

## Safety, Tolerance, and Pharmacokinetic Studies of OPT-80 in Healthy Volunteers following Single and Multiple Oral Doses<sup>∇</sup>

Y. K. Shue,<sup>1\*</sup> P. S. Sears,<sup>1</sup> S. Shangle,<sup>1</sup> R. B. Walsh,<sup>1</sup> C. Lee,<sup>2</sup> S. L. Gorbach,<sup>3</sup>  
F. Okumu,<sup>4</sup> and R. A. Preston<sup>5</sup>

Optimer Pharmaceuticals, San Diego, California<sup>1</sup>; Palm Beach Atlantic University, West Palm Beach, Florida<sup>2</sup>;  
Tufts University School of Medicine, Boston, Massachusetts<sup>3</sup>; Durect Corporation, Cupertino,  
California<sup>4</sup>; and University of Miami School of Medicine, Miami, Florida<sup>5</sup>

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 (MIC<sub>90</sub>) was 0.125 μg/ml, translating to 4- and 16-fold-better potencies than those of metronidazole and vancomycin, respectively, against this species: MIC<sub>90</sub> 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-

tericidal concentration of vancomycin against *C. difficile* ATCC 9689 is fourfold higher than its MIC. The rate of spontaneous development of resistance to OPT-80 for *C. difficile* ATCC 9689 is  $<2.8 \times 10^{-8}$  (15).

OPT-80 effectively protected animals from otherwise fatal infection with *C. difficile* ATCC 9689 in the hamster CDI model (15). Unlike vancomycin, which did not prevent recurrence and death after the conclusion of therapy, OPT-80 provided complete protection at a dose of  $\leq 0.2$  mg/kg of body weight (15).

OPT-80 demonstrated a promising preclinical safety profile (14). Low levels of drug were found in the plasma of hamsters and cynomolgus monkeys following oral administration. In rats, OPT-80 was not detected in the plasma following oral administration because it was extensively metabolized. No drug-related adverse effects were observed in rats or cynomolgus monkeys following oral administration of OPT-80 for 28 consecutive days at doses of  $\leq 90$  mg/kg/day.

Based on preclinical studies, OPT-80 has a distinctive combination of features, including a narrow antimicrobial spectrum, bactericidal activity against *C. difficile*, and minimal systemic exposure, which may offer significant advantages for the treatment of CDI. These features may allow OPT-80 to address this significant limitation of conventional CDI therapy: broad-spectrum antibacterial agents such as metronidazole and vancomycin may exacerbate the disruption of the natural flora that led to CDI. OPT-80, with its narrow spectrum of antibacterial activity, may kill pathogenic *C. difficile* but only minimally affect the normal intestinal flora; this could facilitate the recovery of the normal microbial community in the sub-

\* 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.

<sup>∇</sup> Published ahead of print on 11 February 2008.

ject's colon and reduce the probability of relapse or reinfection. In this report, we present the safety, tolerability, and pharmacokinetics 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 manufacturing 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 containing 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 containing 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, placebo-controlled dose escalation study to evaluate the safety and pharmacokinetics 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 evaluated were 100, 200, 300, and 450 mg administered orally approximately one-half hour after a morning breakfast. Each patient received two escalating doses of the study medication in a crossover manner, with a 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-mg 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. Volunteers were admitted to the study unit the day before each scheduled dosing period; 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 up to 120 h after each administration of OPT-80. Plasma and fecal concentrations of OPT-80 were determined for pharmacokinetic evaluation. Fecal samples were also assayed for concentrations of the metabolite OP-1118 (des-isobutryl OPT-80). A follow-up examination was scheduled on day 7 of each study period.

The phase 1B-MD study was a multiple-dose, double-blind, randomized, placebo-controlled dose escalation study. The tolerability and pharmacokinetics of multiple oral doses of OPT-80 in 24 healthy subjects were evaluated. Healthy nonsmoker volunteers 18 to 65 years of age, 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 study was intended to evaluate a total of 24 subjects. The oral doses of OPT-80 evaluated were 150, 300, and 450 mg, in three groups of eight subjects each, administered daily approximately one-half hour after a morning breakfast for 10 consecutive days. At each dose level, six volunteers were randomized to receive the active drug and two received placebo. The volunteers received a screening examination for entry criteria 7 days prior to dosing of the study medication. Subjects were dosed and monitored on a combined inpatient/outpatient basis. Subjects were admitted to the research unit on day 0 and again on day 9 of the 10-day dosing period and stayed as inpatients for 48 h after each admission. Subjects were discharged on day 2 and day 11 after completing the scheduled events and procedures. During the outpatient period, subjects reported daily to the research unit for dosing and stayed for 3 h under observation. Dosing was initiated at the next higher dose level only after the completion of the evaluation period for the previous dose level.

TABLE 1. Plasma levels of OPT-80 in the multiple-dose study for the 450-mg dose group on days 1 and 10<sup>a</sup>

Subject	Dose (mg/day)	Day	Time (h postdose)	OPT-80 concn (ng/ml)
017	450	10	1	6.13
017	450	10	2	5.74
020	450	1	4	5.45
020	450	10	4	6.61
020	450	10	6	6.41
021	450	10	4	6.70

<sup>a</sup> 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 Medical 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  $-80^{\circ}\text{C}$  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  $-80^{\circ}\text{C}$  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  $-80^{\circ}\text{C}$  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 using 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 (and 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 ( $AUC_{ss}$ ) to the area under the curve after a single dose ( $AUC_{0-inf}$ ). Dose proportionality was tested by comparison of  $AUC_{0-inf}$  and  $AUC_{ss}$  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 monitored 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 to study drug administration, subject's relevant medical history, and whether the finding is likely due to a preexisting condition.

## RESULTS

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

TABLE 2. Peak concentrations and peak times for the metabolite OP-1118 for the 450-mg dose group on days 1 and 10

Subject	Day 1		Day 10	
	C <sub>max</sub> (ng/ml) <sup>a</sup>	Time (h)	C <sub>max</sub> (ng/ml)	Time (h)
017	15.1	2	19.4	2
019	19.2	4	16.1	2
020	33.1	4	28.4	4
021	10.3	4	19.9	4
022	14.5	4	11.8	4
023	8.03	2	<LLOQ	
Mean (SD)	16.7 (8.93)	3 (1)	19.1 (6.11)	3 (1)

<sup>a</sup> C<sub>max</sub> peak concentration.

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 amylase 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 re-

ported 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 semifformed, and one subject who received placebo produced soft stool. Results of the fecal analyses for OPT-80

TABLE 3. Fecal recovery of OPT-80 and its metabolite OP-1118 in the single-dose study<sup>a</sup>

Subject	Dose (mg)	OPT-80		OP-1118		Total % of dose
		Recovery (mg)	% of dose	Recovery (mg)	% of dose	
009	200	63.25	31.63	249.09	124.55	156.17
010	200	49.70	24.85	203.30	101.65	126.50
011	200	45.31	22.66	115.47	57.74	80.39
013	200	90.83	45.42	198.65	99.33	144.74
014	200	55.11	27.56	125.80	62.90	90.46
016	200	54.02	27.01	201.42	100.71	127.72
001	300	36.47	18.24	62.33	31.17	49.40
002	300	86.76	43.38	146.06	73.03	116.41
003	300	47.97	23.99	161.50	80.75	104.74
005	300	39.10	19.55	34.73	17.37	36.92
006	300	148.30	74.15	221.63	110.82	184.97
008	300	123.52	61.76	237.76	118.88	180.64
Mean (SD)			35.01 (17.72)		81.57 (34.15)	116.59 (47.05)

<sup>a</sup> Data are not presented for the 100- and 450-mg dose groups, who completed the study as outpatients.

TABLE 4. Concentrations of OPT-80 and the primary metabolite, OP-1118, versus day of sample collection in the single-dose study<sup>a</sup>

Subject	Dose (mg)	Concn (μg/g feces) of indicated drug or metabolite on day:									
		1		2		3		4		5	
		OPT-80	OP-1118	OPT-80	OP-1118	OPT-80	OP-1118	OPT-80	OP-1118	OPT-80	OP-1118
009	200	17.1	31.6	225.5	832.8	164.5	623.9	79.9	333.6	NC <sup>b</sup>	NC
010	200	<LLOQ	<LLOQ	15.1	57.9	493.7	608.2	161.1	254.8	64.4	NC
011	200	<LLOQ	<LLOQ	252.8	632.1	59.6	136.2	11.5	24.7	<LLOQ	<LLOQ
013	200	139.5	297.4	247.3	466.3	91.9	212.6	12.2	26.0	5.4	13.7
014	200	<LLOQ	<LLOQ	299.8	616.0	115.2	258.1	38.5	97.1	4.2	14.0
016	200	47.6	137.6	221.7	757.4	193.1	750.6	22.3	81.2	5.0	17.1
001	300	269.4	399.5	21.2	46.7	2.1	4.6	<LLOQ	<LLOQ	NC	NC
002	300	<LLOQ	<LLOQ	3.0	<LLOQ	173.1	254.4	101.0	226.5	12.1	36.4
003	300	<LLOQ	<LLOQ	9.8	17.4	107.7	351.8	251.0	805.7	NC	NC
005	300	129.7	67.8	158.4	236.1	4.4	<LLOQ	NC	NC	NC	NC
006	300	17.5	38.7	492.1	680.5	83.2	128.4	2.6	<LLOQ	NC	NC
008	300	58.5	101.5	480.7	908.8	214.1	427.8	11.0	24.1	3.0	<LLOQ

<sup>a</sup> Data are not presented for the 100- and 450-mg dose groups, who completed the study as outpatients.

<sup>b</sup> NC, not collected.

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 concentrations of OP-1118 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 upon multiple dosing based on the plasma concentration data. Due to low OPT-80 plasma levels across the dose range, insufficient plasma data above LLOQ were available for pharmacokinetic analysis. Nevertheless, the phase 1A-SD study appeared to show a dose-related increase in plasma concentrations of OPT-80 over the dose range studied. OPT-80 is eliminated with a half-life of 0.94 to 2.77 h, calculated based on the limited plasma concentration data from the 450-mg dose.

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

Dose (mg)	Concn (μg/g) of:		OPT-80/OP-1118 ratio
	OPT-80	OP-1118	
150	823 ± 436	333 ± 266	2.5
300	1,861 ± 724	553 ± 323	3.4
450	2,983 ± 1,774	610 ± 241	4.9

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.

## REFERENCES

1. Ackermann, G., B. Löffler, D. Adler, and A. C. Rodloff. 2004. In vitro activity of OPT-80 against *Clostridium difficile*. *Antimicrob. Agents Chemother.* **48**:2280–2282.
- 1a. Babakhani, F., J. Seddon, N. Robert, Y. Shue, and P. Sears. 2005. Effect of inoculum, pH, and cations on the in vitro activity of OPT-80 vs. *Clostridium difficile*, abstr. D-1648, p. 135. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
2. Bartlett, J. G. 1992. Antibiotic-associated diarrhea. *Clin. Infect. Dis.* **15**:573–581.
3. 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.
4. Fekety, R., and A. B. Shah. 1993. Diagnosis and treatment of *Clostridium difficile* colitis. *JAMA* **269**:71–75.
5. Finegold, S. M., D. Molitoris, M. L. Vaisanen, Y. Song, C. Liu, and M. Bolanos. 2004. In vitro activities of OPT-80 and comparator drugs against intestinal bacteria. *Antimicrob. Agents Chemother.* **48**:4898–4902.
6. Gerding, D. N. 1989. Disease associated with *Clostridium difficile* infection. *Ann. Intern. Med.* **110**:255–257.
7. 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.
- 7a. 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.
8. Hochlowski, J. E., S. J. Swanson, L. M. Ranfranz, D. N. Whittorn, 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.
9. Johnson, A. P. 2007. Drug evaluation: OPT-80, a narrow-spectrum macrocyclic antibiotic. *Curr. Opin. Investig. Drugs* **8**:168–173.
10. Kelly, C. P., C. Pothoulakis, and J. T. LaMont. 1994. *Clostridium difficile* colitis. *N. Engl. J. Med.* **330**:257–262.
11. 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, abstr. O150. Abstr. 16th Eur. Congr. Clin. Microbiol. Infect. Dis. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.
12. 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.
13. Mylonakis, E., E. T. Ryan, and S. B. Calderwood. 2001. *Clostridium difficile*-associated diarrhea. *Arch. Intern. Med.* **161**:523–533.
14. Okumu, F., R. B. Walsh, P. Sears, and Y. K. Shue. 2004. Safety and pharmacokinetics of OPT-80, a novel antibiotic for treatment of *Clostridium difficile* associated diarrhea (CDAD), abstr. F-726, p. 204. Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
15. 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.
16. Theriault, R. J., 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.
17. Wilcox, M. H., J. G. Cunniffe, C. Trundle, and C. Redpath. 1996. Financial burden of hospital-acquired *Clostridium difficile* infection. *J. Hosp. Infect.* **34**:23–30.
18. 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.