

Clinical Outcomes, Safety, and Pharmacokinetics of OPT-80 in a Phase 2 Trial with Patients with *Clostridium difficile* Infection[∇]

T. Louie,^{1*} M. Miller,² C. Donskey,³ K. Mullane,⁴ and E. J. C. Goldstein⁵

University of Calgary, Calgary, Alberta, Canada¹; Jewish General Hospital, Montreal, Quebec, Canada²; VA Medical Center, Cleveland, Ohio³; University of Chicago, Chicago, Illinois⁴; and R. M. Alden Research Laboratory, Santa Monica, California⁵

Received 6 November 2007/Returned for modification 2 May 2008/Accepted 17 October 2008

OPT-80, a novel, minimally absorbed macrocycle, is a candidate treatment option for *Clostridium difficile* infection (CDI) based on cure without recurrence of CDI in the hamster challenge model, good in vitro activity against *C. difficile*, and relative sparing of commensal gram-negative anaerobes. In this open-label, dose-ranging clinical trial, 48 evaluable subjects were randomized to receive either 50, 100, or 200 mg of OPT-80 orally every 12 h for 10 days as treatment for mild to moderately severe CDI. OPT-80 was well tolerated by all subjects. Plasma concentrations were below the lower limit of quantitation in almost one-half of patients and typically ≤ 20 ng/ml across the dose range; the mean fecal concentrations exceeded the MIC at which 90% of the isolates tested are inhibited by 2,000- to 10,000-fold with increasing dosages. Resolution of diarrhea within 10 days was achieved in 10/14 patients (71%), 12/15 patients (80%), and 15/16 patients (94%), and the median time to resolution of diarrhea was reduced from 5.5 to 3.0 days with increasing dosages. Across all groups, the clinical cure rate, which was defined as resolution of diarrheal disease without the need for further treatment, was 41/45 patients (91%). Recurrence of CDI at ~ 1 month after treatment was observed in two (5%) patients, one each in the 100-mg and 400-mg groups. The apparent high clinical response, good tolerance, low recurrence rate, and more-complete and rapid symptom control with the highest dosage support the selection of the 200-mg twice-daily dose for further clinical development of OPT-80 for treatment of CDI.

Clostridium difficile infection (CDI) is the most common cause of health care-related infectious diarrhea in developed countries (7, 9, 18, 19). This organism accounts for 20 to 30% of the cases of antibiotic-associated diarrhea and nearly all cases of antibiotic-associated colitis (2, 5, 14, 27). While most patients respond to vancomycin or metronidazole treatment, delayed response or failure to respond and recurrence in responders remain serious deficiencies (1, 4, 13, 26). Rising case rates of disease in the United States, Canada, and Europe (3, 15–17, 22), associated with increased disease severity and higher recurrence rates (20, 21), have heightened the urgency to explore new therapeutic options.

OPT-80 (12, 25) is a fermentation product primarily composed of an 18-membered macrocycle. It is highly active against *Clostridium difficile* and most other clostridial species except *Clostridium ramosum* and against anaerobic gram-positive species but has poor activity against gram-negative obligate anaerobes (6, 8, 11, 24), a property that might allow more selective killing of *C. difficile* with retention of components of the resident intestinal microflora (8). The compound was shown to cure CDI in the hamster model of disease without subsequent relapse (24). The MIC at which 90% of the isolates tested are inhibited for *C. difficile* is 0.125 $\mu\text{g/ml}$, compared to 0.5 and 2 $\mu\text{g/ml}$ for metronidazole and vancomycin, respectively. OPT-80 is bactericidal for *C. difficile* with a low propensity to generate resistance (24), has no cross-resistance to existing antimicrobials, and has a prolonged postantibiotic effect. It is hypothesized that the potential to achieve highly active

but narrower-spectrum therapy against *C. difficile* could result in a high response rate to therapy followed by a lower risk of recurrent disease.

OPT-80 has a promising preclinical safety profile. It is minimally absorbed from the gastrointestinal tract. It showed no adverse effects in rats at single oral doses up to 1,000 mg/kg of body weight or in rats or monkeys at repeated doses of up to 90 mg/kg/day for 28 days. Phase 1A single-dose and phase 1B multiple-dose, dose-escalating studies showed that OPT-80 is well tolerated after single oral doses up to 450 mg and multiple doses up to 450 mg for 10 days (23). No medication-related adverse events were reported. Plasma concentrations of OPT-80 were generally below the limit of quantitation (5 ng/ml) following oral administration, while high fecal concentrations were observed.

In this first clinical trial for treatment of CDI, patients presenting with *C. difficile* infection were randomized to receive increasing dosages of OPT-80 to assess possible treatment regimens for registration trials.

(This work was presented in part at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 16 to 19 December 2005 [16a], and at the 16th European Congress of Clinical Microbiology and Infectious Diseases, Nice, France, 1 to 4 April 2006 [16b].)

MATERIALS AND METHODS

Phase 2A study of treatment of *C. difficile* infection. This study was a dose-finding, randomized, open-label study to select a safe and effective dose of OPT-80 for the treatment of mild to moderately severe CDI. Males and females at least 18 years old with three or more diarrheal (liquid or unformed) stools/day or six or more diarrheal stools in a 36-h period and with a positive *C. difficile* toxin result by enzyme immunoassay or cell cytotoxicity assay and treatment with another antimicrobial agent (metronidazole or vancomycin) for less than 24 h were eligible for enrollment. Only patients with a primary episode or first relapse

* Corresponding author. Mailing address: AGW5, Infection Prevention & Control, Foothills Medical Center, 1403 29th St. N.W., Calgary, Alberta T2N 2T9, Canada. Phone: (403) 944-4766. Fax: (403) 944-2484. E-mail: louie@ucalgary.ca.

[∇] Published ahead of print on 27 October 2008.

of disease were eligible. Patients with more than 12 diarrheal bowel movements/day, vomiting, ileus, severe abdominal tenderness, white blood cell (WBC) count of more than 30×10^9 /liter, toxic megacolon, or concern about life-threatening CDI were excluded from study entry. Patients with severe underlying disease who were not expected to survive the study period, who had experienced more than one relapse or recurrence within 3 months, who required concurrent antibiotic therapy, or who had Crohn's disease or ulcerative colitis were also excluded.

Subjects were randomized by interactive voice randomization system to receive either 50, 100, or 200 mg of OPT-80 orally every 12 h (100, 200, or 400 mg/day) for 10 days and were monitored for 6 weeks after completion of treatment to determine relapse or recurrence.

Subjects recorded all symptoms on daily diary cards, including stool frequency and consistency (aided by a chart or diagram), the presence of blood in the stool, nausea, vomiting, and abdominal discomfort. Hematology, biochemistry profiles, and an electrocardiogram were performed at study entry and at the end of treatment (days 10 to 12) or at withdrawal from the study (whichever was sooner). Clinical observation and diary card evaluation were performed at the end of treatment (days 10 to 12). Patient interviews were conducted on treatment days 2 through 9, day 17, weekly after day 17, and on day 52. Patients were instructed to contact the clinical trials coordinator immediately if diarrheal symptoms recurred.

Since no patients with CDI had been treated with OPT-80 prior to this study, a safety guideline was applied as follows: if a subject failed to clinically improve after 4 days of treatment or to achieve resolution of diarrhea, which was defined as more than three bowel movements/day with associated fever, leukocytosis, and abdominal pain 6 days after study entry, the subject was declared a failure and was withdrawn from the study, and alternate treatment was initiated. Patients who were clinically improving were allowed to complete the treatment course. A data safety monitoring board reviewed all adverse events.

Nonresponders and responders who subsequently developed recurrence of CDI were reassessed for clinical features, laboratory profiles, *C. difficile* toxin in fecal filtrates, and the presence of *C. difficile* in stool samples. For these individuals, standard treatment with either oral metronidazole or vancomycin was initiated by the investigator.

Evaluation of clinical outcomes. The safety population included all randomized subjects who received at least one dose of study medication and had safety information available. Efficacy analyses were performed on data from all subjects in the modified intent-to-treat (mITT) population consisting of all randomized subjects who received at least one dose of study medication, had a history of diarrhea, and had three or more loose stools in 24 h, and a positive *C. difficile* toxin test at baseline.

The primary outcome variables for measurement of response to treatment were as follows: (i) clinical cure or failure, (ii) time to resolution of diarrhea, and (iii) total relief of symptoms of CDI. Clinical cure was defined as resolution of diarrhea and abdominal discomfort within the 10-day treatment period and by the patient requiring no additional therapy for CDI during the treatment period. Resolution of diarrhea required conversion of three or more liquid or unformed stools/day to two or fewer semiformal or formed stools/day. For patients who were clinically improved by day 10 but did not meet the definition of resolution of diarrhea, e.g., still had three to six loose bowel movements per 24 h, the investigating physician had the option of close monitoring without immediate initiation of additional therapy. Clinical failure was defined as the need for additional therapy during the treatment period. *C. difficile* culture and toxin testing were performed in subjects who failed to respond. The time to resolution of diarrhea was defined as the time (in days) from the first dose of study medication to the day of resolution of diarrhea, which was defined as the first day that one or two stools (not watery or loose) within a 24-h period occurred and this was sustained for the duration of treatment up to study day 10. Resolution of diarrhea was assessed during a 10- to 12-day period utilizing the subject diary data. Total relief of symptoms of CDI, defined as resolution to three or fewer bowel movements per day (whether solid, semiformal, or liquid as recorded on the patient diary) without other associated signs or symptoms, such as fever ($\geq 37.7^\circ\text{C}$), abdominal pain, and elevated WBC by day 10 of the study. This composite outcome variable was used to discern differences in dose response that might be undetected by clinical cure or failure, since residual colonic inflammation might result in formed stools but higher than normal numbers of bowel movements, abdominal cramps, mild tenderness, or persisting laboratory abnormalities. Persistence of any parameter was termed no relief. If any variable was missing, this outcome was considered indeterminate.

The secondary outcome variable was recurrence of CDI, which was defined as three or more unformed stools (loose or watery) and a positive stool result for *C. difficile* toxin A or B within 6 weeks after the end of treatment.

Pharmacokinetic evaluation for phase 2A study. Pharmacokinetic plasma samples were taken 0.5 h prior to dosing and 2 h after dosing on the first and last days of a 10-day dosing regimen. Plasma and fecal concentrations of OPT-80 and its primary metabolite, OP-1118, were determined by reversed-phase high-performance liquid chromatography (RP-HPLC)-mass spectroscopy (Microconstants, Inc., San Diego, CA).

Plasma samples containing OPT-80 and mono-*O*-methyl OPT-80 (OP-1393) as the internal standard were precipitated with a methanol-acetic acid solution. The supernatant was diluted with water, extracted using Varian SPEC-C18 solid-phase extraction well plates, then analyzed by RP-HPLC using a Supelco HS PEG column maintained at 40°C . The mobile phase was nebulized using heated nitrogen in a Z-spray source or interface, and the ionized compounds were detected using a tandem quadrupole mass spectrometer. The concentration range of the assay for OPT-80 was 5 to 1,000 ng/ml for both OPT-80 and OP-1118.

On day 10, a complete 24-h evacuation of stool was collected and frozen at -80°C for subsequent determination of fecal concentrations. Fecal samples containing OPT-80 and the primary metabolite (OP-1118) were homogenized with an acetonitrile-acetic acid solution. An aliquot of the homogenate was diluted with acetonitrile, and mono-*O*-methyl OPT-80 (OP-1393) was added as the internal standard. RP-HPLC was then used as described above for the plasma assay. The standard concentration range of the assay for OPT-80 was 10 to 2,000 ng/ml, and the range for OP-1118 was 50 to 10,000 $\mu\text{g/ml}$.

Statistical analysis. Computations were done using SAS version 8.2 (SAS Institute, Cary, NC). For time to resolution of diarrhea, Kaplan-Meier product limit method was used to estimate survival function, and the generalized Wilcoxon test was used to compare the three treatment groups. For relief of symptoms of CDI (relief, no relief, or unknown), a two-sided 95% confidence interval around the difference (low dose-high dose) in no relief was constructed for each pairwise comparison (10). Statistical significance was declared if a two-sided *P* value was less than or equal to 0.05. No adjustments were made for multiple comparisons.

RESULTS

Enrollment and demographics. A total of 49 subjects were enrolled in this study. One subject withdrew consent and was dropped from the study prior to receiving study drug. Demographic characteristics of the safety population are shown in Table 1, and baseline clinical parameters of the same population referable to CDI are shown in Table 2. One subject (in the 400-mg/day group) had more than six bowel movements in 36 h but less than three bowel movements in the 24 h prior to enrollment in the study and could not be evaluated for time to resolution of diarrhea but could be evaluated for safety analysis and for clinical cure and recurrence in the per protocol evaluable population. Three patients were removed from the study after one or two doses due to removal of consent (one subject in the 100-mg/day group), requirement for additional antibiotics for pneumonia (one subject in the 100-mg/day group), or withdrawal from the study because of nausea (one subject in the 200-mg/day group). As a result, 14, 15, and 16 subjects in the 100-, 200-, and 400-mg/day groups, respectively, were evaluable for clinical outcomes.

Efficacy. Table 3 summarizes the rates of clinical cure or failure and recurrence. Using resolution of diarrhea by day 10, a clear majority of patients in each group responded to treatment. Responses were not statistically different between groups due to small sample size. Inclusion of patients who did not meet the definition of diarrhea resolution on day 10 but who appear to be clinically well compared to study entry clinical profiles and who normalized their stool pattern in the early follow-up period also as clinical cures shows a high response rate to treatment, regardless of the dosage used. Correspondingly, there were few clinical failures. *C. difficile* could not be cultured from any of the four failures at the time they were

TABLE 1. Demographic characteristics for 48 subjects evaluable for safety in a phase 2A study of OPT-80 treatment of CDI

| Demographic characteristic | Value for subjects in OPT-80 treatment group | | | Value for all subjects in the three treatment groups (n = 48) |
|-----------------------------------------------|----------------------------------------------|---------------------|---------------------|---------------------------------------------------------------|
| | 100 mg/day (n = 16) | 200 mg/day (n = 16) | 400 mg/day (n = 16) | |
| No. of subjects (%) | | | | |
| Sex | | | | |
| Female | 10 (63) | 11 (69) | 9 (56) | 30 (63) |
| Male | 6 (37) | 5 (31) | 7 (44) | 18 (37) |
| Race | | | | |
| Caucasian | 14 (88) | 15 (94) | 14 (88) | 43 (90) |
| Black | 1 (6) | 1 (6) | 0 (0) | 2 (4) |
| Asian | 0 (0) | 0 (0) | 1 (6) | 1 (2) |
| Hispanic | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Other ^a | 1 (6) | 0 (0) | 1 (6) | 2 (4) |
| Age (yr) | | | | |
| Mean ± SD | 56.3 ± 17.8 | 53.1 ± 23.0 | 55.3 ± 17.7 | 54.9 ± 19.3 |
| Median | 54.5 | 55.5 | 56.0 | 56.0 |
| Range | 28.0–89.0 | 18.0–88.0 | 18.0–90.0 | 18.0–90.0 |
| Wt (kg) | | | | |
| Mean ± SD | 69 ± 14 | 68 ± 11 | 68 ± 14 | 68 ± 13 |
| Median | 69 | 66 | 65 | 66 |
| Range | 38–89 | 52–96 | 40–88 | 38–96 |
| Ht (cm) | | | | |
| Mean ± SD | 164 ± 16 | 166 ± 9 | 166 ± 13 | 166 ± 13 |
| Median | 162 | 170 | 164 | 165 |
| Range | 122–188 | 150–178 | 142–193 | 122–193 |
| Calculated body mass index^b | | | | |
| Mean ± SD | 25.8 ± 3.9 | 24.9 ± 4.5 | 24.3 ± 2.5 | 25.0 ± 3.7 |
| Median | 25.0 | 24.0 | 24.5 | 25.0 |
| Range | 17.0–34.0 | 17.0–32.0 | 20.0–28.0 | 17.0–34.0 |

^a Other includes East Indian and Indian.

^b Calculated body mass index is defined as (weight in kilograms)/(height in meters)².

withdrawn from treatment and placed on metronidazole or vancomycin therapy, and only one (in the 200-mg/day group) was toxin positive.

Recurrence was observed in 2 of 41 clinical cure patients,

one each in the 100- and 400-mg/day groups, each at ~4 weeks after treatment. Both patients with recurrence, with five or six bowel movements per day for 2 or 3 days, responded subsequently to metronidazole. Toxin was present, and *C. difficile* was cultivable from the feces of both patients with recurrence.

The time to resolution of diarrhea was defined as the time

TABLE 2. Number of diarrheal bowel movements, WBC count, and temperature at study entry in the safety evaluable population

| Characteristic | Value for subjects in OPT-80 treatment group | | |
|-----------------------------------------|----------------------------------------------|---------------------|------------------------|
| | 100 mg/day (n = 16) | 200 mg/day (n = 16) | 400 mg/day (n = 16) |
| No. of diarrheal bowel movements | | | |
| Mean ± SD | 6.8 ± 2.6 | 6.3 ± 2.6 | 7.1 ± 3.2 |
| Median | 6.5 | 5.5 | 7.0 |
| Range | 3.0–0.0 | 3.0–12.0 | 2.0 ^a –12.0 |
| WBC count (10⁹/liter) | | | |
| Mean ± SD | 7.9 ± 2.6 | 9.7 ± 5.7 | 8.6 ± 3.1 |
| Median | 7.5 | 8.0 | 8.6 |
| Range | 3.8–13.8 | 1.4–24.0 | 4.0–13.2 |
| Temp (°C) | | | |
| Mean ± SD | 36.6 ± 0.4 | 36.5 ± 0.3 | 36.4 ± 0.5 |
| Median | 36.6 | 36.5 | 36.4 |
| Range | 35.9–37.4 | 36.0–37.0 | 35.6–37.5 |

^a One patient had a total of more than six liquid bowel movements in a 36-h period but only 2 in the 24 h prior to enrollment in the study.

TABLE 3. Rates of clinical cure and recurrence in the population treated per protocol

| Parameter or outcome | No. (%) of patients in the following treatment group showing clinical cure, failure, or recurrence: | | |
|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|----------------------|----------------------|
| | 100 mg of OPT-80/day | 200 mg of OPT-80/day | 400 mg of OPT-80/day |
| Total patients | 14 (100) | 15 (100) | 16 (100) |
| Clinical cure | | | |
| Diarrhea resolution by day 10 | 10 (71) | 12 (80) | 15 (94) |
| Diarrhea resolves after day 10 with no additional treatment | 2 (14) | 1 (7) | 1 (6) |
| Clinical failure (requiring change of therapy) | 2 (14) | 2 (13) | 0 (0) |
| Clinical recurrence ^a | 1 (8.3) | 0 (0) | 1 (6.3) |

^a Recurrence of toxin-positive diarrhea within 6 weeks after treatment, evaluated in patients who were clinical successes.

TABLE 4. Complete relief of symptoms of CDAD by end of therapy in the mITT population^a

| Relief of symptoms of CDAD | No. of patients (%) in the following treatment group showing relief of symptoms of CDAD: | | |
|--------------------------------------------|------------------------------------------------------------------------------------------|-------------------------------|-------------------------------|
| | 100 mg of OPT-80/day (n = 16) | 200 mg of OPT-80/day (n = 16) | 400 mg of OPT-80/day (n = 15) |
| Complete relief | 6 (37.5) | 8 (50.0) | 13 (86.7) |
| Incomplete relief | 9 (56.3) | 6 (37.5) | 2 (13.3) |
| Required further treatment | 2 (12.5) | 2 (12.5) | 0 (0) |
| Required no further treatment ^b | 5 (31.3) | 3 (18.8) | 2 (13.3) |
| Dropped from study | 2 (12.5) | 1 (6.3) | 0 (0) |
| Indeterminate ^c | 1 (6.3) | 2 (12.5) | 0 (0) |

^a Complete relief of symptoms of CDAD was defined as resolution to three or fewer total bowel movements per day (formed or unformed), as noted on the patient's diary card) and absence of fever, abdominal pain, and elevated WBC counts by day 10 of the study.

^b These patients had more than three bowel movements on day 10 (range, 4 to 6) but were otherwise asymptomatic and had normal WBC counts and were afebrile. Follow-up analysis showed that bowel movements normalized by day 17.

^c Treatment success but could not be categorized because of missing laboratory data.

for the patient to resolve to one or two unformed stools per day, according to the patient's diary card. In the mITT population, the median times to resolution were 5.5 days, 3.5 days, and 3.0 days for the 100-, 200-, and 400-mg/day treatment groups, respectively. The mean time to resolution of diarrhea (in days) \pm standard deviation was 6.3 ± 3.7 days in subjects treated with 100 mg/day, 4.8 ± 3.6 days in subjects treated with 200 mg/day, and 3.6 ± 2.0 days in subjects treated with 400 mg/day. No statistically significant difference in the time to resolution of diarrhea was observed between the 100- and 200-mg/day treatment groups or between the 200- and 400-mg/day treatment groups; however, the difference between the 100- and 400-mg/day treatment groups approached statistical significance ($P = 0.0506$ by Kaplan-Meier analysis and $P = 0.0503$ by Kruskal-Wallis test).

Total relief of symptoms of *Clostridium difficile*-associated diarrhea (CDAD) by the end of treatment is shown in Table 4. Total relief was achieved by 37.5% of the 100-mg/day recipients, 50.0% of the 200-mg/day recipients, and 86.7% of the 400-mg/day recipients. Patients who did not have total relief by day 10, primarily because the numbers of bowel movements/day were above the threshold of three or fewer per day, were nevertheless treatment successes, having resolved symptoms by day 17 without requiring further treatment. Three patients who were dropped from the study (one for removal of consent, one for the requirement of exclusionary antibiotics, and one for the inability to take oral medications) are also listed as having no relief.

Safety. Adverse events ($n = 17$) were reported in 9/45 subjects, 4 patients in each of the lower dosage groups and 1 in the 400-mg/day group. All adverse events (a fall, shortness of breath, pain in an extremity, renal colic, bronchitis, pneumonia, urinary tract infection, hypotension, fluid overload, pancreatitis, diarrhea, cardiac failure, angina [$n = 2$], cerebral-vascular hemorrhage, gastrointestinal bleeding, and *Staphylococcus aureus* bacteremia) ap-

TABLE 5. Plasma concentrations of OPT-80 and the primary metabolite, OP-1118

| Drug and OPT-80 treatment | Total no. of subjects ^a | No. of subjects with the following plasma drug concn: | | | | Top concn found (ng/ml) ^b |
|---------------------------|------------------------------------|-------------------------------------------------------|------------|--------------|------------|--------------------------------------|
| | | <5 ng/ml | 5–20 ng/ml | 20–100 ng/ml | >100 ng/ml | |
| OPT-80 | | | | | | |
| 100 mg/day | 14 | 12 | 2 | 0 | 0 | 12.3 |
| 200 mg/day | 16 | 7 | 8 | 1 | 0 | 93.7 |
| 400 mg/day | 16 | 3 | 11 | 2 | 0 | 84.9 |
| OP-1118 | | | | | | |
| 100 mg/day | 14 | 1 | 11 | 2 | 0 | 77.2 |
| 200 mg/day | 16 | 2 | 8 | 5 | 1 | 154.3 |
| 400 mg/day | 16 | 0 | 7 | 9 | 1 | 402.3 |

^a Number of subjects with at least one plasma pharmacokinetic sample collected after the first dose.

^b The highest concentration found in any subject within the dosing group.

peared not to be related to study medication. Five subjects reported six of the latter foregoing adverse events as serious adverse events.

Pharmacokinetics. (i) Plasma concentration data. Plasma levels of OPT-80 and its primary metabolite, OP-1118, in all subjects who received OPT-80 and had more than one plasma pharmacokinetic sample collected after the first dose are summarized in Table 5. After administration of multiple doses orally, detectable plasma concentrations were found in 2/14 (14.3%) subjects in the 100-mg/day treatment group (range, 9.45 to 12.3 ng/ml), 9/16 (56.3%) subjects in the 200-mg/day treatment group (range, 5.12 to 93.7 ng/ml), and 13/16 (81.35%) subjects in the 400-mg/day treatment group (range, 5.32 to 84.9 ng/ml). Across all treatment groups, the majority of subjects (43/46 [93.5%]) had plasma concentrations under 20 ng/ml. Concentrations of OPT-80 over 50 ng/ml were observed in two subjects, one each in the 200-mg/day and 400-mg/day dosing groups. In a dose-dependent fashion, low concentrations of OPT-80 were detectable in an increasing proportion of patients. Concentrations of the metabolite OP-1118 tended to be somewhat higher but were still typically at low ng/ml concentrations.

(ii) Fecal concentrations of OPT-80. The mean fecal concentrations of OPT-80 and its primary metabolite, OP-1118, are given in Table 6. The concentrations of OPT-80 on day 10 were 256 ± 136 μ g/g (range, 82 to 558 μ g/g) ($n = 11$) in 100-mg/day recipients, 442 ± 238 μ g/g (range, 12 to 787 μ g/g)

TABLE 6. Mean fecal concentrations of OPT-80 and its primary metabolite, OP-1118, on day 10 of dosing^a

| OPT-80 treatment | No. of subjects ^b | Mean fecal concn (\pm SD) (μ g/g) | |
|------------------|------------------------------|-------------------------------------------|---------------|
| | | OPT-80 | OP-1118 |
| 1000 mg/day | 11 | 256 ± 136 | 393 ± 260 |
| 200 mg/day | 9 | 442 ± 238 | 430 ± 263 |
| 400 mg/day | 13 | 1433 ± 975 | 760 ± 373 |

^a Mean fecal concentrations of OPT-80 and its primary metabolite, OP-1118, on day 10 of dosing or on the final day of dosing for subjects removed from treatment early.

^b Number of subjects with sufficient day 10 fecal samples collected for pharmacokinetic analysis.

($n = 9$) in 200-mg/day recipients, and $1,433 \pm 975 \mu\text{g/g}$ (range, 389 to 3,975 $\mu\text{g/g}$) ($n = 13$) in 400-mg/day recipients. The fecal levels of the metabolite OP-1118 were of similar magnitude, but the ratio of OPT-80 to OP-1118 increased as the dose level increased.

DISCUSSION

In this open-label, randomized, dose-ranging study, OPT-80 was shown to be well tolerated after multiple oral doses up to 400 mg/day in patients with mild to moderately severe *Clostridium difficile* infection. In a dose-dependent fashion, low OPT-80 plasma concentrations (≤ 5 to 20 ng/ml) were detected. Fecal concentrations in 400-mg/day dose recipients exceeded by 10,000-fold the MIC at which 90% of the isolates tested are inhibited (0.125 $\mu\text{g/ml}$) against *C. difficile*. The contribution of OPT-1118, the major metabolite, to clinical outcomes is uncertain. Based on an 8- to 16-fold-lesser activity against *C. difficile* (23) but because of high fecal concentrations, it is presumed that the metabolite could play a therapeutic role.

Resolution of diarrhea or disease within 10 days was achieved in 71%, 80%, and 94% of patients in the three treatment groups given increasing doses of OPT-80. Rapidity of resolution of diarrhea and completeness of symptom control favored the 400-mg daily dose. That several patients did not meet the criteria of diarrhea resolution by 10 days but clinically resolved without further intervention supports the notion that not all patients must meet symptom control by 10 days for clinical resolution. It is not clear if the possible lesser ecologic impact could play a role here. Two subjects in each of the 100-mg/day and 200-mg/day dose groups were clinical failures, although *C. difficile* was not recovered in these patients. Because of the 6-day limit for time to response before declaring failure, it is unknown whether a longer time limit for response would have resulted in a different treatment outcome. The low recurrence rate (5%) is supportive of the possibility that OPT-80 is sufficiently selective to allow the normal microbiota (28) to reestablish itself following treatment of CDAD. Based on the susceptibility profiles against cultivable anaerobes, OPT-80 might be considered a bioprobe on the contribution of the gram-negative obligate anaerobes to colonization resistance. It is unclear, however, to what extent OPT-80 affects the presumed gram-positive noncultivable genera. At present, it should be cautioned that the low recurrence rate could also be due to the limited numbers of patients studied, mild cases as observed by Zar (29), or nonallowance of concomitant antibiotic therapy that is common in hospitalized patients. Confirmation of OPT-80's role in CDI therapy awaits analysis of the recently closed first phase 3 trial comparing 200 mg OPT-80 twice daily to 125 mg vancomycin four times daily for 10 days.

ACKNOWLEDGMENTS

The expert clinical trial support provided by Starr Shangle is gratefully acknowledged. T. Louie is an investigator for Optimer Pharma, Genzyme, and Wyeth. K. Mullane is an investigator for Basilea, Merck, Optimer Pharma, ViroPharma, Pfizer, Schering Plough, and GlaxoSmithKline. E. J. C. Goldstein is a consultant for

ViroPharma and Optimer. M. Miller is an investigator for Optimer Pharma and Merck.

REFERENCES

- Al-Nassir, W. N., A. K. Sethi, M. M. Nerandzic, G. S. Bobulsky, R. L. Jump, and C. J. Donskey. 2008. Comparison of clinical and microbiological response to treatment of *Clostridium difficile* disease with metronidazole and vancomycin. *Clin. Infect. Dis.* **47**:56–62.
- Bartlett, J. G. 1992. Antibiotic-associated diarrhea. *Clin. Infect. Dis.* **15**:573–581.
- Bartlett, J. G. 2006. Narrative review: the new epidemic of *Clostridium difficile* enteric disease. *Ann. Intern. Med.* **145**:758–764.
- Belmares, J., D. N. Gerding, J. P. Parada, S. Miskevics, F. Weaver, and S. Johnson. 2007. Outcome of metronidazole therapy for *Clostridium difficile* disease and correlation with a scoring system. *J. Infect.* **55**:495–501.
- Bouza, E., A. Burillo, and P. Munoz. 2006. Antimicrobial therapy of *Clostridium difficile*-associated diarrhea. *Med. Clin. N. Am.* **90**:1141–1163.
- Credito, K. L., and P. C. Appelbaum. 2004. Activity of OPT-80, a novel macrocycle compared with those of eight other agents against selected anaerobic species. *Antimicrob. Agents Chemother.* **48**:4430–4434.
- Fekety, R., and A. B. Shah. 1993. Diagnosis and treatment of *Clostridium difficile* colitis. *JAMA* **269**:71–75.
- Finegold, S. M., D. Molitoris, M. L. Vaisanen, Y. Song, C. Liu, and M. Bolaños. 2004. In vitro activities of OPT-80 and comparator drugs against intestinal bacteria. *Antimicrob. Agents Chemother.* **48**:4898–4902.
- Guerrant, R. L., J. M. Hughes, N. L. Lima, and J. Crane. 1990. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. *Rev. Infect. Dis.* **12**(Suppl. 1):S41–S50.
- Hauck, W., and S. Anderson. 1986. A comparison of large sample confidence interval methods for the difference of two binomial probabilities. *Am. Statistician* **40**:318–322.
- Hecht, D. W., M. A. Galang, S. P. Sambol, J. R. Osmolski, S. Johnson, and D. N. Gerding. 2007. In vitro activities of 15 antimicrobial agents against 110 toxigenic *Clostridium difficile* clinical isolates collected from 1983 to 2004. *Antimicrob. Agents Chemother.* **51**:2716–2719.
- Hochlowski, J. E., S. J. Swanson, L. M. Ranfranz, D. N. Whittern, A. M. Buko, and J. B. McAlpine. 1987. Tiacumicins, a novel complex of 18-membered macrolides. II. Isolation and structure determination. *J. Antibiot. (Tokyo)* **40**:575–588.
- Hogenaer, C., H. F. Hammer, G. J. Krejs, and E. C. Reisinger. 1998. Mechanisms and management of antibiotic-associated diarrhea. *Clin. Infect. Dis.* **27**:702–710.
- Kelly, C. P., C. Pothoulakis, and J. T. LaMont. 1994. *Clostridium difficile* colitis. *N. Engl. J. Med.* **330**:257–262.
- Kuiper, E. J., B. Coignard, and B. Tull; the ESCMID Study Group for *Clostridium difficile* (ESGCD). 2006. Emergence of *Clostridium difficile*-associated disease in North America and Europe. *Clin. Microbiol. Infect.* **12**(Suppl. 6):2–18.
- Loo, V. G., L. Poirier, M. A. Miller, M. Oughton, M. D. Libman, S. Michaud, A.-M. Bourgault, T. Nguyen, C. Frenette, M. Kelly, A. Vibien, P. Brassard, S. Fenn, K. Dewar, T. J. Hudson, R. Horn, P. Rene, Y. Monczak, and A. Dascal. 2005. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N. Engl. J. Med.* **353**:2442–2449.
- Louie, T., M. Miller, C. Donskey, K. Mullane, E. J. C. Goldstein, M. Corrado, C. F. Okumu, S. Gorbach, P. Sears, S. Shangle, B. Walsh, Y. K. Shue, and R. A. Preston. 2005. Safety, pharmacokinetics and outcomes of PAR-1 in healthy subjects and patients with *Clostridium difficile*-associated diarrhea (CDAD), abstr. LB2-29, p. 226. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
- Louie, T. J., M. Miller, C. Donskey, K. Mullane, E. J. C. Goldstein, D. M. Citron, M. Corrado, S. L. Gorbach, P. Sears, S. Shangle, B. Walsh, and Y.-K. Shue. 2006. A clinical and laboratory evaluation of PAR-101 in patients with *Clostridium difficile*-associated diarrhoea. *Clin. Microbiol. Infect.* **12**(Suppl. 4):39.
- McDonald, L. C., G. E. Killgore, A. Thompson, R. C. Owens, Jr., S. V. Kazakova, S. V. Sambol, S. Johnson, and D. N. Gerding. 2005. An epidemic, toxin gene-variant strain of *Clostridium difficile*. *N. Engl. J. Med.* **353**:2433–2441.
- McFarland, L. V. 1995. Epidemiology of infectious and iatrogenic nosocomial diarrhea in a cohort of general medicine patients. *Am. J. Infect. Control* **23**:295–305.
- Miller, M. A., M. Hyland, M. Ofner-Agostini, M. Gourdeau, and M. Ishak. 2002. Morbidity, mortality, and healthcare burden of nosocomial *Clostridium difficile*-associated diarrhea in Canadian hospitals. *Infect. Control Hosp. Epidemiol.* **23**:137–140.
- Musher, D. M., S. Aslam, N. Logan, S. Nallacheru, I. Bhaila, F. Borchert, and R. J. Hamill. 2005. Relatively poor outcome after treatment of *Clostridium difficile* colitis with metronidazole. *Clin. Infect. Dis.* **40**:1586–1590.
- Peppin, J., M. E. Alary, L. Valiquette, E. Raiche, J. Ruel, K. Fulop, D. Godin, and C. Bourassa. 2005. Increasing risk of relapse after treatment

- of *Clostridium difficile* colitis in Quebec, Canada. Clin. Infect. Dis. **40**:1591–1597.
22. **Pepin, J., L. Valiquette, M. E. Alary, P. Villemure, A. Pelletier, K. Forget, K. Pepin, and D. Chouinard.** 2004. *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ **171**:466–472.
 23. **Shue, Y. K., P. S. Sears, S. Shangle, R. B. Walsh, C. Lee, S. L. Gorbach, F. Okumu, and R. A. Preston.** 2008. Safety, tolerance, and pharmacokinetic studies of OPT-80 in healthy volunteers following single and multiple oral doses. Antimicrob. Agents Chemother. **52**:1391–1395.
 24. **Swanson, R. N., D. J. Hardy, N. L. Shipkowitz, C. W. Hanson, N. C. Ramer, P. B. Fernandes, and J. J. Clement.** 1991. In vitro and in vivo evaluation of tiacumicins B and C against *Clostridium difficile*. Antimicrob. Agents Chemother. **35**:1108–1111.
 25. **Theriault, R. R., J. P. Karwowski, M. Jackson, R. L. Girolami, G. N. Sunga, C. M. Vojtko, and L. J. Coen.** 1987. Tiacumicins, a novel complex of 18-membered macrolide antibiotics. I. Taxonomy, fermentation and antibacterial activity. J. Antibiot. (Tokyo) **40**:567–574.
 26. **Wilcox, M. H., and R. Howe.** 1995. Diarrhoea caused by *Clostridium difficile*: response time for treatment with metronidazole and vancomycin. J. Antimicrob. Chemother. **36**:673–679.
 27. **Wistrom, J., S. R. Norrby, E. B. Myhre, S. Eriksson, G. Granstrom, L. Lagergren, G. Englund, C. E. Nord, and B. Svenungsson.** 2001. Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. J. Antimicrob. Chemother. **47**:43–50.
 28. **Yamamoto-Osaki, T., S. Kamiya, S. Sawamura, M. Kai, and A. Ozawa.** 1994. Growth inhibition of *Clostridium difficile* by intestinal flora of infant feces in continuous flow culture. J. Med. Microbiol. **40**:179–187.
 29. **Zar, F. A., S. R. Bakkanagari, K. M. L. S. T. Moorthi, and M. B. Davis.** 2007. A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. Clin. Infect. Dis. **45**:302–307.