Fidaxomicin Attains High Fecal Concentrations With Minimal Plasma Concentrations Following Oral Administration in Patients With *Clostridium difficile* Infection

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Fidaxomicin has recently been approved for the treatment of *Clostridium difficile* infection (CDI). As part of phase III studies, plasma and fecal samples were analyzed for concentrations of fidaxomicin and its metabolite, OP-1118. Plasma samples were collected before and after dose receipt on the first and last days of therapy, and fecal samples were collected on the last day of therapy. Samples were analyzed for fidaxomicin and OP-1118 (metabolite), using validated liquid chromatography/tandem mass spectrometric methods. Plasma concentrations were low for both fidaxomicin (mean [± standard deviation {SD}], 22.8 ± 26.7 ng/mL and 28.5 ± 33.4 ng/mL on the first and last days of therapy, respectively) and OP-1118 (mean [±SD], 44.5 ± 50.4 ng/mL and 85.6 ± 131 ng/mL, respectively). In contrast, fecal levels were >1000 μg/g for fidaxomicin and >800 μg/g for OP-1118. Fidaxomicin mean fecal levels were >5000 times the minimum inhibitory concentration for *C. difficile* of 0.25 μg/mL.

Fidaxomicin is a new antibiotic, derived from fermentation, that was recently approved for the treatment of *Clostridium difficile* infection (CDI). Currently, few options exist for the treatment of this disease, which has been growing in incidence and severity in recent years [1, 2]. Oral vancomycin is the only antibiotic approved by the US Food and Drug Administration to treat CDI, although the most frequently used therapy is oral metronidazole. Both therapies are associated with high rates of disease recurrence.

Vancomycin is considered poorly absorbed, although sporadic reports have suggested that in the context of CDI, even oral vancomycin can be absorbed to a great enough extent to cause systemic vancomycin reactions, such as red man syndrome and allergic rash [3]. It achieves fecal concentrations >2000 μg/g, far in excess of the 90th percentile of the minimum inhibitory concentration (MIC$_{90}$; 1 μg/mL for *C. difficile*) [4]. However, the fecal levels achieved are high enough that organisms generally considered to be vancomycin insensitive, such as the gram-negative *Bacteroides fragilis* group, can be affected both in vitro [5] and in vivo [6].

In contrast, metronidazole is highly absorbed from the intestinal tract, and fecal levels are reported to be below detection in the absence of diarrhea and to be in the low microgram-per-gram range in the presence of diarrheal disease [7]. The MIC$_{90}$ of metronidazole for *C. difficile* is 1 μg/mL [4], and achieving supra-MICs in the colon during CDI may not be possible if the MIC shifts upward slightly, as has been sporadically reported [8, 9].

Furthermore, metronidazole has a spectrum of activity that encompasses most anaerobes, and the collateral damage to the normal gut flora that are considered necessary for keeping *C. difficile* in check may partially explain why clinical outcomes have been found to be poorer for metronidazole than for vancomycin, particularly in severely ill patients with CDI [10].

An ideal therapy for CDI would be one that has minimal absorption, because systemic levels evidently are not necessary to treat this infection (which is constrained to the gut). Limiting systemic exposure is desirable from a drug safety perspective. Furthermore, such a therapy should be highly active against the pathogen but have limited activity against normal members of the gut flora, to minimize damage to the commensal organisms that are believed to competitively limit *C. difficile* growth and toxin production.

Fidaxomicin is a new narrow-spectrum antibiotic with no discernable activity against gram-negative gut organisms such as *Bacteroides* species, even at milligram-per-gram concentrations, and variable activity against gram-positive organisms, with excellent activity against *C. difficile* [5, 11]. In healthy volunteers and in a small study of patients with CDI, it was shown to attain high fecal concentrations with low plasma concentrations. Its metabolite, OP-1118, is a desisobutyryl hydrolysis product, which also has activity against *C. difficile* [12]. However, the populations in these studies were small, and the method used for plasma had a lower limit of quantification (LLOQ) of 5 ng/mL [13]. This analysis of the pharmacokinetic results of 2 phase III trials provides a larger data set, using a plasma assay with a much higher sensitivity (0.2 ng/mL).

METHODS

Study Design
Subjects with CDI were enrolled in a pair of double-blind, randomized, controlled clinical trials to study the efficacy of 10 days of oral fidaxomicin (200 mg, administered every 12 hours) versus that of oral vancomycin (125 mg, administered every 6 hours). Subjects were eligible to enroll if they had >3 unformed bowel movements in the 24 hours preceding enrollment and had a positive test result for *C. difficile* toxin. Subjects were ineligible if they had had >1 occurrence in the previous 3 months, had immediately life-threatening disease (CDI or other), or had received >24 hours of CDI-effective therapy immediately preceding enrollment. Plasma samples were collected from subjects on the first and last days of therapy, before dose receipt and approximately 3–5 hours (intended) after dose receipt. Fecal samples were collected on the last day of treatment.

Analytical Methods
Plasma and fecal samples were analyzed for fidaxomicin and its main metabolite, OP-1118, by validated liquid chromatography/ tandem mass spectrometry (MS/MS) methods [14]. Methylated fidaxomicin (OP-1393) was synthesized by Optimer Pharmaceuticals and used as an internal standard for both matrices. Fidaxomicin, OP-1118, and (spiked) OP-1393 were extracted from human plasma, using protein precipitation with acetonitrile plus solid-phase extraction (SPE), and evaporated to dryness. The residue was reconstituted and analyzed through reverse-phase high-performance liquid chromatography (HPLC)/MS/ MS (LLOQ, 0.2 ng/mL). Entire fecal samples were homogenized with an acetonitrile/acetic acid solution. An aliquot of the homogenate was diluted with water/acetonitrile (90:10, vol/vol), and OP-1393 was added as internal standard. This mixture then was extracted using SPE well plates and analyzed by reverse-phase HPLC/MS/MS (LLOQ, 50 ng/mL for the homogenate).

Statistical Methods
Descriptive statistics for plasma and fecal concentrations are presented for the safety population, which consisted of all subjects who received ≥1 dose and had ≥1 postdose safety assessment. Mean (± standard deviation [SD]), median, and range are calculated for samples greater than the LLOQ (LLOQ, 0.2 ng/ mL for plasma and 50 μg/g for feces); no values were imputed for samples less than the limit of quantification. Postdose plasma samples were requested to be obtained 3–5 hours following dosing (to approximate the timing of peak plasma concentrations of fidaxomicin and metabolite observed in a prior study), and data are presented for samples collected within the proper window. Fecal samples were collected within 24 hours of receipt of an active dose on the last day of treatment.

Renal insufficiency was evaluated using the baseline estimated creatinine clearance rate as a measure of the glomerular filtration rate, calculated using the Cockcroft-Gault equation, and severity cutoffs suggested by the National Kidney Foundation [15]. Severity of CDI was estimated by applying a modified version of the recent European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines [16] to the data collected (guidelines were modified by including only variables captured in the clinical data set). Specifically, subjects were considered severe if they had ≥1 of the following: leukocyte count $>15 \times 10^9$/L, creatinine level ≥1.5 mg/dL, and temperature >38.5°C. Comparisons of plasma concentrations in specific populations (age and estimated creatinine clearance rate) were made using the Wilcoxon rank sum test.

RESULTS

Population and Samples
A total of 1147 patients received ≥1 dose of drug and were included in the safety population for the 2 phase III trials; 564 patients received fidaxomicin. A total of 3372 unique plasma samples were received from the 2 trials; approximately half

were from the vancomycin arm and were not analyzed. Of samples from the fidaxomicin arm, 427 were collected 3–5 hours after an active dose and had reportable values for fidaxomicin (435 values were reportable for OP-1118). In the first and second trials, 791 fecal samples were obtained, of which approximately half were from the vancomycin arm and not analyzed. Other samples that were obtained outside the desired window also were excluded. Therefore, 175 fecal samples had reportable values for fidaxomicin, and 172 had values reportable for OP-1118.

Demographic and Clinical Characteristics

Among patients who were administered fidaxomicin, the mean age was 62.1 years, most (57.1%) were women, most (63.5%) were inpatients at admission, and most (57.9%) had some degree of renal insufficiency, with 9.2% having severe renal insufficiency (defined as an estimated creatinine clearance rate <30 mL/min, calculated using the Cockcroft-Gault equation). By use of the modified version of ESCMID severity signs for CDI [16] described above, 25.5% of patients had severe CDI.
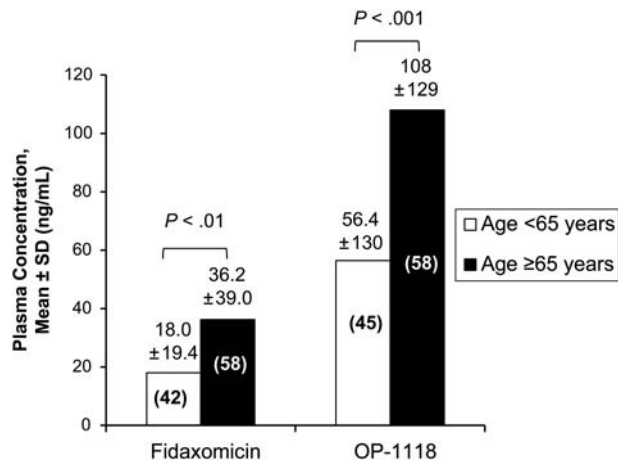
Plasma Concentrations

Plasma concentration data are summarized by time interval in Table 1 for the combined fidaxomicin population. Most subjects had very low plasma concentrations of both fidaxomicin and OP-1118; the overwhelming majority had concentrations <50 ng/mL. Fidaxomicin and OP-1118 concentrations were

Figure 1. Plasma concentrations of fidaxomicin and OP-1118 3–5 hours after dose receipt on the last day of treatment, by age. Sample sizes are specified in parentheses. Abbreviation: SD, standard deviation.

mildly higher on the last day of therapy; this elevation was not significant ($P > .05$) for fidaxomicin but was significant for the metabolite ($P < .001$).

Age and renal function were assessed for their influence on plasma concentrations. Plasma concentrations were mildly but significantly elevated in patients aged ≥65 years (Figure 1), although there was no significant trend toward increasing fidaxomicin plasma concentration with respect to estimated creatinine clearance rate (Table 2). However, the trend was significant for the metabolite OP-1118.

In contrast to the low plasma concentrations, mean fecal concentrations (±SD) of fidaxomicin were 1396 ± 1019 μg/g, and mean fecal concentrations (±SD) of the metabolite OP-1118 were 834 ± 617 μg/g.

Table 1. Fidaxomicin and OP-1118 (Metabolite) Plasma Concentrations, by Analyte and Visit

Visit, metric	Fidaxomicin	OP-1118
Day 1		
Level > LLOQ (no.)	312	316
Level < LLOQ (no.)	13	14
Level < 50 ng/mL (no.)	278	216
Mean level ± SD (ng/mL)	22.8 ± 26.7	44.5 ± 50.4
Median level (ng/mL)	13.9	27.0
Range (ng/mL)	0.364 – 197	0.283 – 363
Last day of treatment		
Level > LLOQ (no.)	100	103
Level < LLOQ (no.)	2	2
Level < 50 ng/mL (no.)	81	55
Mean level ± SD (ng/mL)	28.5 ± 33.4	85.6 ± 131
Median level (ng/mL)	16.4	43.2
Range (ng/mL)	0.305 – 191	1.09 – 871
P^a	.0744	<.001

Abbreviations: LLOQ, lower limit of quantification (0.2 ng/mL); SD, standard deviation.

[a] Comparison of day 1 vs last day of treatment, by the Wilcoxon rank sum test.

Table 2. Plasma Fidaxomicin and OP-1118 Concentrations 3–5 Hours After Dosing on the Last Day of Therapy, by Renal Impairment

Agent	None	Mild	Moderate	Severe	P
Fidaxomicin					
Patients (no.)	37	33	24	4	.2344
Mean level ± SD (ng/mL)	24.13 ± 32.69	24.82 ± 26.54	39.46 ± 43.32	26.12 ± 21.35	
OP-1118					
Patients (no.)	40	32	25	4	.0139
Mean level ± SD (ng/mL)	64.12 ± 131.11	72.17 ± 87.52	137.52 ± 175.74	68.96 ± 50.61	

Renal impairment was assessed using the estimated creatinine clearance rate as an approximation of the glomerular filtration rate. Estimated creatine clearance rates were defined as follows: normal, ≥90 mL/min; mild, ≥60 mL/min to <90 mL/min; moderate, ≥30 mL/min to <60 mL/min; severe, <30 mL/min Abbreviation: SD, standard deviation.

DISCUSSION

Early studies of fidaxomicin in healthy volunteers suggested that fidaxomicin was minimally absorbed, with plasma concentrations in the low nanogram-per-milliliter range or lower, even in patients with CDI [17]. A small amount of the drug (<1% of the dose) is excreted in urine as the metabolite OP-1118 [18], and biliary cannulation studies in dogs have indicated that biliary excretion also occurs (Optimer Pharmaceuticals, unpublished data). The analytical method was improved after these studies to more accurately measure systemic exposure, and this more sensitive method was used in these phase III studies.

The requested timing of the postdose plasma sample (3–5 hours after dosing) was chosen on the basis of results from an early phase I study that indicated that peak plasma concentrations following a 450-mg dose typically appeared approximately 4 hours after dose receipt [17]. Pharmacokinetic analysis in this early study was limited by the sensitivity of the method, which had an LLOQ of 5 ng/mL. More recently conducted phase I studies (Optimer Pharmaceuticals, unpublished data) that used the current high-sensitivity method have achieved better quantification of pharmacokinetic parameters in healthy volunteers and have concluded that the time to maximum plasma concentration (C_{max}) is close to 2 hours, with a half-life (for both fidaxomicin and OP-1118) of 10–11 hours. Although the prespecified 3–5-hour interval is somewhat past the time to C_{max}, half-life considerations suggest that plasma concentrations are unlikely to have diminished greatly from C_{max}. In addition, the 2-hour interval averaged represents approximately one-fifth of the half-life and thus is a reasonable time span to average.

Although fidaxomicin was well tolerated in animal studies, even at high doses producing plasma concentrations typically >4000 ng/mL in dogs dosed for 3 months [19], explorations were made to determine factors that might lead to an elevation of plasma concentrations in some patients. As noted, patients ≥65 years of age had plasma concentrations that were higher than those for patients aged <65 years, and although plasma concentrations of fidaxomicin were not affected significantly by renal impairment, plasma concentrations of its metabolite, OP-1118, were. Because neither fidaxomicin nor OP-1118 is significantly renally excreted [18], it is unclear why OP-1118 concentrations would increase with decreasing CCl; it may be that renal impairment in itself may not be the causative factor for this change but may correlate with other undetermined factors that are responsible for this observation. In contrast to the low plasma concentrations, fecal concentrations of fidaxomicin were >1000 µg/g and >5000-fold higher than the MIC_{90} for *C. difficile* measured in these trials and by others [4, 5, 11]. These fecal levels are well in excess of the MIC for the target organism.

Fidaxomicin, even at the elevated concentrations observed in the gut, does not appear to have a deleterious effect on common members of the intestinal flora. A study by Louie et al [6] indicated that the *Bacteroides* population, as a marker of normal gut flora, remained constant or even increased slightly over the 10-day duration of treatment. Similarly, profiling through molecular methods by Tannock et al [20] indicated that fidaxomicin treatment had minimal impact on the major phylogenetic clusters of the gut microbiota.

CONCLUSION

Following oral administration, fidaxomicin has minimal systemic exposure, even in patients with severe CDI. Despite this, plasma concentrations remain in the low nanogram-per-milliliter range for both fidaxomicin and its primary metabolite, OP-1118. Fidaxomicin achieves fecal concentrations that are well in excess of the MIC_{90} for *C. difficile* and is consistent with a high level of activity toward the target organism at the intended site of action in the colon.

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