cm × 4.6 mm, 5 μm) column. The HPLC system consisted of a Waters 2690 separation module with a Waters dual-mode UV detector set at 228 nm. The assay was linear over the concentration range of 0.1 to 4.0 μg/mL and 10 to 250 μg/mL for plasma and urine, respectively. The within- and between-day coefficients of variation for this assay were less than 10%.

Itraconazole concentrations in plasma were determined using a validated HPLC method.[22] To each 250 μL of plasma samples or standards, 50 μL of 0.3 N $BaOH_2$ and 50 μL of 0.4 N $ZnSO_4$ were added, followed by addition of 1 mL of acetonitrile, mixing, and centrifugation at 3251*g* for 15 minutes. The supernatant was then evaporated to dryness under a gentle stream of nitrogen at 60°C. Samples were reconstituted in 250 μL of mobile phase and injected into the HPLC system.

The HPLC consisted of a Shimadzu LC10AD solvent delivery module, a model SIL10-A automatic sampler attached to a CR-501 integrator. Column effluent was monitored by a Waters 481 LC UV detector set at 263 nm, and separation was achieved using a C_{18} column (250 × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile, 0.05 M phosphate buffer (pH 6.7), and methanol (47:45:8 v:v:v) delivered at 1 mL/min. The assay was linear over a concentration range of 0.02 to 1.5 μg/mL. The inter- and intraday coefficients of variation were less than 10%.

Pharmacokinetic Analysis

The main parameter of itraconazole exposure was AUC obtained during the cimetidine infusion ($AUC_{0\text{-}240\ min}$), which was calculated using the trapezoidal method. The average plasma concentration (C_{avg}) was also determined from the individual concentration-time profiles.

Plasma cimetidine concentration data were modeled by a compartmental analysis with WinNonlin (version 3.1., Pharsight Corporation, Cary, NC). Both one- and two-compartment analyses were evaluated to determine the best-fit model. Various weighting schemes, including a weight of 1, 1/y (y is measured concentration), $1/y^2$, 1/y^ (y^ is predicted concentration), and $1/y\hat{}^2$, were applied. Goodness of fit was assessed on visual inspection of observed versus predicted concentrations, residual distribution versus time, and observed and predicted versus time charts along with the coefficient of variation of parameters (%CV), weighted sum of squares of residuals (WSSR), Akaike's information criteria (AIC), and Schwartz criteria (SC). The model with the lowest %CV, WSSR, AIC, and SC was chosen.

The primary indices of renal excretion of cimetidine were total renal clearance and tubular secretory clearance. Assuming that the mechanisms of renal elimination for cimetidine are filtration and secretion without significant reabsorption, renal clearance can be expressed as

$$CL_r = CL_{gfr} + CL_{sec}, \quad (1)$$

where CL_r is total renal clearance, CL_{gfr} is the clearance due to filtration through the glomerulus (equivalent to renal clearance of iothalamate or GFR), and CL_{sec} is the clearance through tubular secretion. Renal clearance (CL_r) is calculated from plasma and urine data as follows:

$$CL_r = Au(t)/\ AUC(t), \quad (2)$$

where Au is the amount of drug excreted in urine over the time period t, and AUC is the area under the plasma concentration-time curve over the same period. The renal clearance of iothalamate (CL_{gfr}) was calculated by equation (2) using plasma and urine iothalamate data obtained during continuous infusion. The CL_{sec} of cimetidine was then calculated using the CL_r of cimetidine and iothalamate. The CL values were calculated for each urine collection interval (30 min) and presented as mean values obtained from three to five intervals.

Statistical Analysis

Paired *t*-test and Wilcoxon signed ranks test were used to assess differences between the total plasma pharmacokinetic parameters and the renal clearances due to tubular secretion, alone and during itraconazole administration using SPSS (Pharsight Corp., Cary, NC). A sample size of 8 subjects provided 80% power to detect a 15% change in renal clearance for the paired study design, with a 5% chance of a Type I error (two-sided), based on previous observations of within-day renal clearance variability for cimetidine of 14%. Statistical significance was assumed when $p < 0.05$.

RESULTS

Eight healthy subjects completed the study without adverse events. The demographics of the subjects are presented in Table I. All results are reported as mean ± standard deviation.

Plasma Pharmacokinetics of Itraconazole

The mean itraconazole concentration achieved following oral dosing (200 mg twice daily) on day 5 was 0.6 ± 0.09 μg/mL (0.84 μM), which is similar to predicted concentrations based on this dosing regimen. The itraconazole $AUC_{0\text{-}240\ min}$ obtained on day 5 was 137 ± 47 μg•min/mL.

GFR Measurement

The GFR was measured in all subjects using the renal clearance of iothalamate. GFR values obtained on days 1 and 5 were 130 ± 20 mL/min and 125 ± 24 mL/min, respectively ($p > 0.05$).

Table I Demographics and Renal Function Indices of the Study Volunteers

Subject	Sex	Race	Age	Height (cm)	Weight (kg)	Scr (mg/dL)	Cl_{cr} (mL/min)[a]
1	M	I	28	182	70	0.8	136.1
2	M	A	30	165	60	0.7	131.0
3	F	I	23	162	65	0.8	122.2
4	M	C	33	185	84	1.1	113.5
5	M	I	27	175	65	0.8	127.5
6	M	AA	34	185	80	1.3	90.6
7	M	C	28	175	72	1.0	112.6
8	M	I	30	170	73	0.9	123.9
Mean	7 M, 1 F		28	175	68	0.9	120.3
Standard deviation			3	8	4	0.2	14.4

AA, African American; C, Caucasian; I, Indian; A, Asian.
a. Creatinine clearance by Cockcroft-Gault estimation.

Cimetidine Pharmacokinetics

The pharmacokinetic parameters obtained for each subject on days 1 and 5 are listed in Table II. The composite plasma concentration versus time profile is shown in Figure 1. The plasma concentration-time data were fit to a one-compartment open model with zero-order input and first-order elimination. The primary index of exposure during the infusions, $AUC_{0\text{-}240\ min}$, was 136.4 ± 26.7 mg•min/L and 167.3 ± 35.9 mg•min/L on days 1 and 5, respectively ($p > 0.05$). The volume of distribution (Vd) was statistically not different between visits (1.3 ± 0.2 L/kg vs. 1.2 ± 0.3 L/kg). A marginal reduction in the elimination rate constant (K_{el}) was observed (0.007 ± 0.002 min^{-1} vs. 0.006 ± 0.001 min^{-1}, $p > 0.05$).

The total plasma clearance of cimetidine was reduced from 654.5 ± 29.8 mL/min on day 1 to 485.5 ± 23.5 mL/min on day 5 ($p = 0.001$). The renal tubular secretory clearance was reduced in all subjects as shown in Figure 2, with values of 409.8 ± 49.8 mL/min on day 1 and 310.5 ± 67.1 mL/min on day 5, respectively ($p = 0.001$).

DISCUSSION

P-gp is a drug efflux protein with widespread distribution in the body, and many P-gp-mediated drug interactions have been reported.[1,23] For drugs that rely on the kidney's tubular secretory mechanisms, such drug interactions may be significant, leading to altered pharmacokinetics and increased systemic drug exposure. The present study evaluated a renal pharmacokinetic interaction between itraconazole and cimetidine. The results showed that itraconazole reduced the renal tubular secretion of cimetidine by 24%, which confirmed previous reports of this interaction in the in vitro experimental models of renal P-glycoprotein (MDR1-MDCK).[24]

The role of intestinal P-gp in drug absorption and drug interaction has been well characterized, especially for poorly oral bioavailable drugs. In the Caco-2 intestinal cell line, the apical to basolateral transport of cyclosporine was increased in the presence of daunomycin and PSC 833,[25] and vinblastine transport was increased in the presence of verapamil and nifedipine. These findings were confirmed in studies in humans. In 8 cancer patients, coadministration of cyclosporine increased the AUC and decreased the clearance of doxorubicin by 48% and 37%, respectively.[26] In patients receiving oral doxorubicin, concomitant use of PSC 833 resulted in a 50% higher AUC and 50% lower clearance than patients who received doxorubicin alone.[27] The increased systemic exposure of these drugs is most likely due to a combination of increased bioavailability and reduced elimination (hepatic or renal) in these patients.

Renal tubular secretion is the major route of elimination for a wide variety of organic anionic and cationic substances, such as uric acid, penicillin, histamine, and procainamide.[28] The ATP-dependent process requires active transport of substrates into the tubular lumen via membrane-bound transport proteins such as OAT1 and OCT1.[28,29] Affinity of substrates for these transporters is based on their chemical properties and ionization at physiologic pH. P-gp has now been proposed as an additional pathway involved in the renal secretion of lipophilic organic cations. For example, in

Table II Cimetidine Pharmacokinetic Parameters Determined on Days 1 (Baseline) and 5 (following Itraconazole Administration)

	Vd (L/kg)		K_{el} (min^{-1})		CL_T (mL/min)		$CL_{r,sec}$ (mL/min)		$t_{1/2}$ (min)		$AUC_{0\text{-}240\ min}$ (mg•min/L)	
Subject	**Day 1**	**Day 5**	**Day 1**	**Day 5**	**Day 1**	**Day 5**	**Day 1**	**Day 5**	**Day 1**	**Day 5**	**Day 1**	**Day 5**
1	1.5	1.3	0.0060	0.0051	640.9	455.9	433.6	308.9	114	136	123.3	155.5
2	1.6	1.5	0.0067	0.0057	665.5	480.5	568.2	448.9	103	121	106.1	140.2
3	1.6	1.4	0.0064	0.0054	651.5	468.3	419.8	214.2	109	128	139.1	143.5
4	1.4	1.2	0.0052	0.0064	598.1	496.5	408.1	332.4	134	113	110.5	92.5
5	1.3	1.2	0.0083	0.0065	694.3	489.7	498.7	410.2	83	107	136.8	168.2
6	0.8	0.8	0.0108	0.0055	668.4	478.2	335.9	325.1	64	125	156.8	155.3
7	1.1	0.8	0.0087	0.0090	662.7	529.6	299.6	200.1	79	77	182.4	214.3
8	0.9	1.1	0.0114	0.0076	740.1	559.5	314.8	244.3	61	91	172.2	189.0
Mean	1.3	1.2	0.0074	0.0062	654.5	485.5*	409.8	310.5*	98	115	136.4	167.3
Standard deviation	0.2	0.3	0.0019	0.0013	29.8	23.5	49.8	67.1	23	19	26.7	35.9

*$p < 0.05$, day 1 versus day 5.

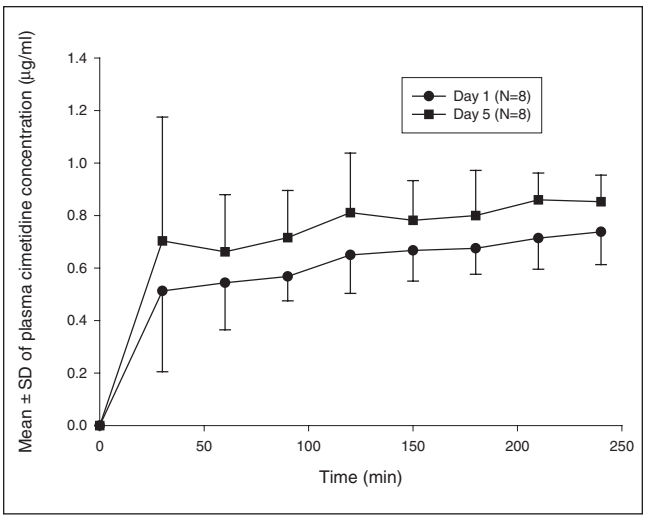

Figure 1. Mean ± standard deviation (SD) of plasma concentrations of cimetidine upon administration of simultaneous bolus (0.2 mg/kg) and 0.6 mg/min continuous infusion in healthy volunteers (n = *8) on day 1 and day 5.*

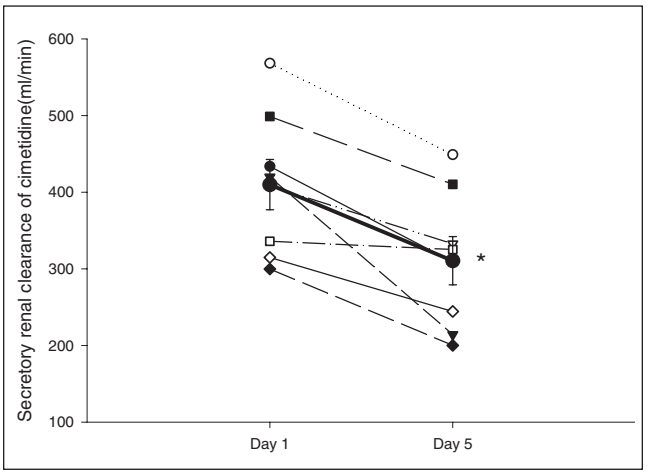

Figure 2. Secretory clearance of cimetidine on days 1 and 5 (n = *8). Solid circles with a dark line indicate the average (± SD) secretory clearance. *p < 0.05, day 1 versus day 5.*

cell culture models of the renal tubule (MDR1-MDCK cells), transport of vinblastine was inhibited by the P-gp inhibitors verapamil and cyclosporin A, suggesting the presence of functional P-gp in renal systems.[30] Recently, fexofenadine was shown to be a substrate of P-gp in the MDR1 overexpressing LLC-PK1 porcine kidney cell line.[31] In P-gp knockout mice (–/– for mdr1), fexofenadine accumulation in kidney was fourfold greater than in the wild type (+/+), suggesting a role of P-gp in the renal elimination of fexofenadine in vivo.[31] These limited data, coupled with the results of the present study, suggest that P-gp is likely involved in the renal excretion of a wide range of drugs that undergo extensive tubular secretion.

Drug interaction studies evaluating the effect of drugs on renal P-gp activity in humans are limited. In healthy volunteers, itraconazole was shown to reduce the renal clearance of the P-gp substrates digoxin and quinidine by 20%[17] and 50%,[18] respectively. However, direct assessment of itraconazole on renal tubular secretion using concurrent GFR measurement was not conducted. We have previously investigated the effect of itraconazole on the transcellular transport of cimetidine in an in vitro system.[24] The MDR1-MDCK cells used in this study were derived from the canine kidney and are known to overexpress P-gp similar to that observed in Caco-2 cells.[32] Pavelic et al[33] have also shown that there is quantitative similarity in P-gp expressed in proximal tubular human tissue samples and the small intestinal samples. We reported that itraconazole, a known inhibitor of P-gp, significantly inhibited the transcellular transport of cimetidine in MDR1-MDCK cells, indicating that P-gp may be involved in its renal excretion. The concentrations of itraconazole used in the study ranged from 0.1 to 2.0 µg/mL, in which maximal inhibition was observed at concentrations above 0.5 µg/mL. Thus, the present study was conducted to evaluate the effect of itraconazole on the renal tubular secretory clearance of the P-gp substrate cimetidine.

The plasma itraconazole concentrations achieved in the present study (approximately 0.6 µg/mL) were similar to concentrations reported to inhibit P-gp in experimental models. This degree of systemic exposure at 400-mg daily dosing was similar to that reported in previous clinical studies assessing itraconazole-mediated drug interactions[18,26] and resulted in significant reductions in $AUC_{0\text{-}240\ min}$ and systemic and renal clearance. These results are similar to those reported by Kaukonen et al,[19] in which a 1.6-fold increase in plasma concentrations and a 2.4-fold increase in the AUC of quinidine were observed following coadministration with itraconazole. Since quinidine is also a CYP3A4 substrate, the observed increase in systemic exposure was likely a result of both CYP3A4 inhibition and decreased renal clearance. In a similar study, itraconazole coadministration decreased the renal clearance of digoxin, a P-gp substrate, by 20%.[18] It is worth noting that metabolites of itraconazole, includ-

ing the antifungally active hydroxylitraconazole, may have some interaction with CYP3A or P-gp, although this remains to be evaluated.

A pure substrate for renal P-gp activity that undergoes extensive renal tubular secretion and can be readily measured in urine has not yet been identified. We chose cimetidine since it is nearly 80% eliminated unchanged in the urine due to extensive tubular secretion and glomerular filtration. Therefore, changes in renal clearance of cimetidine can lead to substantial changes in total systemic clearance. This was observed in the present study, in which itraconazole significantly decreased the renal secretory clearance of cimetidine by 24%. Since there was no change in the glomerular filtration rate between day 1 and day 5 (130 ± 20 mL/min and 125 ± 24 mL/min, respectively, p = 0.44), these findings indicate that increased cimetidine exposure directly resulted from inhibition of renal tubular secretion. Although previous studies with digoxin and quinidine showed decreased renal clearance in the presence of itraconazole, the present study demonstrates a direct effect of itraconazole on renal tubular secretion in light of unchanged filtration.

Although cimetidine is a known inhibitor of CYP enzymes, the formation of its primary S-oxide metabolite is mediated by flavin monooxygenase (FMO3).[34] Therefore, it is unlikely that itraconazole, a known inhibitor of CYP3A, altered the metabolism of cimetidine in the present study. Significant metabolism-based interactions with itraconazole have been reported. Itraconazole caused a 3.3-fold increase in $AUC_{0-\infty}$ and a 68% decrease in systemic clearance of dexamethasone, likely due to CYP inhibition.[35] Similarly, itraconazole decreased the oral clearance of alprazolam from 0.89 to 0.35 mL/min/kg, resulting in a significant increase in $AUC_{0-\infty}$.[36] In the present study, the systemic exposure of cimetidine ($AUC_{0\text{-}240\ min}$) was increased in the presence of itraconazole, which was explained by a significant reduction in renal tubular secretion.

Since use of P-gp modulators in cancer and HIV regimens is becoming increasingly prevalent, it is important to comprehensively evaluate the pharmacokinetic implications that can result from P-gp inhibition. Drug therapy aimed at blocking P-gp-mediated drug absorption may also impair renal (and systemic) drug clearance mechanisms, leading to increased plasma concentrations and potential toxicity. This will be especially important for drugs that are P-gp substrates with narrow therapeutic indices. Cimetidine was chosen in this study, in part, due to its wide therapeutic index and excellent safety profile. However, elevated plasma concentrations of cimetidine have been associated with neurotoxicities such as mental confusion and convulsions.[37] In the present study, we administered cimetidine at a low dose (0.6 mg/min) to minimize the risk of hypotension, bradycardia, and neurotoxicity. However, it is possible that higher doses of cimetidine and itraconazole, in patients with renal impairment, likely would have resulted in a greater increase in systemic exposure than that reported here.

Results from this study highlight the need to consider renal drug interactions as a potential cause of drug toxicity during P-gp modulation therapy. We provide an innovative in vivo research tool that allows simultaneous measurement of GFR, total renal clearance, and renal tubular secretion of a target probe drug (cimetidine) alone and in the presence of an interacting drug (itraconazole). Cimetidine is a safe alternative to digoxin and quinidine for in vivo quantification of renal P-gp activity and for evaluating mechanism-based renal drug interactions. Further studies evaluating the effects of renal disease, drugs and metabolites, and nephrotoxins on P-gp expression and activity are necessary to understand the function of this protein in the kidney and its effect on the pharmacokinetics of drugs.

The authors wish to thank the nursing and support staff at the University of Maryland GCRC for their assistance in conducting this study. The authors have no conflicts of interest.

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