In Vitro Activity of OPT-80 Tested against Clinical Isolates of Toxin-Producing Clostridium difficile

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Agar dilution antimicrobial susceptibility testing (CLSI, M11-A7, 2007) performed for 208 toxin-producing clinical isolates of Clostridium difficile resulted in OPT-80 MICs ranging from 0.06 to 1 μg/ml, with 90% of the isolates inhibited by a concentration of 0.5 μg/ml. The in vitro activity of OPT-80 was independent of the susceptibilities of isolates to nine other antimicrobial agents.

Clostridium difficile is the most frequent infectious cause of nosocomially acquired diarrhea in North America and accounts for the majority of cases of antimicrobial-associated colitis (2, 11, 25). C. difficile-associated disease (CDAD) arises primarily in patients who are currently receiving or who have recently received antimicrobial therapy. CDAD prolongs hospital stays and increases patient costs (12). Management of patients with CDAD includes discontinuation of predisposing antimicrobial agents, if possible, as well as supportive therapy, and empirical therapy with either metronidazole or oral vancomycin if initial measures fail to alleviate symptoms. Metronidazole and vancomycin are effective at treating CDAD; however, both agents have been associated with treatment failure and recurrence rates of approximately 20% (6, 14, 15, 18). Vancomycin use is widely discouraged in an effort to minimize the potential emergence and spread of vancomycin resistance in enterococci and staphylococci (10). Metronidazole use has been associated with significant side effects, including nausea, a disulfiram reaction, and peripheral neuropathies with prolonged treatment (6).

OPT-80, also known as PAR-101 (Optimer Pharmaceuticals, Inc., San Diego, CA), is an investigational agent that is being developed for the treatment of CDAD (1, 5, 7, 13, 22, 23, 24). The chemical structure of OPT-80 has been published previously (5, 22). OPT-80 is a narrow-spectrum agent reported to be selectively active against gram-positive anaerobes including Clostridium spp., particularly C. difficile and C. perfringens (1, 22, 24); to be less active against gram-positive, non-spore-forming bacilli (e.g., Propionibacterium spp. and lactobacilli) and peptostreptococci (5); and to be poorly active against anaerobic gram-negative bacilli (7). OPT-80’s spectrum of activity also includes staphylococci and enterococci, with poor activity against streptococci and aerobic and facultative gram-negative bacilli (7). OPT-80 acts by inhibiting RNA polymerase-mediated transcription but, unlike rifampin, preferentially inhibits holoenzyme transcription at a much higher rate than that at which it inhibits the transcription of core enzyme (8, 17, 19, 21). OPT-80 is bactericidal, with a low propensity for resistance development (frequency, <2.8 × 10⁻⁶ at four and eight times the MIC) (22).

Currently, most clinical microbiology laboratories do not routinely culture diarrheal stools for C. difficile or perform antimicrobial susceptibility testing on such isolates because of the time requirement, complexity, and expense of these procedures. However, because the inadequacy of current empirical therapies may be increasing and as the epidemiology and pathogenicity of C. difficile evolve (18), periodic or routine surveillance of clinical isolates to determine their in vitro susceptibility profiles and studies determining the activities of investigational agents such as OPT-80 seem warranted. The purpose of the current study was to assess the in vitro activities of OPT-80 against a recent collection of 208 toxin-producing clinical isolates of C. difficile.

Bacterial isolates studied. Two hundred eight isolates of C. difficile from diarrheal toxin-positive stool specimens were cultured on Clostridium difficile moxalactam norfloxacin (CDMN) Selective Supplement agar (Oxoid Canada, Nepean, Ontario, Canada) following a 95% ethanol shock step (equal volumes of stool and 95% ethanol, gently mixed at 22 to 25°C for 1 h). Toxin production was determined using either the Triage Toxin A C. difficile panel enzyme immunoassay (Biodesit Diagnostics, San Diego, CA) or the C. difficile Toxin/Antitoxin tissue culture assay (TechLab, Blacksburg, VA). All stool samples were collected from patients admitted to or seen at 1 of 2 urban tertiary-care teaching hospitals, 8 urban community hospitals, or 10 rural hospitals in the province of Manitoba, Canada, from January to April 2007. Each isolate’s identity was confirmed by Gram staining, typical odor, latex agglutination (Microgen Bioproducts Ltd., Surrey, United Kingdom), and chartreuse fluorescence under UV light (16).

Antimicrobial susceptibility testing. Susceptibility to 10 antimicrobial agents was tested using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) (4). OPT-80 powder was provided by Optimer Pharmaceuticals, Inc.; powders of the other agents tested were provided by their respective manufacturers or were obtained from Sigma Chemical Company (St. Louis, MO). The solvent used for OPT-80 powder was dimethyl sulfoxide (DMSO); distilled sterile water was used as the diluent. C. difficile ATCC 4597. Fax: (204) 787-4699. E-mail: jkarlowsky@hsc.mb.ca.

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700057 and/or Bacteroides fragilis ATCC 25285 was used as the control strain for amoxicillin-clavulanate, ceftriaxone, clindamycin, meropenem, metronidazole, moxifloxacin, piperacillin-tazobactam, and vancomycin testing (4). The reference MIC range for OPT-80 for C. difficile ATCC 700057 was 0.06 to 0.25 \( \mu \text{g/ml} \) (Optimer Pharmaceuticals, Inc.). The reference MIC range used for testing of rifampin against C. difficile ATCC 700057 was set at 0.004 to 0.03 \( \mu \text{g/ml} \), since no CLSI standard range exists (3). MICs were interpreted, when possible, using CLSI-recommended MIC breakpoints (4).

Antimicrobial agent MIC ranges, modal MICs, concentrations inhibiting 50% (MIC\(_{50}\)) and 90% (MIC\(_{90}\)) of isolates tested, and MIC distributions for each agent tested are shown in Table 1. OPT-80 MICs fell within a narrow range from 0.06 to 0.25 \( \mu \text{g/ml} \) for the 208 isolates tested, with 90.9% (189/208) of isolates susceptible and resistant to moxifloxacin (data not shown). OPT-80 MICs for the six rifampin-resistant (MIC, >2 \( \mu \text{g/ml} \)) isolates ranged from 0.125 to 0.5 \( \mu \text{g/ml} \), with a MIC of 0.25 \( \mu \text{g/ml} \) for four isolates.

The MIC\(_{50}\) (0.25 \( \mu \text{g/ml} \)) and MIC\(_{90}\) (0.5 \( \mu \text{g/ml} \)) results generated by the current study of OPT-80 against clinical isolates of C. difficile are similar to those published in three earlier studies (MIC\(_{50}\), 0.125 to 0.25 \( \mu \text{g/ml} \); MIC\(_{90}\), 0.125 to 0.25 \( \mu \text{g/ml} \)) (7, 9, 22). In contrast, studies performed by Ackermann et al. (MIC\(_{50}\), 0.0019 \( \mu \text{g/ml} \); MIC\(_{90}\), 0.0078 \( \mu \text{g/ml} \)) and Credito and Appelbaum (MIC\(_{50}\), ≤0.016 \( \mu \text{g/ml} \); MIC\(_{90}\), 0.125 \( \mu \text{g/ml} \)) demonstrated OPT-80 to be more active (1, 5) (Table 2). The observed variation may be due to differences in the solubility of OPT-80 in the diluent used in each study. In the study reported the lowest MICs, OPT-80 was dissolved and diluted in DMSO; in the other studies, in which OPT-80 was dissolved in DMSO and diluted in water, higher MICs were obtained (1).

Another, less likely source of reported MIC differences may be agar dilution (5, 7, 9, 22; also the current study) versus broth

### Table 1. Distribution of the MICs of antimicrobials tested against 208 toxin-positive isolates of Clostridium difficile

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Range</th>
<th>Mode</th>
<th>50%</th>
<th>90%</th>
<th>No. of isolates for which the antimicrobial agent MIC (( \mu \text{g/ml} )) was:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin</td>
<td>0.008–&gt;2</td>
<td>0.008</td>
<td>0.015</td>
<td>0.015</td>
<td>101</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.25–4</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.5–2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>1–16</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4–16</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>1–&gt;16</td>
<td>&gt;16</td>
<td>8</td>
<td>&gt;16</td>
<td>14</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.5–256</td>
<td>1</td>
<td>16</td>
<td>&gt;256</td>
<td>1</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>16–256</td>
<td>32</td>
<td>32</td>
<td>128</td>
<td>2</td>
</tr>
</tbody>
</table>

a MICs for all six isolates were >2 \( \mu \text{g/ml} \).

b MICs for all 101 isolates were >16 \( \mu \text{g/ml} \).

c MICs for 67/69 (moxifloxacin) and 64/86 (ceftriaxone) isolates were >4 \( \mu \text{g/ml} \).

d MICs for 67/69 (moxifloxacin) and 64/86 (ceftriaxone) isolates were <4 \( \mu \text{g/ml} \).

### Table 2. Comparison of the antimicrobial susceptibility testing results from the current study with those from other publications describing the in vitro activities of OPT-80 against isolates of Clostridium difficile

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Solvent</th>
<th>Diluent</th>
<th>MIC determination method (edition or yr)</th>
<th>No. of isolates</th>
<th>MIC (( \mu \text{g/ml} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study</td>
<td>DMSO</td>
<td>Water</td>
<td>CLSI agar dilution (M11-A7)</td>
<td>208</td>
<td>0.06–1</td>
</tr>
<tr>
<td>Hecht et al., 2007 (9)</td>
<td>DMSO</td>
<td>Water</td>
<td>CLSI agar dilution (M11-A6)</td>
<td>110</td>
<td>0.015–0.25</td>
</tr>
<tr>
<td>Ackermann et al., 2004 (1)</td>
<td>DMSO</td>
<td>DMSO</td>
<td>Deutsches Institut fuer Normung (DIN) broth microdilution using Wilkins-Chalgren broth (2000)</td>
<td>207</td>
<td>≤0.0009–0.06 ≤0.0009 0.0019 0.0078</td>
</tr>
<tr>
<td>Finegold et al., 2004 (7)</td>
<td>NA</td>
<td>NA</td>
<td>CLSI agar dilution (M11-A5)</td>
<td>23</td>
<td>0.06–2</td>
</tr>
<tr>
<td>Credito and Appelbaum, 2004 (5)</td>
<td>NA</td>
<td>NA</td>
<td>CLSI agar dilution (M11-A5)</td>
<td>21</td>
<td>≤0.016–0.25 ≤0.016 ≤0.016 0.125 0.25</td>
</tr>
<tr>
<td>Swanson et al., 1991 (22)</td>
<td>NA</td>
<td>NA</td>
<td>CLSI agar dilution using Wilkins-Chalgren agar (M11-A)</td>
<td>15</td>
<td>0.125–0.25 NA 0.25 0.25</td>
</tr>
</tbody>
</table>

a NA, information not available in the published article.
microlution (1) methodology; agar dilution using Wilkins-Chalgren agar (22) versus enriched Brucella blood agar medium (5, 7, 9; also the current study) seems less likely to be a source of OPT-80 MIC differences.

OPT-80 is currently in phase 3 clinical trials for the treatment of C. difficile-associated diarrhea and colitis (13). In clinical trials, OPT-80 has been dosed at 200 mg twice daily (400 mg/day). OPT-80 has been reported to be poorly absorbed by the gastrointestinal tract, with a maximum concentration in serum of 26.5 ng/ml reached after 1.5 h, and an apparent elimination half-life of 0.94 to 2.77 h was reported following a 400-mg dose (22); 92.6% of OPT-80 is recovered in feces (fecal drug level, 3000 μg/mg of dose) (7) primarily as its major metabolite, OP-1118, which is also biologically active (20). In a hamster model for pseudomembranous colitis, OPT-80 completely prevented the lethality of the animals and prevented the occurrence of relapses (22). The potential of OPT-80 to affect the resistance of normal gut flora to colonization is anticipated to be low (7).

In conclusion, OPT-80 demonstrated potent in vitro activity (MIC90, 0.5 μg/ml) against toxin-positive clinical isolates of C. difficile from Manitoba, Canada. OPT-80 may represent a future alternative to metronidazole and vancomycin for the treatment of CDAD.

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REFERENCES