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## Abstract

**BACKGROUND** OPT-80 is a narrow spectrum antibiotic currently under clinical evaluation for the treatment of *Clostridium difficile*-associated diarrhea.

**METHODS** The safety and pharmacokinetics of OPT-80 were evaluated *in vitro* and after oral and intravenous (IV) administration to Sprague-Dawley rats and cynomolgus monkeys.

**RESULTS** In rats the 50% lethal dose by the IV route was approximately 200 mg/kg. No treatment-related effects were observed when male and female rats were administered an oral dose of OPT-80 at 1,000 mg/kg, the highest dose tested. No drug-related adverse effects associated with OPT-80 were found in repeated oral administration to rats or monkeys for 28 consecutive days with OPT-80 doses of 90 mg/kg, the highest repeat dose level tested. The OPT-80 levels in rat plasma after oral administration were below 0.5 µg/mL. After IV administration, OPT-80 was essentially cleared from rat plasma within 10 minutes following an intravenous dose at 20 mg/kg, and the C<sub>max</sub> following this dose was between 2 and 7 µg/mL. The C<sub>max</sub> for OPT-80 in monkey plasma after oral administration of 30 mg/kg and 90 mg/kg was 36.5 - 128 ng/mL and 119 - 525 ng/mL, respectively. Standard *in vitro* and *in vivo* genotoxicity tests were negative.

**CONCLUSIONS** OPT-80 produced minimal or no toxic effects. No treatment-related effects were observed at any dose level up to 90 mg/kg (the highest dose tested) in rats or cynomolgus monkeys when OPT-80 was administered orally for 28 days. OPT-80 is minimally absorbed following oral administration.

## Introduction

*Clostridium difficile* is a significant cause of nosocomial diarrhea.(1, 2) Disruption of normal colonic flora, mainly from antibiotic use, followed by overgrowth of toxigenic strains of *C. difficile* results in illness that can range from mild watery diarrhea to severe life threatening pseudomembranous colitis. The frequency and severity of disease has been reported to be increasing with estimated annual health care costs of over \$1 billion in the United States.(3, 4) Recurrent diarrhea due to re-infection or relapse has been reported in 20-30% of patients receiving treatment for *C. difficile* diarrhea. The two most commonly utilized specific therapies are vancomycin and metronidazole. Although both agents are effective in treating the infection, they can lead to recurrence of disease, development of ecologically undesirable organisms (in the case of vancomycin), or frequent adverse effects (in the case of metronidazole). Both vancomycin and metronidazole are, themselves, capable of inducing *C. difficile* intestinal disease.(5, 6).

OPT-80 is a new macrocyclic compound under development by Optimer Pharmaceuticals as a therapeutic regimen for *Clostridium difficile*-associated diarrhea (CDAD).

OPT-80 has minimal activity against gram-negative bacteria, moderate activity against most gram-positive bacteria, and excellent activity toward *Clostridium*.(7, 8, 9) The narrow-spectrum activity of OPT-80 may be important in reducing the recurrence rate of CDAD, as it is probably the continuing disturbance of the gastrointestinal flora caused by other CDAD treatments that leads to recolonization and overgrowth by *Clostridium*.

This study reports the results of preclinical pharmacokinetic and toxicology studies in which Sprague-Dawley rats and cynomolgus monkeys were administered OPT-80 by intravenous or oral routes.

## Materials & Methods

**Pharmacokinetics of OPT-80 in Rats.** OPT-80 was administered to Sprague-Dawley rats (3/sex/time point) as a Labrasol solution via oral gavage at a dose of 5 mg/kg. Blood samples were collected at 7 time points following dosing.

**Acute Oral Toxicity in Rats.** Rats (5/sex/group) were given a single oral dose (167, 500, or 1,000 mg/kg OPT-80 in Labrasol, or vehicle control) and observed for 14 days, after which they were sacrificed and examined by necropsy.

**Acute Intravenous Toxicity and Toxicokinetics in Rats.** Four groups of rats (5/sex/group) were dosed at 0, 20, 62.5, or 200 mg/kg of OPT-80 administered in a constant 1 mL/kg volume of 10% dimethyl acetamide, 20% ethanol, and 70% PEG 400 vehicle. The animals were observed for 14 days following dose administration, then sacrificed and examined. An additional 6 animals/sex/group were included to provide blood samples for pharmacokinetic analysis. An additional group of 3 males and 3 females with jugular cannulae were dosed at 20 mg/kg in order to provide pharmacokinetic samples at very early time points. Plasma samples were collected from these animals at 2, 5, 10, 20, 30, 60, 120, 240, and 480 minutes post dosing.

**28 Day Oral Toxicity and Toxicokinetics in Rats.** OPT-80 was administered to Sprague-Dawley rats (10/sex/group) as a Labrasol solution via oral gavage for 28 consecutive days at doses of 0, 10, 30, or 90 mg/kg. Clinical signs, food consumption, and body weights were assessed once per week. Pre-study and pre-terminal ophthalmic and pre-terminal serum chemistry and hematology evaluations were performed on all study animals. Animals were sacrificed on day 29, and gross (including organ weights), histologic, and pathologic examinations were undertaken. An additional 12 rats/sex/group were included to provide toxicokinetic samples, which were collected on day 0 and 28 before dosing and at 5 time points after dosing.

**28 Day Oral Toxicity and Toxicokinetics in Monkeys.** This study was conducted to assess the toxicity and to determine the toxicokinetic profile of OPT-80 when the drug was administered to cynomolgus monkeys as a daily oral dose for 28 consecutive days at doses of 0, 10, 30, or 90 mg/kg. A total of twenty-four (24) Cynomolgus monkeys (12 males and 12 females) were randomly assigned to 4 treatment groups (3 sex/group) and received OPT-80 at nominal dose levels of 10, 30, and 90 mg/kg/day. A Control

group (3 animals/sex) received the vehicle article (Labrasol®) only. Clinical signs, food consumption, and body weights were assessed once per week. Pre-study and pre-terminal ophthalmic and pre-terminal serum chemistry and hematology evaluations were performed on all study animals. Animals were sacrificed on day 29, and gross (including organ weights), histologic, and pathologic examinations were undertaken. The following parameters were evaluated: clinical observations, body weights, appetite, ophthalmoscopy, ECG, hematology, coagulation, clinical chemistry, urinalysis, toxicokinetics, organ weights, gross macroscopic, and microscopic observations. Serial blood samples were collected on day 0 and 28 before dosing and at 8 time points after dosing for toxicokinetic analysis.

**Quantitation of OPT-80 in Plasma.** OPT-80 levels were analyzed via a validated HPLC method. The limit of quantitation was 0.5 µg/mL. Plasma samples for the 28 day monkey study were later reanalyzed using an LC-MS-MS method, which has a lower limit of quantitation of 5 ng/mL.

**Bacterial Reverse Mutation Test.** The mutagenic potential of OPT-80 was assessed by measuring its ability to induce reverse mutations in selected loci of several *Salmonella typhimurium* strains or in the *trp* locus of *Escherichia coli* VP2 *uvrA* in the presence or absence of a metabolically active (S9) fraction of a rat liver extract. Standard techniques were used (10,11), and the dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg per plate.

**In Vitro Mammalian Chromosomal Aberration Test.** The propensity of OPT-80 to induce chromosomal aberrations was evaluated in Chinese hamster ovary cells by established techniques (12) at various concentrations: 12.5, 25, 50, 100, 125, 150, 200 µg/mL in the non-activated system, and 3.125, 6.25, 12.5, 25, 50, 75, 100 µg/mL in the S9 (rat liver extract) activated system.

**In Vivo Mammalian Erythrocyte Micronucleus Test.** Rats were dosed with 0, 18.75, 37.5, and 75 mg/kg of OPT-80 (in 10% dimethyl acetamide, 20% ethanol, and 70% PEG 400) in a constant volume of 1 mL/kg body weight by a single injection into the lateral tail vein. Cyclophosphamide monohydrate was used as the positive control article at a dose of 40 mg/kg. Bone marrow cells (polychromatic erythrocytes and normochromatic erythrocytes), collected 24 and 48 hours after treatment, were examined microscopically for the presence of micronuclei.

## Results

### IN VIVO SINGLE DOSE TOXICITY & TOXICOKINETIC STUDIES

#### Acute Oral Toxicity in Rats

- There were no abnormal treatment-related findings at necropsy.
- Oral administration of OPT-80 produced no treatment-related effects in male or female rats at doses of 1,000 mg/kg (the highest dose tested).

#### Acute Intravenous Toxicity and Toxicokinetic Study in Rats

- At the 200 mg/kg dose, 5 of 10 animals died acutely, and one additional female in the group showed labored breathing; the LD<sub>50</sub> via the intravenous route was approximately 200 mg/kg.
- Evidence of minor toxicities in the 20, 62.5, and 200 mg/kg treatment groups included colored urine (red to red-brown, or bright yellow; 40, 30, and 20% by group), which was reversed within 4 hours of dosing, and some anogenital staining. In addition, 10% and 80% of the animals in the intermediate and high-dose groups, respectively, exhibited mild to severe lesions in their tails, where the poorly-soluble compound was injected.
- No treatment-related effects were noted at necropsy.
- OPT-80 was rapidly cleared from circulation. In rats dosed at 20 mg/kg, the OPT-80 levels were near or below the LLOQ (0.5 µg/mL) by the time the first sample was drawn at 30 minutes. For animals dosed at 200 mg/kg, very low levels (<2 µg/mL) were observed through 8 hours with no notable peak. In cannulated rats dosed with 20 mg/kg, OPT-80 levels were near or below the LLOQ (0.5 µg/mL) within 10 minutes post dose.
- In cannulated rats dosed with 20 mg/kg, OPT-80 levels were near or below the LLOQ (0.5 µg/mL) within 10 minutes post dose, with a C<sub>max</sub> at 2 minutes post-dosing of 2.6 - 6.5 µg/mL.

### IN VIVO REPEATED DOSE TOXICITY & TOXICOKINETIC STUDIES

#### 28 Day Oral Toxicity and Toxicokinetic Study in Rats

- No treatment-related effects were observed following 28 consecutive daily oral doses of OPT-80 at 90 mg/kg/day, the highest dose level.
- No clinical signs that were considered treatment-related were observed in males or females that received 90 mg/kg/day of OPT-80.
- There were no treatment-related effects noted for any of the parameters examined: body weights, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, necropsy, and pathology.
- Plasma levels of OPT-80 were below the LLOQ of 0.5 µg/mL.

#### 28 Day Oral Toxicity and Toxicokinetic Study in Monkeys

- No treatment-related effects were observed following 28 consecutive daily oral doses of OPT-80 at any dose level.
- The oral administration of OPT-80 for 28 consecutive days to cynomolgus monkeys was well tolerated.
- The highest dose level in this study (90 mg/kg) is considered to be the No Observable Effect Level (NOEL).
- Plasma levels of OPT-80 were low, reaching a peak at 1-2 hour post dose of 36.5 - 128 ng/mL (30 mg/kg dose) and 119-525 ng/mL (90 mg/kg dose).

### SUMMARY OF PRECLINICAL TOXICITY & TOXICOCOKINETIC STUDIES

- Oral administration of OPT-80 produced no treatment-related effects in male or female rats at doses of 1,000 mg/kg.
- OPT-80 produced no treatment-related effects at any dose level up to 90 mg/kg (the highest dose tested) when administered orally to rats or cynomolgus monkeys for 28 days.
- Plasma levels of OPT-80 following oral administration to rats and cynomolgus monkeys were below the LLOQ of 0.5 µg/mL at a dose level up to 90 mg/kg for 28 days.
- OPT-80 (dissolved in 10% dimethyl acetamide, 20% ethanol, and 70% PEG 400) caused acute mortality in rats at intravenous dose levels of 200 mg/kg; it may also produce some acute toxicity at lower intravenous dose levels. Intravenous doses below 20 mg/kg have not been tested, and subacute and tissue effects have not been determined for intravenous dosing.
- In pharmacokinetic studies of OPT-80 in rats, concentration-time data after intravenous administration exhibited a flat profile after an early C<sub>max</sub>. The low levels of OPT-80 measured in plasma after intravenous administration in a non-aqueous vehicle may be due to the compound's poor aqueous solubility (less than 0.01 mg/L at pH 7).
- No blood levels were observed in the plasma of rodents and low levels were observed in cynomolgus monkeys following oral administration. This is consistent with the literature results: OPT-80 was not detected in the plasma of hamsters after oral administration of non-formulated OPT-80. (8)

### GENOTOXICITY STUDIES

The purpose of these experiments was to detect and assess possible genetic hazards from compounds, which may damage nucleic acids. The standard *in vitro* and *in vivo* genotoxicity testing required for IND submission were performed under ICH guidelines at BioReliance, Rockville, MD.

#### GLP: Bacterial Reverse Mutation Assay

- OPT-80 was negative in the Bacterial Reverse Mutation Assay: the compound did not produce positive mutagenic responses with any of the tested strains at any dose.

#### GLP: In Vitro Mammalian Chromosome Aberration Test

- No significant increase in structural aberrations was observed at any dose in the non-activated 20 hour treatment group.
- OPT-80 caused more frequent, mild chromosomal aberrations than the vehicle in a short-term, high dose, non-activated assay, but not in a corresponding activated assay or in longer-term activated or non-activated assays. In these experiments, the percent aberrant cells at high dose in the non-activated 4 hour treatment group was high relative to the historical control range.

#### GLP: In Vivo Mammalian Erythrocyte Micronucleus Test

- No biologically significant reductions in the ratio of polychromatic erythrocytes to total erythrocytes were observed in the test article-treated groups relative to the vehicle control groups.
- No significant increases and no evidence of a dose-related increase in the number of micronucleated polychromatic erythrocytes were observed in any treatment group, regardless of dose level or bone marrow collection time.
- OPT-80 was negative in the rat micronucleus assay.

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