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# A First-in-Human, Randomized, Double-Blind, Placebo-Controlled, Single- and Multiple-Ascending Oral Dose Study To Assess the Safety and Tolerability of LFF571 in Healthy Volunteers

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***Clostridium difficile* is the leading cause of hospital-acquired infectious diarrhea. LFF571 is a novel inhibitor of the prokaryotic translation elongation factor Tu and is active against a range of bacterial species, including *C. difficile*. This first-in-human study investigated the safety and pharmacokinetics of single and multiple ascending oral doses of LFF571 in healthy subjects. This was a randomized, double-blind, placebo-controlled study. Except for one cohort, LFF571 was given with a high-fat meal to all single-dose cohorts (25 mg, 100 mg, 400 mg, and 1,000 mg). In the multiple-dose cohorts (25 mg, 100 mg, or 200 mg every 6 h for 10 days), LFF571 was given without regard to food. A total of 56 subjects completed the study, with 32 and 25 receiving single and multiple doses, respectively. There were no deaths, no serious adverse events, and no subject withdrawals due to an adverse event. The most common adverse event was diarrhea; gastrointestinal pain or distension was also noted. Diarrhea did not develop more frequently among subjects who received LFF571 than among those who received a placebo. LFF571 had limited systemic exposure and high steady-state fecal concentrations. The highest concentration of LFF571 in serum (3.2 ng/ml) was observed after the last dose in a subject who received 200 mg every 6 h for 10 days. LFF571 was generally safe and well tolerated in single and multiple oral doses in healthy subjects. The minimal serum and high fecal concentrations support the further development of LFF571 for the treatment of *C. difficile* infections.**



*Clostridium difficile* is a Gram-positive, anaerobic, spore-forming bacterium and the leading cause of antibiotic-associated diarrhea (1). *C. difficile* infection is a serious, toxin-mediated disease commonly associated with fever, abdominal pain, diarrhea, and leukocytosis. Complications include fulminant pseudomembranous colitis, toxic megacolon, bowel perforation, sepsis, and death (7). *C. difficile* infections typically occur after therapy with antibiotics, which may provide an environment conducive to *C. difficile* growth by disrupting the normal intestinal flora. Increasingly, however, the disease is recognized in individuals with no recent antibiotic exposure and in previously low-risk groups, such as children, pregnant women, and those with irritable bowel syndrome (17). The incidence and severity of *C. difficile* infections have been increasing over the past decade, in part because of the emergence of hypervirulent strains such as NAP1/027/B1 (13, 15). In the United States, *C. difficile* infection-related hospitalizations have risen from 82,000 in 1996 to about 350,000 in 2008 (1), with approximately 15,000 to 20,000 deaths attributed to *C. difficile* each year (17). Although treatments for *C. difficile* infections are available, recurrences are relatively frequent after initial successful therapy, especially with vancomycin or metronidazole (9). Fidaxomicin has been shown to reduce the overall recurrence rates compared with vancomycin, although no significant reduction in relapse was noted for patients infected with the NAP1/027/B1 strain (14, 16).

The macrocyclic thiopeptide LFF571 (Fig. 1) is an investigational antimicrobial agent with potent activity against Gram-positive aerobic and anaerobic bacteria, including *C. difficile* (3, 5, 6, 10). LFF571 inhibits protein synthesis by binding to bacterial elongation factor Tu (EF-Tu) (4, 11). *In vitro*, the MIC of LFF571 for 90% of the *C. difficile* isolates tested ($MIC_{90}$) ranged from 0.25 to 0.5 μg/ml, which makes it more potent than vancomycin ($MIC_{90}$ of 1 to 2 μg/ml) or metronidazole ($MIC_{90}$ of 0.5 to 2 μg/ml) (3, 5, 6). LFF571 is also active *in vitro* against other aerobic and anaerobic Gram-positive organisms, including *Enterococcus faecalis*, *E. faecium*, and *Staphylococcus aureus* but has moderate-to-weak activity against most Gram-negative gastrointestinal tract organisms (3, 5, 6). In a hamster model of *C. difficile* infection, LFF571 (5 mg/kg) was more efficacious than vancomycin (20 mg/kg) and resulted in fewer disease recurrences (18). Here we present a first-in-human study that evaluated the safety, tolerability, and pharmacokinetics (PK) of LFF571 in healthy volunteers after the administration of single and multiple ascending doses.

## MATERIALS AND METHODS

**Study design.** This first-in-human study employed a randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose design to determine the safety, tolerability, and PK of LFF571 in healthy subjects. The study was divided into two sequential parts, with completion of the part I single-ascending-dose study prior to the start of the part II multiple-ascending-dose cohort study. Each ascending dose was given after safety assessment of the previous dose level. In each cohort, eight

Received 26 April 2012 Returned for modification 14 June 2012
Accepted 1 September 2012
Published ahead of print 10 September 2012
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doi:10.1128/AAC.00867-12

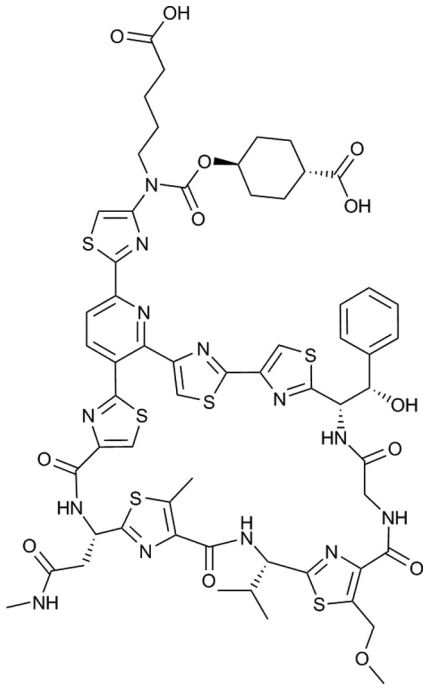

**FIG 1** Chemical structure of LFF571. LFF571 is a semisynthetic thiopeptide derived from the natural product GE2270 A.

subjects were randomized in a ratio of 3:1 to receive LFF571 (six subjects) or a placebo (two subjects). LFF571 was supplied as capsules at dose strengths of 25 mg and 100 mg. The placebo contained pregelatinized starch, microcrystalline cellulose, and magnesium stearate. The study was conducted at Charles River Clinical Services Northwest, Inc., Tacoma, WA, in accordance with the Declaration of Helsinki and Good Clinical Practice. The study protocol was approved by the Institutional Review Board of the center. All subjects provided written informed consent.

Part I assessed four single oral dose levels (25 mg, 100 mg, 400 mg, and 1,000 mg) of LFF571. After at least 10 h of overnight fasting, subjects received a single dose of LFF571 or a placebo with an FDA high-fat, high-calorie breakfast (19). After a 6-day washout from the previous dosing period, subjects in the 400-mg group received a second single dose of LFF571 (400 mg) or a placebo under fasting conditions (with water alone after at least 10 h of overnight fasting) to assess the effects of food on absorption.

Part II assessed LFF571 (25 mg, 100 mg, or 200 mg administered every 6 h) for 10 days, without regard for meals. Eligible subjects were admitted to the study center, remained domiciled for a 10-day multiple-dosing period, and were discharged on day 11; all subjects returned on day 15 ± 1 day for evaluation. Subjects received a total of 40 doses of LFF571 or a placebo over the course of 10 days.

**Subjects.** Healthy male and nonpregnant female subjects 18 to 65 years of age were eligible for the study. Good health was determined by medical history, physical examination, and laboratory tests at screening. Subjects had to weigh at least 50 kg and have a body mass index of 18 to 32 kg/m$^2$. Only females with no child-bearing potential were allowed to enroll, and all female subjects were required to have a negative pregnancy test result at screening and at the baseline. Exclusion criteria included significant illness within 2 weeks prior to initial dosing; use of tobacco products in the previous 3 months; drug or alcohol abuse within the past 12 months; use of prescription drugs or herbal supplements within the previous 4 weeks or over-the-counter medication (except for incidental acetaminophen), dietary supplements, or vitamins within 2 weeks; participation in any clinical investigation within 4 weeks; and donation or loss of ≥400 ml of blood within 8 weeks.

**Safety assessment.** Safety assessments consisted of collecting all adverse events with their severity and relationship to study drug, physical examinations, PK monitoring, and laboratory evaluations, including monitoring for pregnancy. In part II only, stool samples from screening and day 10 ± 1 were examined for the presence of occult blood by Hemoccult/HemoQuant assays. The number of bowel movements per day, the time of each bowel movement, and the subject-reported Bristol stool scale type (12) were also recorded from the first dose on day 1 to discharge on day 11.

**PK parameters and assessment.** LFF571 levels in blood and feces were assessed throughout the study to evaluate the PK of the drug. Blood was collected in part I on day 1 predose and at 0.5 h, 1 h, 2 h, 6 h, 12 h, and 24 h postdose; this schedule was repeated for the fasting 400-mg cohort. In part II, blood was collected on day 1 before the 06:00 dose (before the first dose) and at 0.5 h, 1 h, 2 h, 6 h (before the 12:00 dose), 12 h (before the 18:00 dose), and 24 h (before the 06:00 dose) after the first dose; on days 5 and 7 before the 06:00 dose (before the first dose of the day); on day 9 before the 06:00 dose, before the 12:00 dose, and before the 18:00 dose; on day 10 before the 06:00 dose and at 0.5 h, 1 h, 2 h, and 6 h (before the 12:00 dose) postdose; and on day 11 at 6 h after the last dose. Blood (3 ml) was collected in a Serum Separator Vacutainer tube and processed by centrifugation for 10 min at 1,000 × *g* at 3 to 5°C; serum was stored below −20°C until analysis. Fecal samples were collected and total weights were recorded in parts I and II for the entire domiciled period after administration of the study medication. In part I (0 to 48 h) and part II (last 3 days domiciled), samples from one individual over a 24-h period were combined and diluted in Dulbecco's phosphate-buffered saline. Aliquots were frozen below −60°C. LFF571 levels in serum and feces were quantified by liquid chromatography-tandem mass spectrometry. The lower limits of quantification (LLOQs) by this method were 0.5 ng/ml for serum and 100 ng/g for feces. All randomized subjects who received at least one dose of LFF571 were included in the PK analysis population. Conventional serum PK parameters (area under the concentration-time curve from time zero to infinity [$AUC_\infty$], AUC from time zero to the last quantifiable concentration [$AUC_{0\text{-}t}$], maximum concentration [$C_{max}$], time to maximum concentration [$T_{max}$], and accumulation ratio [AUC and $C_{max}$ on day 10/AUC and $C_{max}$ on day 1]) and amounts of drug recovered in feces were evaluated where possible by noncompartmental methods using WinNonlin Professional version 5.2 (Pharsight Corp., St. Louis, MO). Concentrations below the LLOQ were treated as zero.

**Statistical methods.** PK parameters were summarized by descriptive statistics. To assess food effects, fed and fasting data from the same subjects were pooled and $AUC_\infty$, $AUC_{last}$, $AUC_{0\text{-}t}$, and $C_{max}$ were analyzed by mixed-model analysis of variance with fasted or fed as a fixed effect and a compound symmetry correlation within each subject. It was recognized that by this analysis the fed/fasted effect could be confounded with the period effect. Ratios of the geometric means of the fasted and fed conditions were calculated together with 90% confidence intervals.

## RESULTS

**Subject demographics.** A total of 57 subjects were enrolled in this study. Thirty-two subjects were randomized and completed part I; 24 subjects received LFF571. In part II, 25 subjects were randomized and 24 completed the study; 1 subject (placebo group, 100 mg every 6 h) discontinued after withdrawing consent, and that subject was replaced. The demographic data for all of the subjects enrolled are shown in Table 1. In parts I and II, no subject had any medical history or condition or received any concomitant medications (such as proton pump inhibitors, $H_2$ receptor antagonists, laxatives, antacids, or any other agents that could affect gastrointestinal motility) that were deemed to impact safety or the PK analyses.

**Safety and tolerability.** There were no deaths or serious adverse events, and no subject discontinued the study medication

**TABLE 1** Demographic summary of parts I and II of this study

| Parameter | Part I | | | Part II | | |
|---|---|---|---|---|---|---|
| | LFF571 ($n = 24$) | Placebo ($n = 8$) | Total ($n = 32$) | LFF571 ($n = 18$) | Placebo ($n = 7$) | Total ($n = 25$) |
| Mean age, yr (range) | 27.2 (18–51) | 34.0 (19–44) | 28.9 (18–51) | 31.5 (18–52) | 32.7 (20–48) | 31.8 (18–52) |
| No. (%) of males | 22 (91.7) | 7 (87.5) | 29 (90.6) | 17 (94.4) | 6 (85.7) | 23 (92.0) |
| No. (%) of: | | | | | | |
| Caucasians | 13 (54.2) | 4 (50.0) | 17 (53.1) | 9 (50.0) | 5 (71.4) | 14 (56.0) |
| Blacks | 2 (8.3) | 2 (25.0) | 4 (12.5) | 3 (16.7) | 1 (14.3) | 4 (16.0) |
| Asians | 1 (4.2) | 1 (12.5) | 2 (6.3) | 1 (5.6) | 0 (0.0) | 1 (4.0) |
| Native Americans | 1 (4.2) | 1 (12.5) | 2 (6.3) | 1 (5.6) | 0 (0.0) | 1 (4.0) |
| Others | 7 (29.2) | 0 (0.0) | 7 (21.9) | 4 (22.2) | 1 (14.3) | 5 (20.0) |
| Mean wt, kg (range) | 74.10 (55.8–99.7) | 82.61 (62.9–99.1) | 76.23 (55.8–99.7) | 80.1 (60.4–98.7) | 75.10 (60.9–86.0) | 78.70 (60.4–98.7) |
| Mean ht, cm (range) | 175.4 (162–185) | 177.0 (169–186) | 175.8 (162–186) | 175.2 (160–186) | 173.4 (161–185) | 174.7 (160–186) |

because of an adverse event. Eight subjects developed a total of 13 adverse events after receiving a single dose of the study drug after a high-fat, high-calorie meal, i.e., 7 (29.2%) of 24 subjects who received LFF571 and 1 (12.5%) of 8 subjects who received a placebo. Eleven (84.6%) of the 13 adverse events were mild in severity, while 1 (7.7%) was assessed as moderate and 1 (7.7%) was considered severe. The severe event was an elevated lipase level (201 U/liter) 2 days after the subject received 400 mg of LFF571. Since the subject had no associated symptoms or signs and a repeat lipase value the next day was within the normal range (27 U/liter), it was felt that this represented a false-positive laboratory result. The moderate adverse event was constipation, which started on the day that the subject was dosed with 400 mg LFF571 and resolved 6 days later without therapy. This subject also had mild flatulence on the day that he received LFF571. Both the constipation and flatulence were suspected to be related to the study drug. Most of the adverse events occurred sporadically among the dosing arms. Only three adverse events were noted in more than one subject, i.e., constipation (one subject who received 100 mg of LFF571 and one who received 400 mg of LFF571), upper viral respiratory infection (one subject who received 1,000 mg of LFF571 and one who received a placebo), and cough (the same two subjects who developed viral infections). No subject developed diarrhea.

Four subjects in the fasting, 400-mg dose cohort developed a total of four adverse events, i.e., 2 (33.3%) of 6 subjects who received LFF571 and 2 (100%) of 2 subjects who received a placebo. All adverse events were mild in severity. One adverse event, headache, was noted in two subjects (one subject who received LFF571 and one who received a placebo).

In part II, 19 subjects developed a total of 47 AEs, i.e., 13 (72.2%) of 18 subjects who received LFF571 and 6 (85.7%) of 7 subjects who received a placebo (Table 2). Forty-six (97.9%) of the 47 AEs were assessed as mild in severity, while 1 (2.1%) was moderate. The moderate event was a laceration above the right eye and was not suspected to be related to the study drug. Diarrhea (including frequent bowel movements) was the most common adverse event reported in part II and was reported by 13 subjects. Diarrhea occurred more frequently in subjects who received a placebo (85.7%) than in those who received 25 mg (50.0%), 100 mg (16.7%), or 200 mg (50.0%) of LFF571 every 6 h. All episodes of diarrhea were assessed as mild. The relatively high rates of diarrhea are likely related to the use of the specific case definition based on the Bristol stool chart (at least one bowel movement classified as type 5, 6, or 7) and frequency of bowel movements (three or more bowel movements in a 24-h period, regardless of type). No bowel movement was described as liquid (type 7), and 8 subjects had only a single bowel movement that was consistent with diarrhea on the basis of the Bristol stool chart (type 5 or 6).

Among the subjects who received LFF571, adverse events such as gastrointestinal pain or distension developed in 3 (50.0%) of 6 who received 200 mg of LFF571 every 6 h, in contrast to 1 (16.7%) of 6 and 0 who received 25 mg and 100 mg every 6 h, respectively. However, 2 (28.6%) of 7 subjects who received a placebo also reported at least one of these adverse events. Of note, the three subjects who had symptoms that lasted 2 or more days all received 200 mg of LFF571 every 6 h. Although higher doses of LFF571 may be associated with a slightly higher rate and duration of gastroin-

**TABLE 2** Adverse events after multiple doses of LFF571 (part II)

| Adverse event[a] | No. (%)[c] who received the following every 6 h: | | | |
|---|---|---|---|---|
| | 25 mg LFF571 | 100 mg LFF571 | 200 mg LFF571 | Placebo |
| Any | 5 (83.3) | 3 (50.0) | 5 (83.3) | 6 (85.7) |
| Gastrointestinal | | | | |
| Pain or distension | 1 (16.7) | 0 | 3 (50) | 2 (28.6) |
| Constipation | 1 (16.7) | 0 | 0 | 0 |
| Diarrhea[b] | 3 (50.0) | 1 (16.7) | 3 (50.0) | 6 (85.7) |
| Flatulence | 0 | 0 | 0 | 1 (14.3) |
| Nausea | 0 | 0 | 1 (16.7) | 1 (14.3) |
| Others that occurred in >1 subject | | | | |
| Fatigue | 0 | 1 (16.7) | 1 (16.7) | 0 |
| Headache | 0 | 0 | 1 (16.7) | 1 (14.3) |

[a] Adverse events were coded using Medical Dictionary for Regulatory Activities terminology. Shown are adverse-event preferred terms. Gastrointestinal pain or distension includes the preferred terms abdominal discomfort, abdominal distension, abdominal pain, abdominal pain lower, dyspepsia, and gastrointestinal pain.
[b] Only one instance of diarrhea is shown if a subject had more than one episode. This occurred in one subject who received 25 mg of LFF571 every 6 h (three separate diarrhea episodes) and in two subjects who received the placebo (two separate episodes of diarrhea in each subject).
[c] There were six subjects in each LFF571 group and seven in the placebo group.

**TABLE 3** Summary of serum PK parameters of part I (single dose)

| Dose (mg), condition | No. of subjects[a] | $AUC_{0-24}$ (ng · h/ml) | $C_{max}$ (ng/ml) | $T_{max}$ (h) |
|---|---|---|---|---|
| 25, fed | 0 | NA[b] | NA | NA |
| 100, fed | 1 | 3.09 (NA)[c] | 0.62 (NA) | 6.00 (NA) |
| 400, fed | 4 | 2.93 (2.89–2.97) | 0.59 (0.58–0.59) | 6.00 (6.00–6.03) |
| 400, fasted | 6 | 13.11 (6.55–35.6) | 2.24 (0.90–3.17) | 2.00 (2.00–6.00) |
| 1,000, fed | 5 | 3.81 (2.53–5.41) | 0.54 (0.51–0.76) | 6.02 (6.00–12.00) |

[a] Number of subjects with adequate serum drug concentrations to evaluate $AUC_{0-24}$ and $C_{max}$.
[b] NA, not available because of lack of measurable drug concentration data.
[c] Data are presented as median (range).

testinal pain or distension than the placebo, all gastrointestinal adverse events were assessed as mild and the small numbers of subjects preclude determining if any definitive association with LFF571 exists. Only two other adverse events, fatigue and headache, were noted in more than one subject (Table 2).

**PK parameters of LFF571 in serum.** LFF571 had limited systemic exposure after single doses (up to 1,000 mg) and multiple doses (up to 200 mg four times daily for 10 days) in healthy subjects. In part I, a single administration of 25 mg, 100 mg, 400 mg, or 1,000 mg of LFF571 typically led to serum drug concentrations that were near or below the lower limit of quantification, 0.5 ng/ml. The highest serum drug concentration observed in part I was 3.17 ng/ml and was seen in a subject who received 400 mg under fasted conditions. The ratios of the geometric least-squares means (90% confidence intervals) of fed to fasted LFF571 $AUC_{last}$ and $C_{max}$ were 0.13 (0.06 to 0.29) and 0.29 (0.18 to 0.47), respectively. Most serum PK parameters could not be calculated because of insufficient measurable concentrations of LFF571; the measureable PK parameters for the single-ascending-dose cohorts are summarized in Table 3. Maximum serum drug concentrations were generally observed 6 h postdose under fed conditions and 2 h postdose under fasted conditions. After the 25-mg and 100-mg doses, all of the subjects except one had serum drug concentrations below the LLOQ. As the dose of LFF571 increased, the number of subjects with detectable serum drug concentrations increased (Table 3). Concentration profiles of the 400-mg fasting cohort, which had the highest serum LFF571 exposure, are provided in Fig. 2 to illustrate the minimal LFF571 concentration achieved systemically.

After 10 days of dosing, serum LFF571 concentrations were generally at or slightly above the LLOQ (0.5 ng/ml), as reflected by the low $C_{max}$ observed (Table 4). The highest serum drug concentration observed was 3.2 ng/ml, which was seen on day 11 (6 h after the last dose) in a subject who had received 200 mg every 6 h. Most PK parameters could not be calculated because of an insufficient number of measurable concentration data; measureable PK parameters for the multiple-ascending-dose cohorts for days 1 and 10 are summarized in Table 4. Maximum serum LFF571 concentrations were generally observed 2 h after dosing. At the highest LFF571 dosing regimen studied (200 mg every 6 h), the median (range) accumulation ratios were 1.46 (0.80 to 4.51) and 1.88 (0.83 to 8.69) for $C_{max}$ and $AUC_{0-6}$, respectively.

**Fecal recovery of LFF571.** In part I, pooled fecal samples were collected for 48 h postdose. The recovery of LFF571 in fecal samples varied from about 10 to 30%, as estimated by dividing the amount of drug recovered in feces by the dose administered. In part II, three 24-h fecal samples were collected on the last 3 days of dosing, approximating steady-state conditions. Under these conditions, 60 to 100% of the daily dose of LFF571 was recovered within a 24-h period. In the multiple-dosing cohorts, the median (range) fecal concentrations of LFF571 were 340.5 (181 to 878), 2,080 (820 to 5,890) and 5,410 (2,200 to 8,720) μg/g for the cohorts that received 25 mg every 6 h, 100 mg every 6 h, and 200 mg every 6 h, respectively. The high fecal recovery and low serum drug concentration suggest that LFF571 remains in the gastrointestinal tract after dosing.

## DISCUSSION

The increased prevalence and changing epidemiology of *C. difficile* infections highlight the need for more effective treatments, particularly for severe and relapsing infections. Ideal candidates for *C. difficile* therapy are orally administered drugs that achieve high concentrations in the gastrointestinal tract while being minimally absorbed into the systemic circulation. Drugs should show high efficacy and low relapse rates, especially for the treatment of virulent strains, and side effects should be minimal. Ideally, the dosing regimen should be as convenient as possible to minimize the patient treatment burden. It is also desirable that new drugs for treatment of *C. difficile* infections do not select for cross-resistance to available antibacterial agents and have limited activity against normal flora in the gut, as repopulation by these organisms is thought to be important in preventing a relapse (8).

LFF571 is a novel inhibitor of bacterial EF-Tu that has potent *in vitro* activity against *C. difficile*. This first-in-human study in-

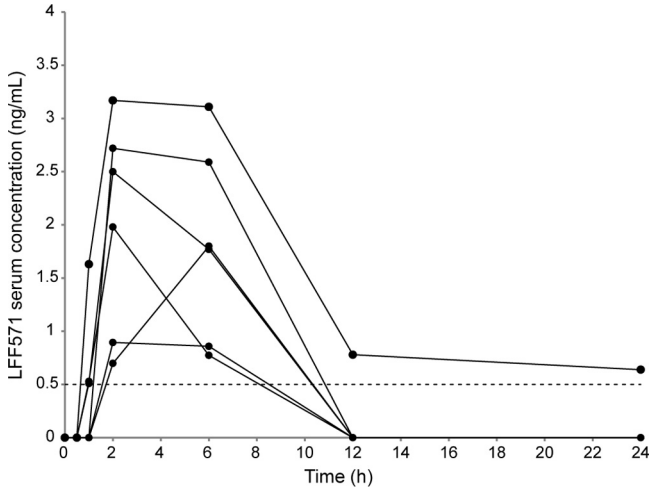


**FIG 2** Serum LFF571 concentrations in healthy subjects over time. Serum LFF571 concentration-versus-time profiles of subjects in the 400 mg single-dose fasting cohort ($n = 6$) are shown. The dashed line indicates the LLOQ.

**TABLE 4** Summary of serum PK parameters of part II (multiple dosing)

| Time and dose (mg) taken every 6 h | No. of subjects[a] | $AUC_{0-6}$ (ng · h/ml) | $C_{max}$ (ng/ml) | $T_{max}$ (h) |
|---|---|---|---|---|
| Day 1 | | | | |
| 25 | 0 | NA[b] | NA | NA |
| 100 | 2 | 1.73 (1.24–2.22)[c] | 0.70 (0.51–0.89) | 2.00 (2.00–2.00) |
| 200 | 6 | 3.99 (1.38–8.17) | 1.24 (0.56–2.14) | 2.00 (2.00–5.92) |
| Day 10 | | | | |
| 25 | 2 | 2.80 (2.01–3.59) | 0.63 (0.57–0.69) | 1.00 (1.00–1.00) |
| 100 | 6 | 3.12 (1.28–6.03) | 0.80 (0.52–1.33) | 2.01 (2.00–5.92) |
| 200 | 6 | 9.42 (6.75–12.0) | 2.19 (1.40–2.58) | 2.00 (1.00–5.92) |

[a] Number of subjects with adequate serum drug concentrations to evaluate $AUC_{0-6}$ and $C_{max}$.
[b] NA, not available because of lack of measurable drug concentration data.
[c] Data are presented as median (range).

vestigated the safety and PK parameters of LFF571 in healthy adults. The PK parameters of orally administered single doses (up to 1,000 mg) and multiple doses (up to 200 mg every 6 h for 10 days) of LFF571 indicated that the drug was minimally absorbed, with serum drug concentrations close to or below the LLOQ (0.5 ng/ml). The highest serum drug concentration observed (3.2 ng/ml) was seen in a subject who received the maximum dose studied (200 mg for 10 days), although a limitation of defining the peak exposure ($C_{max}$ and $T_{max}$) was that the collection of PK samples was limited to 0.5, 1, 2, 6, 12, and 24 h postdose. Because of insufficient quantifiable serum LFF571 concentrations, most PK parameters could not be evaluated. Although the serum LFF571 concentrations observed under fasting conditions were slightly higher than those observed under fed conditions, levels remained low and the differences observed after fasting are unlikely to be clinically relevant.

High concentrations of LFF571 in feces were detected in all cohorts. After a single dose, recovery of the drug was relatively low and variable. This was likely attributable to the short fecal collection time period of 0 to 48 h postdose (a limitation of the study). In fact, LFF571 was not detectable in the majority of the fecal samples collected during the first day (0 to 24 h postdose). Depending on the bowel movement habits of subjects, it is likely that the majority of the administered dose would be excreted after 48 h. In part II, in which multiple fecal samples were collected over the last 3 days of therapy (approximating steady-state conditions), 60 to 100% of the daily dose of LFF571 was recovered within a 24-h period. Although the possibility of first-pass biliary-hepatic elimination cannot be formally ruled out, the high fecal recovery and low serum drug concentrations suggest that LFF571 remains in the gastrointestinal tract after dosing. This hypothesis is supported by the high molecular weight of the compound and the low membrane-permeating ability of LFF571 observed in Caco-2 cells (lower than the low-permeating-ability marker mannitol; data not shown). Furthermore, <0.5% of LFF571 is absorbed in rats and *C. difficile*-infected hamsters (10). In rats, there was minimal distribution to the liver and no measurable distribution to other tissues (data not shown) and LFF571 was excreted in feces as the unchanged drug after oral and intravenous dosing (data not shown). Accumulation of LFF571 at the site of *C. difficile* infection supports its potential use against this disease. With multiple doses, concentrations of LFF571 in the feces of healthy volunteers (which ranged from a low of 181 μg/g in a subject who received 25 mg to a high of 8,720 μg/g in a subject who received 200 mg) were approximately 362 to 34,800 times the *in vitro* $MIC_{90}$ of LFF571 for *C. difficile*.

In this study, LFF571 was generally safe and well tolerated among subjects who received single and multiple doses. Among subjects who received a single dose of LFF571, the overall incidence of adverse events did not seem to increase with the dose level (up to 1,000 mg) and did not differ from that among subjects who received a placebo. The adverse events that were noted appeared to occur sporadically. Consistent with an oral antibiotic, subjects who received higher multiple doses of the study drug may have slightly higher rates and longer durations of gastrointestinal pain or distension than those who received a placebo. The small numbers of subjects in this study, however, preclude determining if any definitive association with LFF571 exists. Of note, diarrhea developed frequently among subjects who received multiple doses of both LFF571 and the placebo. This most likely represented the stringent case definition of diarrhea, which included frequent bowel movements. No bowel movement was described as liquid. Overall, gastrointestinal adverse events were assessed as mild in severity and no subject stopped taking the study drug because of an adverse event.

In conclusion, LFF571 is a novel EF-Tu inhibitor that shows potent *in vitro* activity against *C. difficile* and accumulated to high levels in the feces of healthy adults. LFF571 was generally safe and well tolerated, with limited systemic exposure. Gastrointestinal adverse events were noted but were mild in severity, and no subject stopped taking the study drug because of an adverse event. These findings support the future development of LFF571 as a potential oral treatment for *C. difficile* infections.

## ACKNOWLEDGMENTS

Financial support was provided by Novartis for the conduct of this study and the preparation of the manuscript.

We thank Catherine Jones of Novartis Institutes for BioMedical Research for providing writing and editorial assistance.

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