Safety, Tolerance, and Pharmacokinetic Studies of OPT-80 in Healthy Volunteers following Single and Multiple Oral Doses


Optimer Pharmaceuticals, San Diego, California; Palm Beach Atlantic University, West Palm Beach, Florida; Tufts University School of Medicine, Boston, Massachusetts; Direcxt Corporation, Cupertino, California; and University of Miami School of Medicine, Miami, Florida

Received 8 August 2007/Returned for modification 2 October 2007/Accepted 4 February 2008

Current therapies for Clostridium difficile infection (CDI) are encumbered by treatment failures and recurrences. Due to its high in vitro activity against C. difficile but low activity against the typical intestinal flora, minimal absorption, and durable cure in the hamster model of C. difficile infection, OPT-80 was considered for clinical development as a therapy for CDI. This trial consisted of two phases. Four single oral doses of OPT-80 (100, 200, 300, and 450 mg) were administered in a crossover manner to 16 healthy volunteers in a double-blind, placebo-controlled phase 1A study; a 1- to 2-week washout interval separated the treatments. In the double-blind phase 1B study, 24 healthy subjects were randomized to receive OPT-80 (150, 300, or 450 mg) or placebo for 10 days. In both studies, OPT-80’s safety and tolerability were evaluated and the concentrations of OPT-80 and its primary metabolite (OP-1118) in plasma and feces were determined. OPT-80 levels in the urine were also analyzed for the phase 1A study. In both the single-dose and the multiple-dose studies, OPT-80 was well tolerated by all subjects in all dose groups. Maximal plasma concentrations were near or below the limit of quantification (5 ng/ml) across the dose range; urine concentrations were below the detection limit. The fecal total recovery of OPT-80 plus its major metabolite, OP-1118, approximated 100%. The tolerability, high fecal concentration, and low systemic exposure data from these studies support the further clinical development of OPT-80 as an oral therapy for CDI.

Clostridium difficile-associated diarrhea (CDAD) is a significant problem in hospitals and long-term care facilities and in the community. Clostridium difficile is the most common cause of nosocomial diarrhea in developed countries (7, 12, 17). The organism accounts for approximately 20% of cases of antibiotic-associated diarrhea and the majority of cases of antibiotic-associated colitis (2, 6, 10, 18). The rising incidence of CDAD has been attributed to the frequent prescription of broad-spectrum antibiotics to hospitalized patients (4).

OPT-80 is a naturally occurring 18-member macrocycle derived from fermentation (8, 16). The antimicrobial activity of OPT-80 versus anaerobic species including C. difficile has been examined (1, 3, 5, 9). OPT-80 displays a narrow antimicrobial spectrum with excellent activity against many clostridia including C. difficile and moderate activity against certain gram-positive cocci. It is inactive against gram-negative organisms and Candida spp. In an experiment in which 110 genetically distinct strains of C. difficile were tested, the MIC at which 90% of isolates tested were inhibited (MIC90) was 0.125 μg/ml, translating to 4- and 16-fold-better potencies than those of metronidazole and vancomycin, respectively, against this species: MIC90 values of metronidazole and vancomycin for this panel of organisms were 0.5 and 2.0 μg/ml, respectively (7a). Furthermore, OPT-80 is bactericidal, with a minimum bactericidal concentration against C. difficile ATCC 9689 that is equal to its MIC. In comparison, the minimum bac-

* Corresponding author. Mailing address: Optimer Pharmaceuticals, Inc., 10110 Sorrento Valley Road, San Diego, CA 92121. Phone: (858) 909-0736. Fax: (858) 909-0737. E-mail: ykshue@optimerpharma.com.

† Published ahead of print on 11 February 2008.
ject’s colon and reduce the probability of relapse or reinfec-
tion. In this report, we present the safety, tolerability, and pharma-
cokinetics of OPT-80 in healthy volunteers following single and multiple oral doses.

(This study was presented in part at the 45 Interscience
Conference on Antimicrobial Agents and Chemotherapy,
Washington, DC, 16 to 19 December 2005 [1a], and at the 16th
European Congress of Clinical Microbiology and Infectious
Diseases, Nice, France, 1 to 4 April 2006 [11].)

MATERIALS AND METHODS

Study drugs. Clinical trial materials (CTMs), including the investigational
product and a placebo, were manufactured according to current good manufac-
turing practice procedures and dispensed in individually sealed and labeled
envelopes according to a randomization code.

CTMs for the phase 1A-SD study were supplied as 50-mg capsules, each
containing 50 mg OPT-80 in 0.5 g Labrasol and placebo capsules, each contain-
ing 0.5 g Labrasol only. CTMs for the phase 1B-MD study were supplied as
50-mg capsules, each containing 50 mg of OPT-80 formulated in 110 mg of
Avicel PH-102 (microcrystalline cellulose; NF) and placebo capsules, each con-
taining 162 mg of Avicel PH-102 only. The respective placebo capsules were
fabricated with identical appearance to the active drug.

Study design. The phase 1A-SD study was a double-blind, randomized, pla-
cebo-controlled dose escalation study to evaluate the safety and pharma-
cinetics of single oral doses of OPT-80. A total of 16 healthy, nonsmoking volunteer
subjects, 18 to 65 years of age and with body mass indices between 18.5 and 29.9,
who tested negative for drugs of abuse and were able to give written informed
consent were enrolled in the study; even numbers of male and female subjects
were enrolled to provide gender balance. The four single doses of OPT-80
were administered orally approximately once daily 1- to 2-week washout
interval separating the treatments; the next-higher dose level was administered
only after the completion of the evaluation period for the previous dose level. At
each dose level, six volunteers were randomized to receive active drug and two
received placebo. The 100- and 450-mg dose groups were monitored on a
combined inpatient/outpatient basis; the 200- and 300-mg groups were dosed and
monitored exclusively as inpatients to facilitate collection of fecal samples. Vol-
unteers were admitted to the study unit the day before each scheduled dosing
day; they remained at the study site until the 24-hour plasma, urine, and fecal
samples were collected. During the outpatient period, subjects reported daily to
the study unit for scheduled events and procedures.

Serial blood, urine, and fecal samples were collected at various time intervals
to study drug administration, subject’s relevant medical history, and whether the
relationship of adverse events to the study drug was assessed by a qualified
medical examiner. Study subjects were monitored carefully throughout each
dosing period for adverse experiences. The

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (mg/day)</th>
<th>Day</th>
<th>Time (h postdose)</th>
<th>OPT-80 concn (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>017</td>
<td>450</td>
<td>10</td>
<td>1</td>
<td>6.13</td>
</tr>
<tr>
<td>017</td>
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<tr>
<td>020</td>
<td>450</td>
<td>10</td>
<td>4</td>
<td>5.45</td>
</tr>
<tr>
<td>021</td>
<td>450</td>
<td>10</td>
<td>4</td>
<td>6.61</td>
</tr>
<tr>
<td>021</td>
<td>450</td>
<td>10</td>
<td>6</td>
<td>6.41</td>
</tr>
<tr>
<td>021</td>
<td>450</td>
<td>10</td>
<td>4</td>
<td>6.70</td>
</tr>
</tbody>
</table>

a All samples above the LLOQ are shown. The LLOQ was 5 ng/ml.

Serial blood, urine, and fecal samples were collected at various time intervals
during the multiple-dosing periods. Plasma, urine, and fecal concentrations of
OPT-80 and its metabolite OPT-1118 were determined.

Both studies were conducted in accordance with Good Clinical Practice and
International Council on Harmonization guidelines. Both study protocols were
approved by the Medical Sciences Committee of the University of Miami Med-
ical School. Study personnel obtained written informed consent directly from all
subjects prior to their entry into the study.

Collection of blood, urine, and fecal samples for pharmacokinetic evaluation.

For the phase 1A-SD study, blood samples were collected at 0 (predose), 0.5, 1,
2, 3, 4, 6, 8, 10, 12, 14, 24, 48, 72, 96, and 120 h postdosing. For the phase 1B-MD
study, blood samples were collected at 0 (predose), 1, 2, 4, 6, 12, and 24 h
postdose on day 1 and day 10. Plasma samples were separated within 30 min of
blood collection and stored frozen at –80°C until shipment to the bioanalytical
laboratory.

Subjects voided naturally for urine collection. For the phase 1A-SD study,
cumulative urine samples were collected over the time intervals of 0 to 24, 24 to
48, 48 to 72, 72 to 96, and 96 to 120 h postdosing. For the phase 1B-MD study,
cumulative urine samples were collected over 4- to 8-h intervals after the first
dose (day 1) and again after the last dose (day 10). An aliquot (20 ml) of each
urine sample was transferred into a polypropylene vial labeled with subject-
and sample-specific information and stored frozen at –80°C until shipment to the
analytical laboratory for assay.

For the phase 1A-SD study, cumulative fecal samples were collected over the time
intervals of 0 to 24, 24 to 48, 48 to 72, 72 to 96, and 96 to 120 h postdosing.
For the phase 1B-MD study, fecal samples were collected after the last dose at
day 10 or the first bowel movement thereafter. Immediately after each fecal
collection, the sample was weighed and thoroughly mixed; an aliquot (ca. 20% of
total weight) was transferred into a polypropylene vial labeled with subject-
and sample-specific information and stored frozen at –80°C until shipment to the
analytical laboratory for assay. For subjects with multiple bowel movements
during any 24-hour period, the fecal samples were combined.

Pharmacokinetic and statistical analysis. Summary statistics were generated
by WinNonlin and/or Excel for OPT-80 concentrations and pharmacokinetic
parameters. Given sufficient plasma levels over the lower limit of quantitation
(LLOQ), the data are analyzed as follows. Median (range) and mean (and
standard deviation) OPT-80 plasma concentrations at each nominal time point
were calculated, as appropriate for each dose group. Concentrations reported as
below the LLOQ were not included. Drug accumulation is defined as the ratio
of the area under the curve at steady state (AUCss) to the area under the curve
after a single dose (AUC0-12): Dose proportionality was tested by comparison of
AUC0-12 and AUCss over the studied dose range. Likewise, pharmacokinetic
linearity was tested by comparison of the half-lives of elimination over the same
dose range.

Tolerability and safety evaluation. The tolerability and safety of OPT-80 were
evaluated based on adverse-event reports, vital signs, electrocardiograms, clinical
laboratory values, and results of physical examination. Study subjects were mon-
itored carefully throughout each dosing period for adverse experiences. The
relationship of adverse events to the study drug was assessed by a qualified
physician and is in general based on such considerations as temporal relationship

RESULTS

Enrollment and demographics. The phase 1A-SD study en-
rolled a total of 16 healthy subjects. The phase 1B-MD study

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enrolled a total of 24 healthy subjects. Subjects for these studies were similar in age (49.3 ± 8.6 years), weight (71.5 ± 9.4 kg), height (166.0 ± 9.4 cm), and body mass index (25.9 ± 1.6).

Adverse events. No clinically meaningful changes were observed in the electrocardiograms, laboratory evaluations, or vital signs in either phase 1 study.

In the phase 1A-SD study, OPT-80 was well tolerated by all subjects at all doses. Five adverse events were reported, three at the 100-mg dose level in two subjects and two at the 200-mg dose level in one subject. All the reported adverse events were mild; they included headache (1), rhinorrhea (1), open wound in the left upper leg (1), elevated lipase (1), and elevated amylase (1). One subject in the 200-mg group exhibited elevated amylose and lipase predose; this subject was allowed to complete the 200-mg dose, but he was withdrawn from the scheduled 450-mg crossover dose. None of the adverse events was considered to be drug related.

In the phase 1B-MD study, OPT-80 was also well tolerated by all subjects at all doses. Thirteen adverse events were reported: 6 in the 150-mg group, 2 in the 450-mg group, and 5 in the placebo group. All of the reported adverse events were mild. The adverse events in the 150-mg dose group included headache (1), weakness (1), difficulty swallowing (1), pharyngitis (1), conjunctivitis (1), and eosinophilia (1). The adverse events in the 450-mg dose group included headache (1) and upper respiratory infection (1). The mild adverse events reported in the placebo group included fatigue (1), nasal congestion (1), rash (1), pruritis (1), and upper respiratory infection (1). None of the adverse events were considered to be drug related.

Pharmacokinetics of OPT-80 in plasma. The concentration of OPT-80 in plasma was generally low or below the LLOQ following single-dose or multiple-dose oral administration. Following multiple-dose oral administration, plasma concentrations of OPT-80 were mostly below the LLOQ (5 ng/ml) across the dosing range; plasma levels observed in the top dosing group are presented in Table 1.

Levels of OP-1118, the major metabolite, were slightly higher than those of the parent drug but still near the limit of quantification. Observed peak levels of the metabolite and the times at which the peak was observed are presented in Table 2.

Urinary excretion of OPT-80. Due to the low concentrations of OPT-80 detected in the plasma, no appreciable levels of intact OPT-80 could be found in the collected urine.

Fecal recovery of OPT-80. In the phase 1A single-dose study, approximately one-third of the oral dose was recovered from the feces of inpatient subjects as the parent drug; fecal recovery data for these subjects are presented in Table 3. In these subjects, most of the dose was excreted as an OPT-80 metabolite, OP-1118, which is characterized by a molecular weight of 988. The total fecal recovery of OPT-80 as the parent drug plus OP-1118 approximated 116.6% (±47.1%) of the 200- and 300-mg doses; this total may exceed 100% due to the inhomogeneity of the fecal sample. Peak concentrations of OPT-80 approximated 490 μg/g feces in the 200- to 300-mg dose range (Table 4). No attempt was made to calculate the fecal recovery of the 100-mg and the 450-mg doses due to concern with incomplete fecal collection from these two outpatient groups.

Stool consistency was not evaluated.

In the phase 1B-MD study, fecal samples were collected after the last dose at day 10 or the first bowel movement thereafter. Most subjects produced formed stool samples on day 10; stool from one subject in the 450-mg dose group was soft and semiformed, and one subject who received placebo produced soft stool. Results of the fecal analyses for OPT-80

<table>
<thead>
<tr>
<th>TABLE 2. Peak concentrations and peak times for the metabolite OP-1118 for the 450-mg dose group on days 1 and 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>017</td>
</tr>
<tr>
<td>019</td>
</tr>
<tr>
<td>020</td>
</tr>
<tr>
<td>021</td>
</tr>
<tr>
<td>022</td>
</tr>
<tr>
<td>023</td>
</tr>
<tr>
<td>Mean (SD)</td>
</tr>
</tbody>
</table>

* C<sub>max</sub> peak concentration.

<table>
<thead>
<tr>
<th>TABLE 3. Fecal recovery of OPT-80 and its metabolite OP-1118 in the single-dose study*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>009</td>
</tr>
<tr>
<td>010</td>
</tr>
<tr>
<td>011</td>
</tr>
<tr>
<td>013</td>
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<td>014</td>
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<tr>
<td>016</td>
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<tr>
<td>001</td>
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<tr>
<td>002</td>
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<tr>
<td>003</td>
</tr>
<tr>
<td>005</td>
</tr>
<tr>
<td>006</td>
</tr>
<tr>
<td>008</td>
</tr>
</tbody>
</table>

* Data are not presented for the 100- and 450-mg dose groups, who completed the study as outpatients.
and OP-1118 are tabulated in Table 5. Fecal OPT-80 concentrations on day 10 of the multiple-dose study were higher than those in the single-dose study, and increases in fecal concentrations of OPT-80 appeared to be dose related. In addition, fecal concentrations of OP-1118 approximately doubled between the 150-mg and 300-mg doses and appeared to have plateaued between the 300-mg and 450-mg doses, indicating possible saturation of OPT-80 metabolism. Fecal OPT-80 concentrations were 823, 1,861, and 2,983 µg/g in the 150-, 300-, and 450-mg dose groups, respectively. Meanwhile, fecal OP-1118 concentrations were 333, 553, and 610 µg/g, respectively, for the corresponding doses.

### DISCUSSION

Overall, these studies show that OPT-80 is well tolerated after single-dose and multiple-dose oral administrations up to 450 mg daily for 10 consecutive days. No serious adverse events were reported. The mild adverse events that were observed were not considered to be related to the study drug.

After either single- or multiple-dose oral administration, low OPT-80 levels were detected in plasma, most of which fell below the limit of quantitation. No accumulation of the drug was found based on the plasma data. The new solid-dosage form of OPT-80 was formulated in microcrystalline cellulose (Avicel PH-102). However, a further pharmacokinetic analysis was not possible for the phase 1B-MD study, due to insufficient plasma data above LLOQ across the dose range. The difference in absorption characteristics between the single dose and the multiple doses could be attributed to formulations of the study drug: in the phase 1A-SD study, OPT-80 was formulated with Labrasol in a liquid-filled capsule, but in the phase 1B-MD study the study drug was formulated in microcrystalline cellulose (Avicel PH-102).

Differences in formulation may also be responsible for the stability of OPT-80 upon oral administration. In the phase 1A-SD study, the fecal total recovery of OPT-80 plus its major metabolite, OP-1118, approximated 100%, but only about one-third of the dose was recovered as intact OPT-80; most of the dose was recovered as the major metabolite, OP-1118, which has an antimicrobial spectrum similar to that of the parent but typically 8- to 16-fold lower activity. By contrast, four-fifths of the recovered material was excreted as the parent drug at the 450-mg dose level in the phase 1B-MD study, in which OPT-80 was formulated with Avicel PH-102 as a solid-dosage form. As OP-1118 formation could occur via hydrolysis by gastric acid or enzymatic activity of intestinal microsomes, this difference in fecal recovery of the parent compound may reflect the greater exposure of the Labrasol-formulated OPT-80 to gastric acid or saturation of an intestinal enzyme at the higher dose.

In conclusion, OPT-80 was well tolerated after administration as a single dose or multiple oral doses across the 100- to 450-mg dose range. No serious adverse events were reported; no adverse events were study drug related. No accumulation of drug was found based on the plasma data. The new solid-dosage form produced minimal plasma concentrations but very high OPT-80 stool concentrations, which is desirable for the therapeutic indication under investigation: OPT-80 is intended for the local treatment of *C. difficile* infection, which occurs primarily in the large intestine (13). OPT-80 was safe and well tolerated at even the highest dose level: 450 mg daily for 10 consecutive days. Results from these two studies support the further clinical development of OPT-80 as an oral therapy for *C. difficile* infection.

### TABLE 4. Concentrations of OPT-80 and the primary metabolite, OP-1118, versus day of sample collection in the single-dose study

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (mg)</th>
<th>Day</th>
<th>Conc (µg/g) of indicated drug or metabolite on day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>OPT-80</td>
</tr>
<tr>
<td>009</td>
<td>200</td>
<td>1</td>
<td>17.1</td>
</tr>
<tr>
<td>010</td>
<td>200</td>
<td>&lt;LLOQ</td>
<td>&lt;LLOQ</td>
</tr>
<tr>
<td>011</td>
<td>200</td>
<td>&lt;LLOQ</td>
<td>&lt;LLOQ</td>
</tr>
<tr>
<td>013</td>
<td>200</td>
<td>&lt;LLOQ</td>
<td>&lt;LLOQ</td>
</tr>
<tr>
<td>014</td>
<td>200</td>
<td>&lt;LLOQ</td>
<td>&lt;LLOQ</td>
</tr>
<tr>
<td>016</td>
<td>200</td>
<td>47.6</td>
<td>137.6</td>
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<td>001</td>
<td>300</td>
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<td>&lt;LLOQ</td>
<td>&lt;LLOQ</td>
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<td>005</td>
<td>300</td>
<td>129.7</td>
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<td>006</td>
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<td>17.5</td>
<td>38.7</td>
</tr>
<tr>
<td>008</td>
<td>300</td>
<td>58.5</td>
<td>101.5</td>
</tr>
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</table>

*Data are not presented for the 100- and 450-mg dose groups, who completed the study as outpatients.

*NC, not collected.

### TABLE 5. Fecal recovery data of the multiple-dose study

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Conc (µg/g) of:</th>
<th>OPT-80(OP-1118) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OPT-80</td>
<td>OP-1118</td>
</tr>
<tr>
<td>150</td>
<td>823 ± 436</td>
<td>333 ± 266</td>
</tr>
<tr>
<td>300</td>
<td>1,861 ± 724</td>
<td>553 ± 323</td>
</tr>
<tr>
<td>450</td>
<td>2,983 ± 1,774</td>
<td>610 ± 241</td>
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REFERENCES


