The Pharmacokinetics of the CYP3A Substrate Midazolam After Steady-state Dosing of Delafloxacin

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ABSTRACT

Purpose: Delafloxacin is a novel anionic fluoroquinolone in Phase III development for the treatment of serious skin infections. The objective of this study was to evaluate the effects of delafloxacin on the pharmacokinetics of midazolam, a cytochrome P450 (CYP) 3A substrate.

Methods: CYP3A activity using midazolam as a probe was assessed before and after multiple doses of delafloxacin to reach steady state. In this nonrandomized, open-label, single-sequence, Phase I study, 22 healthy male and female subjects were administered a single 5-mg oral dose of midazolam on days 1 and 8, with oral delafloxacin 450 mg every 12 hours administered from days 3 to 8. Full pharmacokinetic profiles were obtained on days 1 and 8 (midazolam and 1-hydroxymidazolam) and days 3 and 7 (delafloxacin).

Findings: The geometric mean ratios (90% CIs) for $AUC_{0-\infty}$ and C_{max} of midazolam coadministered with delafloxacin versus midazolam alone were 89.4 (83.2–96.0) and 93.6 (83.7–104.6). Similarly, the geometric ratio for the $AUC_{0-\infty}$ of 1-hydroxymidazolam, the primary metabolite of midazolam, was 105.7 (97.7–114.3); the ratio of C_{max} was not equivalent at 116.1 (101.7–132.4), which was outside the CI of 80% to 125%. Multiple doses of oral delafloxacin for 6 days were generally well tolerated.

Implications: Steady-state dosing of delafloxacin produced no significant changes in midazolam pharmacokinetics, except for a small but not clinically relevant change in the C_{max} of 1-hydroxymidazolam. ClinicalTrials.gov identifier: NCT02505997. (*Clin Ther*. 2017;39:1182–1190) © 2017 The Authors. Published by Elsevier HS Journals, Inc.

Key words: CYP3A, delafloxacin, fluoroquinolones, midazolam, pharmacokinetics.

INTRODUCTION

Awareness of potential drug–drug interactions is important in drug development, notably with antibiotics because they are often concomitantly administered with other drugs such as pressors, other antibiotics for empiric therapy, and, in the case of fluoroquinolones, antacids and other multivalent cation-containing drugs.[1] These interactions may increase or decrease the action of either drug and change the rate and extent of absorption and plasma protein binding displacement; microbiologically, they may alter the ability of cell membranes or receptor sites to bind to either drug. Drug–drug interactions can be either pharmacokinetic or pharmacodynamic in nature, which could lead to a change in efficacy and/or toxicity.

Fluoroquinolones are widely used in both inpatient and outpatient settings; thus, clinicians ought to be aware of any drug–drug interactions. Apart from the aforementioned antacids, which reduce the oral absorption of many fluoroquinolones, other interactions have been described in the literature for fluoroquinolones with xanthines, including theophylline and caffeine, warfarin, probenecid, phenytoin, and digoxin.[2–4] Delafloxacin, a novel anionic fluoroquinolone for the treatment of gram-positive and gram-negative infections (including atypicals and anaerobes), is undergoing clinical development for acute bacterial skin and skin

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structure infections and community-acquired bacterial pneumonia.[5–7]

The US Food and Drug Administration's Draft Guidance on Drug Interaction Studies[8] recommends that pharmacokinetic interactions be defined during drug development as part of the drug's safety and effectiveness. Delafloxacin has been studied in in vitro metabolic studies and is not an inhibitor of cytochrome P450 (CYP) 1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4/5, nor is it an inducer of CYP1A2 or CYP2B6. However, delafloxacin is a mild in vitro inducer of CYP3A in cultures of human hepatocytes (data on file, Melinta Therapeutics, Lincolnshire, IL). Midazolam, a benzodiazepine sedative-hypnotic agent, is metabolized by CYP3A and has been adopted as a metabolic probe of CYP3A in humans.[9,10] We therefore studied the in vivo impact of delafloxacin on midazolam pharmacokinetics in healthy subjects to assess any potential for clinical drug–drug interactions. This study also study evaluated the pharmacokinetics of multiple doses of oral delafloxacin.

SUBJECTS AND METHODS

This Phase I, nonrandomized, open-label study was designed to evaluate the effect of multiple oral doses of delafloxacin on the pharmacokinetic profile of a single oral dose of midazolam. The protocol was approved by the investigator's institutional review board (IntegReview IRB, Austin, Texas) before study initiation, and the study was conducted by PPD Phase I Clinic (Austin, Texas) according to the International Conference on Harmonisation of Good Clinical Practice Guidelines. All subjects signed informed consent before admission into study.

Study Population

Twenty-two male and female subjects between 18 and 55 years of age with no history of significant medical problems were enrolled in the study. Subjects abstained from alcohol-, caffeine-, and methylxanthine-containing beverages or food for 96 hours before entry into the clinical study on day −1 until discharge on day 9. Subjects were either nonsmokers or abstained from any nicotine-containing products for a minimum of 180 days before admission. Subjects were excluded if they had received any investigational drug within 8 weeks before administration of the first dose of the study drug, within 6 months for biologic therapies, or within 5 half-lives of the investigational drug, whichever time period was longer; or previously received delafloxacin in a clinical study; had a positive urinary test result for amphetamines, barbiturates, benzodiazepines, cocaine metabolites, and other illegal substances at screening or on day −1; had a positive screening test result for hepatitis B, C, and/or HIV; and were taking prescription or over-the-counter medication (except for acetaminophen) within 2 weeks of the start of the study drug (4 weeks with drugs known to inhibit or induce CYP enzymes). Additional exclusion criteria included oral/intravenous antibiotics within 4 weeks of the first dose; routine use of >2 g of acetaminophen daily; any medical or surgical condition that might have interfered with the absorption, distribution, metabolism, or excretion of either drug; consumption of any food or drink that could influence CYP enzyme activity or transporters within 7 days; blood donation (400 mL) within 30 days; strenuous activity within 4 days; clinically significant gastrointestinal disease; and allergies or reactions to the study drugs.

Study Design

Subjects underwent screening evaluations to determine eligibility within 28 days before admission into the clinical unit on day −1 for baseline assessments. On day 1, subjects received a single oral 5-mg dose of midazolam after an overnight fasting period of 10 hours, which continued for 4 hours after drug administration. On day 3, subjects were administered oral delafloxacin 450 mg every 12 hours for 5 days, concluding with a single dose in the morning of day 8. A single oral dose of midazolam was coadministered on day 8 under the same fasting conditions as day 1. Subjects were confined to the clinical unit until discharge on day 9. As with other quinolones, concurrent administration of oral delafloxacin with cations (eg, calcium, magnesium, or aluminum antacids) was avoided or delafloxacin was administered at least 2 hours before or 6 hours after taking these products.

Safety and tolerability were assessed throughout the study period by monitoring and recording adverse events, clinical laboratory results (hematology [including coagulation parameters], serum chemistry, and urinalysis), vital sign measurements, 12-lead ECG results, and physical examination findings.

Pharmacokinetic Blood Samples

Heparinized blood samples for the determination of plasma concentrations of midazolam and 1-hydroxymidazolam were collected before dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after dosing on days 1 and 8. Serial blood samples for the determination of plasma concentrations of delafloxacin were collected in chilled (2°C–8°C) tubes containing dipotassium EDTA before the morning dose and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours after the morning dose on days 3 and 7. The 12-hour sample was obtained before the evening dose of delafloxacin. Blood samples were also collected just before the morning dose of delafloxacin on days 4, 5, 6, and 8.

All plasma samples were assayed (PPD Bioanalytical Laboratory, Richmond Virginia) using validated LC-MS/MS assays for delafloxacin, midazolam, and 1-hydroxymidazolam (data on file, Melinta Therapeutics, Lincolnshire, IL). Briefly, delafloxacin was quantitated in plasma samples (dipotassium EDTA) using a validated LC-MS/MS method with a nominal concentration range of 5 to 5000 ng/mL. Sample preparation was performed by supported liquid phase extraction on Isolute 96-well SLE+ plates (Biotage, Uppsala, Sweden). Analysis of the final extract was performed with HPLC by using an XBridge C_{18} column (Waters Corporation, Milford, Massachusetts) and MS/MS detection using positive ion electrospray. The method demonstrated acceptable linearity, accuracy, and precision. Delafloxacin stability was demonstrated in standard freeze/thaw and room temperature tests and in samples frozen at –20 °C and lower for up to 484 days. Lower and upper limits of quantitation were 0.005 μg/mL and 5.00 μg/mL for delafloxacin, respectively. The assay precision for quality control (QC) samples ranging from 0.015 to 10.0 μg/mL was 2.00% to 15.0%. The difference from theoretical value (accuracy) for the same QC sample range was –1.50% to 3.99%.

Midazolam and 1-hydroxymidazolam were quantitated in heparinized plasma samples by using a validated LC-MS/MS method with a nominal concentration range of 0.1 to 100 ng/mL for midazolam and 0.1 to 50 ng/mL for 1-hydroxymidazolam. The analytes were isolated by liquid–liquid extraction using methyl t-butyl ether, followed by evaporation under nitrogen stream and reconstitution with ammonium acetate/water/methanol. The reconstituted sample was analyzed via HPLC and MS/MS detection by using positive ion electrospray. A linear, 1/concentration squared, weighted, least-squares regression algorithm was used to quantitate unknown samples. Lower and upper limits of quantitation were 0.1 and 100 ng/mL for midazolam and 0.1 ng/mL and 50 ng/mL for 1-hydroxymidazolam, respectively. The assay precision for the midazolam QC samples ranging from 0.300 to 75.0 ng/mL was 1.72% to 3.05%. The difference from theoretical value (accuracy) for the same midazolam QC sample range was –1.64% to 5.09%. The assay precision for 1-hydroxymidazolam QC sample ranging from 0.300 to 37.5 μg/mL was 1.26% to 5.83%. The difference from theoretical value (accuracy) for the same QC sample range was 0.398% to 2.67%.

Pharmacokinetic Analyses

The following pharmacokinetic parameters were calculated from plasma concentration data for each subject by using standard noncompartmental methods: C_{max} and T_{max}; terminal elimination rate constant (λ_z) and terminal $t_{½}$; AUC, including $AUC_{0-\infty}$, AUC_{0-t}, AUC_{0-12}, and AUC_{0-24}; CL/F from the ratio of dose to $AUC_{0-\infty}$; and V_z/F based on λ_z.

Statistical Analysis

All analyses were conducted by using SAS version 9.2 (SAS Institute, Inc, Cary, North Carolina) or Phoenix WinNonlin Version 6.2.1 (Pharsight Corporation, St. Louis, Missouri). In general, continuous data were summarized by presenting the number of subjects, mean, SD, median, and range. Categorical data were summarized by presenting the number (frequency) and percentage of subjects at each level of response. Demographic information collected at screening was summarized and listed.

To assess the effect of delafloxacin on the pharmacokinetics of midazolam and 1-hydroxymidazolam, an ANOVA was performed on the natural log-transformed AUC_{0-t}, AUC_{0-24}, $AUC_{0-\infty}$, and C_{max} of midazolam and 1-hydroxymidazolam to estimate the ratio of geometric least squares means between the treatments and their 90% CIs. The ANOVA model included treatment as a fixed effect and subject as a random effect. Absence of the effect of delafloxacin on the pharmacokinetics of midazolam was concluded if the 90% CIs for the test-to-reference ratio (midazolam + delafloxacin/midazolam alone) of geometric means

of $AUC_{0-\infty}$ and C_{max} were entirely contained within the criterion interval of 80% to 125%.

Delafloxacin steady-state pharmacokinetics were assessed based on the predose concentrations from days 4, 5, 6, 7, and 8. A linear mixed model using day as fixed effect and subject as a random effect on the natural log-transformed predose values was performed to evaluate whether steady state was achieved by using the Helmert transformation approach. The comparison began with day 4 versus days 5 through 8. The ratio of geometric least squares means and its 95% CI were presented for the comparison.

RESULTS

Subject Disposition and Demographic Characteristics

Eight female and 14 male healthy subjects received delafloxacin and midazolam, had evaluable pharmacokinetic data, and were included in the midazolam and delafloxacin pharmacokinetic analysis populations. Subject demographic and baseline characteristics are summarized in Table I. No previous or concomitant medications, nor medical or treatment procedures, were reported. All 22 subjects completed the study.

Table I. Summary of subject demographic and baseline characteristics.

Characteristic	Overall (N = 22)
Age, y	
Mean (SD)	37.1 (10.4)
Range	18–50
Sex	
Female	8 (36.4%)
Male	14 (63.6%)
Race	
White	12 (54.5%)
Black or African American	10 (45.5%)
Ethnicity	
Hispanic or Latino	11 (50.0%)
Not Hispanic or Latino	11 (50.0%)
Height, cm	
Mean (SD)	170 (12.0)
Range	149–193
Weight, kg	
Mean (SD)	74.7 (14.4)
Range	50.1–97.1
Body mass index, kg/m^2	
Mean (SD)	25.6 (2.82)
Range	19.9–30.7

Midazolam and 1-Hydroxymidazolam Pharmacokinetics

The mean plasma concentrations of midazolam and 1-hydroxymidazolam versus time are plotted in Figure 1. After a single dose of midazolam, mean plasma concentrations of midazolam and 1-hydroxymidazolam were similar regardless of whether midazolam was coadministered with delafloxacin, except the terminal phase of midazolam alone displayed a longer biphasic elimination of 1-hydroxymidazolam. Mean plasma concentrations rapidly reached a peak at ~0.5 hour followed by a biphasic decrease in concentrations over 24 hours.

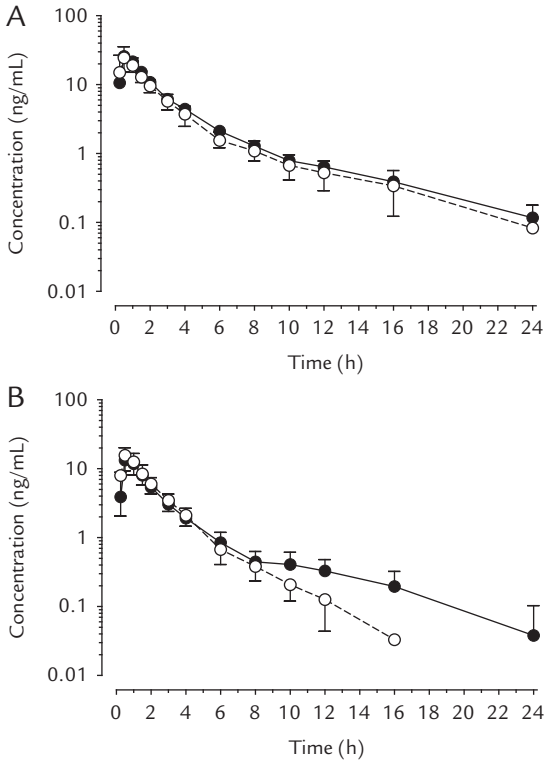

Figure 1. Mean (SD) plasma concentrations of (A) midazolam and (B) 1-hydroxymidazolam versus time when administered alone (•) or coadministered with delafloxacin (○).

Table II. Mean (%CV) pharmacokinetic parameters of midazolam and 1-hydroxymidazolam.

Parameter	Midazolam		1-Hydroxymidazolam	
	Midazolam Alone (n = 22)	Midazolam + Delafloxacin (n = 22)	Midazolam Alone (n = 22)	Midazolam + Delafloxacin (n = 22)
AUC_{0-t}, h · ng/mL	64.4 (31.2)	57.6 (29.4)	31.1 (36.9)	32.3 (28.2)
AUC_{0-24}, h · ng/mL	64.7 (31.0)	57.9 (28.9)	31.6 (36.2)	32.6 (28.2)
$AUC_{0-\infty}$, h · ng/mL	65.9 (31.7)	58.8 (29.3)	31.6 (37.9)*	32.8 (28.1)
C_{max}, ng/mL	27.5 (35.7)	25.8 (37.9)	15.1 (42.3)	16.7 (32.8)
T_{max}, h†	0.50 (0.25, 1.00)	0.50 (0.25, 1.50)	0.50 (0.50, 1.00)	0.50 (0.50, 1.50)
$t_{½}$, h	4.67 (28.5)	4.63 (26.3)	4.95 (34.1)*	2.68 (33.2)
CL/F, L/h	82.7 (30.6)	92.6 (31.7)	NA	NA
V_z/F, L	535 (31.0)	598 (29.7)	NA	NA

NA = not applicable.
*n = 20.
†Median (minimum, maximum) values are presented.

The mean plasma pharmacokinetic parameters of midazolam and 1-hydroxymidazolam are listed in Table II. After a single dose of midazolam, individual $AUC_{0-\infty}$ values of midazolam did not seem to be meaningfully changed by coadministration of delafloxacin from day 1 to day 8 (Figure 2). Mean C_{max} values and median T_{max} and $t_{½}$ values were similar, and the mean CL/F and V_z/F values were not noticeably higher when midazolam was coadministered with delafloxacin compared with midazolam alone.

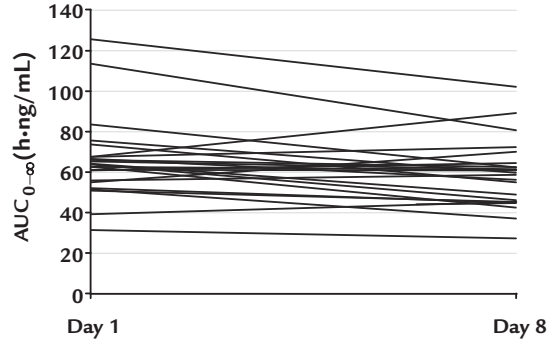

Figure 2. Individual midazolam AUC values before (day 1) and after (day 8) administration of delafloxacin.

The results presented in Table III indicate that delafloxacin did not affect the C_{max} and $AUC_{0-\infty}$ of midazolam; the 90% CIs of the geometric mean ratios were all included within the prespecified no-effect bounds (80%–125%). Similar conclusions were found with the 1-hydroxymidazolam pharmacokinetic parameters (Table IV) with the exception of C_{max}, whereas the 90% CI of the geometric LS means (116.05 [101.700–132.430]) was not contained within the 80% to 125% interval.

Delafloxacin Pharmacokinetics

The mean plasma delafloxacin concentration–time profiles on days 3 and 7 are plotted in Figure 3. After the initial oral dose of 450-mg delafloxacin on day 3 in the morning, delafloxacin plasma concentrations peaked rapidly, resulting in mean C_{max} and median T_{max} values of 7.17 μg/mL and 0.75 hour, respectively, followed by a slower elimination phase over 12 hours. After multiple dosing from days 3 to 7, mean delafloxacin plasma concentrations increased with a measurable predose concentration and slightly higher terminal phase concentrations. The overall shape of the steady-state concentration–time profiles of delafloxacin on day 7 was comparable to that of day 3

Table III. Statistical analysis of midazolam pharmacokinetic parameters.

Parameter	Treatment	N	Geometric LS Means	Ratio (%) of Geometric LS Means	90% CI of the Ratio
AUC_{0-t}, h · ng/mL	Midazolam	22	61.8		
	Midazolam + delafloxacin	22	55.2	89.4	83.2–96.1
AUC_{0-24}, h · ng/mL	Midazolam	22	62.1		
	Midazolam + delafloxacin	22	55.6	89.5	83.4–96.1
$AUC_{0-\infty}$, h · ng/mL	Midazolam	22	63.1		
	Midazolam + delafloxacin	22	56.4	89.4	83.2–96.0
C_{max}, ng/mL	Midazolam	22	25.7		
	Midazolam + delafloxacin	22	24.1	93.6	83.7–105

LS = least squares.

(single dose). Visual inspection of the mean trough plots and the individual trough plots of delafloxacin indicates that mean predose delafloxacin concentrations were ~0.5 to 1.5 μg/mL between days 4 and 8. Statistical analysis of the trough concentrations from day 4 to day 8 revealed that levels were statistically different from the previous day through day 7 but not day 7 compared with day 8 troughs; these findings suggest steady state had been achieved after 4 days of dosing (data not shown).

Mean delafloxacin pharmacokinetic parameters on day 3 (single dose) and day 7 (steady state) are listed in Table V. Slight accumulation was observed after 4 days of dosing; mean AUC_{0-12} values were 35% greater compared with single-dose exposure. Terminal phase kinetics changed slightly at steady state with CL/F of 16.8 L/h on day 7 compared with 20.6 L/h after a single dose of delafloxacin. C_{max} and V_z/F values were relatively similar between single-dose exposure and those values at steady state.

Safety Assessment

All 22 subjects were assessed for safety after administration of single doses of midazolam and multiple dosing of delafloxacin. Overall, both drugs were safe and well tolerated. There were 6 treatment-emergent

Table IV. Statistical analysis of 1-hydroxymidazolam pharmacokinetic parameters.

Parameter	Treatment	N	Geometric LS Means	Ratio (%) of Geometric LS Means	90% CI of the Ratio
AUC_{0-t}, h · ng/mL	Midazolam	22	28.9		
	Midazolam + delafloxacin	22	30.8	107	98.8–115
AUC_{0-24}, h · ng/mL	Midazolam	22	29.4		
	Midazolam + delafloxacin	22	31.1	106	98.0–114
$AUC_{0-\infty}$, h · ng/mL	Midazolam	20	29.6		
	Midazolam + delafloxacin	22	31.3	106	97.7–114
C_{max}, ng/mL	Midazolam	22	13.3		
	Midazolam + delafloxacin	22	15.5	116	102–132

LS = least squares.

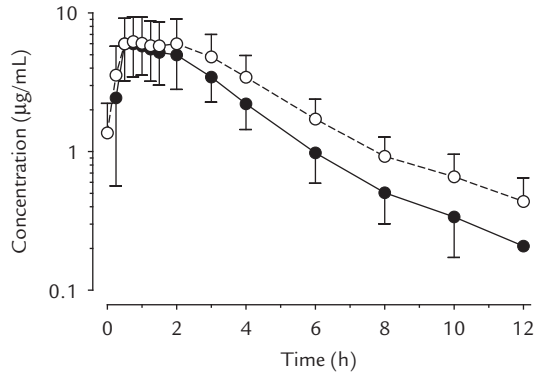

Figure 3. Mean (SD) plasma concentrations of 450-mg delafloxacin on day 3 (•) and on day 7 (○) after 4 days of 450-mg delafloxacin administered every 12 hours.

adverse events reported in 5 of 22 subjects; 3 subjects (13.6%) reported diarrhea, which was mild in severity and considered probably related to delafloxacin. One subject did not receive the second daily dose of delafloxacin on day 8 per investigator discretion due to an out-of-range serum creatinine level, which subsequently resolved; the subject still participated in the delafloxacin and midazolam pharmacokinetic analyses and was not discontinued from the study. There were no deaths, serious adverse events, or treatment-related adverse events leading to study discontinuation. Furthermore, there were no treatment-emergent adverse events after the coadministration of delafloxacin and midazolam.

DISCUSSION

Fluoroquinolones, in particular ciprofloxacin, levofloxacin, grepafloxacin, norfloxacin, and clinafloxacin, have been associated with inhibition of CYP1A2.[4,11,12] In vitro studies conducted with human liver microsomes showed that delafloxacin is not an inhibitor of CYP1A2 nor any of the other isozymes (CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4/5). However, delafloxacin was a mild inducer of CYP3A in cultures of human hepatocytes. Because delafloxacin may be coadministered with drugs that are substrates of CYP3A, such as midazolam, we evaluated the effect of multiple doses of oral delafloxacin on the pharmacokinetic profile of a single oral dose of midazolam.

Midazolam systemic exposures as measured by $AUC_{0-\infty}$ and C_{max} were equivalent when oral midazolam was administered after 5 days of oral delafloxacin BID. The 90% CIs for the ratio of mean midazolam $AUC_{0-\infty}$ and C_{max} were contained within 80% and 125%, satisfying the criterion for lack of effect. Analysis of delafloxacin trough levels showed that delafloxacin was generally at steady state after 4 days (day 7 of the study), which is adequate to assess the interaction. Furthermore, the dose-corrected midazolam $AUC_{0-\infty}$ and C_{max} values calculated in our study are similar to values found in the literature.[13–17] The geometric ratio of C_{max} for 1-hydroxymidazolam at 116.1 (101.7–132.4) was not equivalent, as it was just outside the CI of 80% to 125%; however, total

Table V. Mean (%CV) pharmacokinetic parameters of delafloxacin.

Parameter	Day 3 (Single Dose) (N = 22)	Day 7 (Steady State) (N = 22)
AUC_{0-t}, h . µg/mL	22.7 (27.4)	30.8 (37.1)
AUC_{0-12}, h . µg/mL	22.7 (27.4)	30.8 (37.1)
$AUC_{0-\infty}$, h . µg/mL	23.5 (26.1)	NA
C_{max}, µg/mL	7.17 (28.1)	7.45 (42.4)
T_{max}, h*	0.75 (0.50, 4.00)	1.00 (0.50, 6.00)
$t_{½}$, h	2.46 (20.9)	2.92 (25.5)†
C_{trough}, µg/mL	NA	1.36 (63.5)
CL/F, L/h	20.6 (29.4)	16.8 (38.9)
V_z/F, L	74.4 (41.0)	70.8 (47.9)†

NA = not applicable.
*Median (minimum, maximum) values are presented.
†n = 21.

exposures (AUC) were equivalent, which suggests that, overall, delafloxacin did not increase metabolism to 1-hydroxymidazolam. In addition, the mean terminal phase kinetics were unchanged when midazolam was coadministered with delafloxacin. Interestingly, the mean $t_{½}$ of 1-hydroxymidazolam, the primary metabolite of midazolam, decreased by ~46% (2.68 versus 4.95 hours) when midazolam was coadministered with delafloxacin. The reason for the difference is not completely understood but may be a result of a number of samples below the lower limit of quantitation (0.1 ng/mL).

The secondary objective of the present study was to evaluate the pharmacokinetics, safety, and tolerability of multiple oral doses of 450-mg delafloxacin to reach steady state in healthy male and female subjects. After multiple dosing of oral delafloxacin, steady state was reached after 4 days of dosing, and mean $AUC_{0–12}$ increased 35% compared with the mean $AUC_{0–12}$ after a single oral dose. The terminal $t_{½}$ as determined in this study (~2.5 hours) would predict a shorter time to steady state. The delafloxacin $t_{½}$ was determined based on data available over the first 12 hours after dosing. The limited data may account for the shorter $t_{½}$ observed in this study compared with a previous study in which 48 hours of data were available to characterize delafloxacin pharmacokinetics, with delafloxacin's terminal $t_{½}$ ranging from 5.5 to 7.7 hours.[18]

There were modest decreases in delafloxacin CL/F and V_z/F and increases in C_{max} and $t_{½}$ after multiple dosing. As mentioned earlier, steady state with regard to delafloxacin had been achieved after 4 days of dosing, indicating that the effect of delafloxacin on the pharmacokinetics of midazolam is deemed maximal. Other than $t_{½}$, the pharmacokinetics of delafloxacin and safety in our study are consistent with those reported previously.[18] Mean $AUC_{0–\infty}$, C_{max}, T_{max}, and CL/F values were similar whether after single or multiple doses of delafloxacin. Self-limiting, mild diarrhea was the most commonly reported treatment-emergent adverse event in ~14% of the subjects.

Delafloxacin has been shown to be effective in the treatment of serious gram-positive acute bacterial skin and skin structure infections[19,20] and is undergoing study in the treatment of patients with community-acquired bacterial pneumonia. In all of these disease states, an understanding of drug–drug interactions is critically important from the perspectives of safety, efficacy, and health economics. The number of drugs known to be substrates, inhibitors, or modifiers of CYP is considerable, and CYP3A represents 40% to 60% of all CYP isozymes.[11] It is critically important to clinicians to have information demonstrating a lack of a clinically relevant pharmacokinetic interaction by delafloxacin on CYP3A and other CYP isozymes.

CONCLUSIONS

We evaluated the clinical relevance of the earlier in vitro research with human hepatic enzymes that showed weak inhibition of CYP3A by delafloxacin. Using midazolam, an established CYP3A probe, clinical pharmacokinetics were similar with and without coadministration of therapeutic doses of delafloxacin, demonstrating no clinically meaningful induction of CYP3A.

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CONFLICTS OF INTEREST

Melinta Therapeutics was responsible for the design, conduct, analysis, and interpretation of the data.

Dr. Wood-Horrall was the principal investigator of the study. Drs. Paulson and Hoover are clinical pharmacology consultants on behalf of Melinta Therapeutics. Ms. Quintas, Ms. Lawrence, and Dr. Cammarata are employed by Melinta Therapeutics. The authors have indicated that they have no other conflicts of interest regarding the content of this article.

REFERENCES

1. Hartshorn EA, Lomaestro BM, Bailie GR. Quinolone-cation interactions: a review. *Ann Pharmacother*. 1991;25:1249–1258.
2. Marchbanks CR. Drug-drug interactions with fluoroquinolones. *Pharmacother*. 1993;13:23S–28S.
3. Fish DN. Fluoroquinolone adverse effects and drug interactions. *Pharmacother*. 2001;21:253S–272S.
4. Bolhuis MS, Panday PN, Pranger AD, et al. Pharmacokinetic drug interactions of antimicrobial drugs: a systemic review on oxazolidinones, rifamycines, macrolides, fluoroquinolones, and beta-lactams. *Pharmaceut*. 2011;3:865–913.

5. Van Bambeke F. Delafloxacin, a non-zwitterionic fluoroquinolone in Phase III of clinical development: evaluation of its pharmacology, pharmacokinetics, pharmacodynamics, and clinical efficacy. *Future Microbiol*. 2015;10:1111–1123.
6. Bassetti M, Della Siega P, Pecori D, et al. Delafloxacin for the treatment of respiratory and skin infections. *Expert Opin Investig Drugs*. 2015;24:433–442.
7. Bassetti M, Righi E, Carnelutti A. New therapeutic options for respiratory tract infections. *Curr Opin Infect Dis*. 2016;29:178–186.
8. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (US). *Draft Guidance for Industry: Drug interaction studies—study design, data analysis, implications for dosing, and labeling recommendations*. 2012. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf. Accessed August 17, 2016.
9. Thummel KE, Shen DD, Podoll TD, et al. Use of midazolam as a human cytochrome P450 3A probe. I. In vitro-in vivo correlations in liver transplant patients. *J Pharmacol Exp Ther*. 1994;271:549–556.
10. Galetin A, Ito K, Hallifax D, Houston JB. CYP3A4 substrate selection and substitution in the prediction of potential drug-drug interactions. *J Pharmacol Exp Ther*. 2005;314:180–190.
11. Johnson MD, Newkirk G, White JR. Clinically significant drug interactions. *Postgrad Med*. 1999;105:193–195.
12. McLellan RA, Drobitch RK, Monshouwer M, Renton KW. Fluoroquinolone antibiotics inhibit cytochrome P450-mediated microsomal drug metabolism in rat and human. *Drug Metab Dispos*. 1996;24:1134–1138.
13. Schmitt C, Hofmann C, Riek M, et al. Effect of saquinavir-ritonavir on cytochrome P450 3A4 activity in healthy volunteers using midazolam as a probe. *Pharmacother*. 2009;29:1175–1181.
14. Abel S, Russell D, Whitlock LA, et al. Effect of miraviroc on the pharmacokinetics of midazolam, lamivudine/zidovudine, and ethinyloestradiol/levonorgestrel in healthy volunteers. *Br J Clin Pharmacol*. 2008;65(Suppl 1):19–26.
15. Shord SS, Lingtak-Neander C, Camp JR, et al. Effects of oral clotrimazole troches on the pharmacokinetics of oral and intravenous midazolam. *Br J Clin Pharmacol*. 2010;69:160–166.
16. Pentikis HS, Connolly M, Trapnell CB, et al. The effect of multiple-dose, oral rifaximin on the pharmacokinetics of intravenous and oral midazolam in healthy volunteers. *Pharmacother*. 2007;27:1361–1369.
17. Jang G, Kaufman A, Lee E, et al. A clinical therapeutic protein drug-drug interaction study: coadministration of denosumab and midazolam in postmenopausal women with osteoporosis. *Pharma Res Per*. 2014;2:1–11.
18. Hoover R, Hunt T, Benedict M, et al. Single and multiple ascending-dose studies of oral delafloxacin: effects of food, sex, and age. *Clin Ther*. 2016;38:39–52.
19. Cammarata S, Gardovskis J, Farley B, et al. Results of a global Phase 3 study of delafloxacin (DLX) compared to vancomycin with aztreonam (VAN) in acute bacterial skin and skin structure infections (ABSSSI). *Open Forum Infect Dis*. 2015;2(suppl 1):776. Abstr 776. Program and abstracts of the Infectious Diseases of America. San Diego, CA.
20. O'Riordan W, McManus A, Teras J, et al. A global phase 3 study of delafloxacin compared to vancomycin/aztreonam in patients with acute bacterial skin and skin structure infections. *Open Forum Infect Dis*. 2016;3(suppl 1):1347.

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