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Pharmacokinetics of LFF571 and Vancomycin in Patients with Moderate *Clostridium difficile* Infections

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***Clostridium difficile* infection causes diarrheal disease with potentially fatal complications. Although treatments are available, including vancomycin, metronidazole, and fidaxomicin, the recurrence of disease after therapy remains a problem. LFF571 is a novel thiopeptide antibacterial that shows *in vitro* potency against *C. difficile* that is comparable to or greater than that of other clinically used antibiotics. Here, we compare the pharmacokinetics (PK) of LFF571 and vancomycin in patients with *C. difficile* infection as part of an early efficacy study. This multicenter, randomized, evaluator-blind, and active-controlled study evaluated the safety, efficacy, and pharmacokinetics of LFF571 in adults with primary episodes or first relapses of moderate *C. difficile* infections. Patients were randomized to receive 200 mg of LFF571 or 125 mg of vancomycin four times daily for 10 days. The PK parameters were calculated from drug concentrations measured in serum and fecal samples. The systemic exposure following oral administration of 200 mg of LFF571 four times per day for 10 days in patients with *C. difficile* infection was limited. The highest LFF571 serum concentration observed was 41.7 ng/ml, whereas the levels in feces at the end of treatment were between 107 and 12,900 μg/g. In comparison, the peak vancomycin level observed in serum was considerably higher, at 2.73 μg/ml; the levels of vancomycin in feces were not measured. Similar to healthy volunteers, patients with *C. difficile* infections exhibited high fecal concentrations and low serum levels of LFF571. These results are consistent with the retention of LFF571 in the lumen of the gastrointestinal tract. (This study has been registered at ClinicalTrials.gov under registration no. NCT01232595.)**

Clostridium difficile infection is a leading cause of hospital-acquired diarrheal disease. The infection is potentially severe, with complications, including pseudomembranous colitis, toxic megacolon, bowel perforation, renal failure, sepsis, and death (1). The emergence of a more virulent strain of the bacterium, *C. difficile* NAP1/BI/027, has contributed to the increasing prevalence and severity of the disease over the last decade (2, 3). Several drugs are available to treat the infection, including fidaxomicin, vancomycin, and metronidazole. However, a hallmark of *C. difficile* infection is the frequent recurrence of symptoms after initial successful treatment. Although recurrence after infection with strains other than NAP1/BI/027 is reduced after treatment with fidaxomicin compared to that after treatment with vancomycin, relapse remains a problem with all drug treatments (4, 5).

LFF571 is a novel semisynthetic thiopeptide antibiotic (6) with potent *in vitro* activity against *C. difficile* (7, 8). The compound has also shown efficacy in a Golden Syrian hamster model of *C. difficile* infection (9). The pharmacokinetics (PK) of single and multiple doses of LFF571 have been studied in healthy volunteers, in whom the drug was shown to be safe and well tolerated. In this population, doses of LFF571 up to 200 mg every 6 h for 10 days showed limited systemic exposure and high fecal concentrations of the drug (10). These results indicated that LFF571 remains primarily in the gastrointestinal tracts of healthy subjects. In the present study (registered at ClinicalTrials.gov under registration no. NCT01232595), we evaluated the pharmacokinetics of LFF571 and vancomycin in patients with moderate *C. difficile* infection as part of a proof-of-concept efficacy study (11). In this study, LFF571 was effective and well tolerated when used for the treatment of *C. difficile* infections. To our knowledge, this is the only randomized *C. difficile* efficacy study to systematically collect serum vancomycin PK data.

MATERIALS AND METHODS

Patients and study design. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and current good clinical practice. The protocol and amendments were approved by all required institutional review boards, and each patient provided written informed consent. The eligibility criteria and study design are described elsewhere (11). Briefly, this was a multicenter, randomized, evaluator-blind, and active-controlled trial comparing the safety, efficacy, and PK of LFF571 and vancomycin in adult patients with moderate *C. difficile* infections. Eligible patients were males and females between 18 and 90 years of age diagnosed with a primary episode or first recurrence of a moderately severe *C. difficile* infection. Moderately severe infections were defined by the presence of ≥3 to ≤12 liquid or unformed stools in the 24 h prior to enrollment, positive *C. difficile* toxin A/B or B assay result, and one or more of the following in the 24 h prior to enrollment: abdominal pain, peripheral blood leukocyte count of $>10 \times 10^9$/liter but $<30 \times 10^9$/liter, or fever. Patients were randomized (initially 1:1 and then 5:1) to receive

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TABLE 1 LFF571 serum concentrations at each time point

Study day	Time (h)	n^a	Concn (μg/ml) (median [range])	No. (%) of concn below lower limit of quantification
1	Predose	43	0 (0–0.627)	42 (97.7)
	1 after dose	16	0 (0–1.58)	10 (62.5)
	2–3 after dose	41	1.13 (0–22.2)	13 (31.7)
	4–6 after dose	13	3.69 (0–7.60)	2 (15.4)
3	Predose	43	4.00 (0–41.6)	2 (4.7)
	1 after dose	18	3.65 (0–24.1)	2 (11.1)
	2–3 after dose	43	2.54 (0–41.7)	2 (4.7)
	4–6 after dose	15	3.17 (0–17.1)	1 (6.7)
8 or 9	Predose	24	2.48 (0–30.5)	1 (4.2)
10 or 11	Predose	17	1.97 (0–28.1)	2 (11.8)
	1 after dose	12	2.39 (0–26.3)	2 (16.7)
	2–3 after dose	13	2.14 (0–19.4)	1 (7.7)
	4–6 after dose	10	2.16 (0.566–18.1)	0
12 or 13	12–72 after dose	43	0 (0–11.9)	25 (58.1)

[a] *n*, number of subject profiles that had adequate concentration data for evaluation. One patient had detectable LFF571 at baseline. The value was near the lower limit of quantification and was confirmed by repeat testing.

TABLE 2 LFF571 serum PK parameters

Study day	Statistic[a]	AUC_{last} (h · ng/ml)[b]	C_{max} (ng/ml)[c]	T_{max} (h)
1	*n*	10	29	29
	Median	9.24	2.77	3.00
	Mean (SD)	11.6 (9.47)	4.17 (4.64)	3.24 (1.04)
	Range	0.313–27.2	0.514–22.2	1.78–5.57
3	*n*	13	42	42
	Median	17.8	4.32	1.00
	Mean (SD)	23.5 (20.1)	7.22 (8.34)	1.55 (1.62)
	Range	5.48–66.0	0.691–41.7	0–5.00
10	*n*	11	14	14
	Median	9.65	3.21	1.81
	Mean (SD)	20.7 (31.0)	5.26 (7.02)	2.26 (1.99)
	Range	0.571–107	0.566–28.1	0–5.45

[a] *n*, number of subject profiles that had adequate concentration data for evaluation; SD, standard deviation.
[b] The day 3 and 10 values represent the AUC at time τ (AUC_τ) at steady state.
[c] The day 3 and 10 values represent the C_{max} at steady state.

200 mg of LFF571 (two 100-mg capsules) or 125 mg of vancomycin (one 125-mg capsule) orally four times daily for 10 days.

Serum collection for PK assessment. Blood samples were taken by direct venipuncture or an indwelling cannula inserted in a forearm vein. After clotting for approximately 30 min at room temperature, the samples were centrifuged, and serum was transferred into polypropylene screw-cap tubes, which were frozen within 90 min of venipuncture and kept at or below −20°C until analysis. All samples were given a unique sample and collection number.

The collection times were as follows: on day 1, before and 2 to 3 h after the first dose, and if the patient agreed, there was an optional collection at 1 h postdose and immediately prior to the second dose (trough); and on day 3 or 4, before and 2 to 3 h after the most convenient dose, with optional collections at 1 h postdose and immediately prior to the subsequent dose (trough). Optional samples were collected on day 8 or 9 before the most convenient dose (trough), as well as on day 10 or 11 before and 1, 2 to 3, and 4 to 6 h (or just prior to the subsequent dose) after the most convenient dose. The final collection was at the end of therapy, on day 12 or 13, during a 12- to 72-h window after the last dose.

Fecal collection for PK assessment. At the end-of-therapy visit, the first fecal sample after the last dose administration was collected. The samples were delivered to the site at the end-of-therapy visit (day 12 or 13) or when the sample was collected.

Bioanalytical methods. LFF571 in serum was quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (lower limit of quantification, 0.5 ng/ml). Vancomycin in serum was quantified using the Siemens ADVIA Centaur test system and reagents (lower limit of quantification, 0.91 μg/ml). LFF571 in stool was quantified by LC-MS/MS (lower limit of quantification, 100 ng/g).

Assessment of PK parameters. Serum concentration-time profiles were used to measure the area under the serum drug concentration-time curve from time zero until the last measurable time point (AUC_{last}), as well as the maximum concentration of drug in serum (C_{max}) and the time to C_{max} (T_{max}) during the 6-h dosing interval. Up to four PK specimens were collected on day 1, day 3 or 4, and day 10 or 11. All patients had four PK samples, except for two who had three samples. For these two patients, the 4- to 6-h sample on day 10 or 11 was missing.

Statistical methods. Summary statistics are provided for the primary PK parameters.

RESULTS

Patient demographics. A total of 72 patients were enrolled and randomized, of whom 46 received LFF571 and 26 received vancomycin. The majority of the patients (89.1% in the LFF571 group and 88.5% in the vancomycin group) completed the study. The baseline demographic and disease characteristics were largely comparable between the LFF571 and vancomycin treatment groups in terms of mean age (59.8 versus 54.9 years, respectively), gender (males, 37.0% versus 30.8%, respectively), and race (Caucasian, 95.7% versus 88.5%; black, 2.2% versus 11.5%; and Asian, 2.2% versus 0%, respectively) (11). However, more LFF571-treated than vancomycin-treated patients had an initial relapse (28.3% versus 16.0%, respectively) or severe clinical symptoms (23.9% versus 12.0%, respectively), while more vancomycin-treated than LFF571-treated patients had been given prior *C. difficile* therapy (48.0% versus 28.3%, respectively) or were infected with the NAP1/BI/027 strain (32.0% versus 19.6%, respectively). The population was overweight, with a mean body mass index (BMI) of 29.26 kg/m^2. The PK analysis set consisted of all patients who had received at least one dose of the study drug and who had sufficient specimens for analysis.

PK parameters of LFF571 in serum. The patients with *C. difficile* infection had limited systemic exposure to LFF571 after dosing with 200 mg four times daily for 10 days (Table 1). At 1 h after the first dose, 62.5% of the patients had LFF571 levels below the lower limit of quantitation (0.5 ng/ml). This proportion decreased to 31.7% at 2 to 3 h and 15.4% at 4 to 6 h after the first dose. Subsequently, 5 to 15% of the patients had undetectable levels during therapy with LFF571. Twelve to 72 h after the last dose, 58.1% of the patients had LFF571 levels below the lower limit of quantification. The LFF571 levels in serum were consistently low, with the highest observed value of 41.7 ng/ml detected on day 3 of dosing (Table 1), although a second patient had a value of 41.6 ng/ml, also on day 3.

A sufficient number of patients administered LFF571 had adequate serum concentration data to allow for calculations of the AUC_{last}, C_{max}, and T_{max} on day 1, day 3 or 4, and day 10 or 11 (Table 2). The maximum concentrations of the drug in serum were generally observed 1 to 3 h after administration.

TABLE 3 Vancomycin serum concentrations at each time point

Study day	Time (h)	n^a	Concn (μg/ml) (median [range])	No. (%) of concn below lower limit of quantification
1	Predose	21	0 (0–1.96)	19 (90.5)
	1 after dose	7	0 (0–1.66)	5 (71.4)
	2–3 after dose	21	0 (0–1.85)	19 (90.5)
	4–6 after dose	7	0 (0–1.90)	6 (85.7)
3	Predose	22	0 (0–2.37)	17 (77.3)
	1 after dose	7	0 (0–2.54)	4 (57.1)
	2–3 after dose	22	0 (0–1.93)	16 (72.7)
	4–6 after dose	8	0 (0–2.73)	5 (62.5)
8 or 9	Predose	13	0 (0–2.51)	10 (76.9)
10 or 11	Predose	10	0 (0–2.57)	8 (80.0)
	1 after dose	7	0 (0–2.38)	5 (71.4)
	2–3 after dose	6	0 (0–2.06)	4 (66.7)
	4–6 after dose	6	0 (0–2.34)	5 (83.3)
12 or 13	12–72 after dose	23	0 (0–2.46)	19 (82.6)

[a] *n*, number of subject profiles that had adequate concentration data for evaluation. Two patients had detectable vancomycin at baseline. The first, who had a concentration of 1.73 μg/ml, had recently received intravenous vancomycin. The second, who had a concentration of 1.96 μg/ml, had received intravenous vancomycin, but the last dose was >14 days prior to enrollment.

PK parameters of vancomycin in serum. The vancomycin levels in serum were approximately 100 times greater than those of LFF571. The highest serum vancomycin concentration detected was 2.73 μg/ml, 6 h after dosing on day 3 of treatment (Table 3). The maximum concentrations of vancomycin were typically seen 1 to 2 h after dosing. A considerable percentage of the patients receiving vancomycin, however, had serum drug levels below the lower limit of quantification (0.91 μg/ml). On day 1, 71.4% of the patients had undetectable vancomycin levels 1 h after the first dose; this increased to 85.7% by 4 to 6 h postdose. The total percentage of patients with undetectable vancomycin levels in serum ranged from 57.1 to 90.5% throughout the treatment period. Twelve to 72 h after the end of treatment, 82.6% of the patients had serum vancomycin levels below the lower limit of quantitation. An insufficient number of patients administered vancomycin had adequate serum concentration data to allow for an accurate calculation of the AUC_{last}, C_{max} and T_{max} parameters.

Fecal recovery of LFF571. Fecal samples were collected from 41 patients during a 0- to 24-h window after the administration of the final dose to determine the LFF571 concentrations in the gastrointestinal tract. The levels of vancomycin in feces were not measured. The mean (standard deviation) level of LFF571 in feces was 3,950 μg/g (2,810 μg/g), with a median value of 3,240 μg/g and a range of 107 to 12,900 μg/g. No associations between fecal LFF571 concentrations and clinical outcome shortly after completing therapy and during the 30-day follow-up period were noted. Among the 41 patients with available fecal drug concentration data, two were classified as treatment failures shortly after completing a full 10-day course of therapy, and 10 developed recurrent disease during the 30-day follow-up period. The fecal LFF571 concentrations for the two patients with treatment failures were 3,150 μg/g and 3,730 μg/g. For the 10 patients with recurrent disease, the median fecal LFF571 concentration was 4,010 μg/g. Both the lowest (107 μg/g) and highest (12,900 μg/g) fecal concentrations of LFF571 were seen in patients classified as clinical relapses.

DISCUSSION

Patients with *C. difficile* infections had limited systemic exposure to LFF571 after dosing with 200 mg four times per day for 10 days. These results are similar to those noted after dosing in healthy volunteers (10), although slightly higher LFF571 serum concentrations were noted in the patients infected with *C. difficile* in the current study. These higher concentrations may reflect an enhanced absorption of LFF571 due to gastrointestinal tract inflammation and damage associated with the infection. However, the absolute serum concentrations of LFF571 remained low throughout the 10-day dosing regimen, with the highest value of 41.7 ng/ml detected on day 3. A limitation of defining the peak exposure was that the collection of PK samples was limited to 1, 2 to 3, and 24 h postdosing on day 1 and day 3 or 4, with some of the collection times being optional.

The fecal concentrations of LFF571 ranged from 107 μg/g to 12,900 μg/g of feces. These concentrations are considerably higher than the MIC_{90} values (0.25 to 0.5 μg/ml) for LFF571 against *C. difficile* (7, 8, 12). These fecal drug concentrations are similar to those attained after oral dosing of vancomycin and fidaxomicin (1, 13). It is hypothesized that these high concentrations contribute to the efficacies of these agents. At doses used to treat patients with *C. difficile* infections, 25 to 35% of an oral fidaxomicin dose is converted to the 8- to 64-fold-less-active major metabolite OP-1118, although the concentrations of active drug still remain well above the MIC_{90} (13–15). Metronidazole is rapidly absorbed, and fecal concentrations are typically much lower (<10 μg/g of feces); yet, this antibiotic remains an effective therapy for mild to moderate *C. difficile* infections (2). Fecal LFF571 concentrations did not correlate with clinical outcomes when assessed shortly after completing therapy and during the 30-day follow-up period.

The high fecal concentrations and low serum concentrations of LFF571 are consistent with most LFF571 remaining in the lumen of the gastrointestinal tract in patients with *C. difficile* infections. In *C. difficile*-infected hamsters, the absorption of LFF571 was <0.5% (6). However, the possibility of first-pass biliary-hepatic elimination in humans cannot be formally ruled out. In rats, LFF571 was excreted in feces as unchanged drug after intravenous and oral administration (data not shown). Orally administered drugs that achieve high concentrations in the gastrointestinal tract at the site of infection and that are minimally absorbed in the systemic circulation are potentially ideal candidates for *C. difficile* therapy.

Oral vancomycin is poorly absorbed, and serum vancomycin concentrations are typically <1 μg/ml if vancomycin is dosed at 125 mg every 6 h. Detectable but subtherapeutic serum vancomycin concentrations ranging from 1 μg/ml to 5.1 μg/ml have been reported in patients with *C. difficile* infections treated with 500 mg of oral vancomycin every 6 h (16, 17). A systematic collection of the serum samples for the PK analyses was not done in these studies. In a prospective study of 57 patients treated with 125 mg of oral vancomycin every 6 h, only one patient had a detectable concentration of 6.7 μg/ml based on an assay range of 3.4 μg/ml to 90 μg/ml (18). The details on how the serum PK samples were collected were not reported. Therapeutic serum vancomycin concentrations are generally not seen unless the patient has underlying

renal failure, in which case levels of >30 μg/ml have been reported (19–21). However, even among patients with impaired renal function, serum vancomycin concentrations typically remain undetectable or reach only subtherapeutic levels. In the current study, detectable serum vancomycin concentrations were noted in 9.5 to 42.9% of the patients throughout the treatment interval, with the highest noted value being 2.73 μg/ml. The lower number of LFF571-treated patients with undetectable serum drug concentrations compared with that of vancomycin-treated patients likely reflects the relative insensitivity of the vancomycin detection assay (i.e., the lower limit of detection is almost 20-fold greater than that for LFF571). All patients received 125 mg of oral vancomycin every 6 h, and no patient with a calculated creatinine clearance of <30 ml/min or in need of dialysis was enrolled in the current study.

In conclusion, these results confirm that systemic exposure to LFF571 after oral dosing remains low, even in patients with *C. difficile* infections. This suggests the retention of LFF571 in the lumen of the gastrointestinal tract. The systemic exposure to vancomycin after oral dosing tended to be higher. However, no patient had maximum serum drug concentrations in the therapeutic range for pathogens, such as *Staphylococcus aureus* or *Enterococcus faecalis*.

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