In Vitro Activities of CB-183,315, Vancomycin, and Metronidazole against 556 Strains of Clostridium difficile, 445 Other Intestinal Anaerobes, and 56 Enterobacteriaceae Species

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MICs of CB-183,315, a novel lipopeptide antibiotic, vancomycin, and metronidazole were determined for intestinal anaerobes and Enterobacteriaceae. The MIC₉₀ for Gram-negative anaerobes were >8,192, 8,192, and 4 µg/ml for CB-183,315, vancomycin, and metronidazole, respectively. Against Enterobacteriaceae, the MIC₉₀ were >8,192 µg/ml, 1,024 µg/ml, and 1,024 µg/ml, respectively. The CB-183,315 MIC₉₀ for Clostridium difficile was 0.5 µg/ml. Its lack of activity against normal fecal organisms makes it a promising new agent for treating C. difficile.

Clostridium difficile infection (CDI) is the most common cause of health care-related diarrhea, usually associated with prior exposure to antimicrobial agents. The current theory is that the antibiotics disrupt the normal fecal microbiota, changing its complexity and diversity as a primary event, with the subsequent acquisition of toxigenic C. difficile as a secondary event in the development of the disease (6, 10, 13). CDI has increased in prevalence and severity during the last decade, resulting in increased mortality and complications, recurrent disease, and prolonged hospital stays (7, 17). Non-health-care–related cases of CDI are now being reported (7, 16). Treatment options have been limited by the unsatisfactory efficacy of current therapeutic agents, the high recurrence rates of disease, the disruption of normal intestinal microbiota, and the colonization by vancomycin-resistant enterococci (VRE) (1, 2, 14). Although a recently introduced macrocyclic antibiotic, fidaxomicin, shows equivalent primary cure rates and reduced recurrence rates for CDI compared to those of vancomycin (15), it shows rates equivalent to those of vancomycin against restriction endonuclease analysis (REA) type BI strain–associated disease.

CB-183,315 is a novel lipopeptide antibiotic with Gram-positive activity which is bactericidal against C. difficile. To study the spectrum of its activity, including its effect on the normal components of fecal microbiota, we determined the MICs of CB-183,315 and vancomycin against C. difficile and other intestinal anaerobes, as well as against Escherichia coli, Klebsiella spp., and Enterobacter spp. For comparison, metronidazole was tested against the Gram-negative anaerobes and the Enterobacteriaceae spp.

The C. difficile strains were recent clinical isolates (2005 to 2008) recovered from stools of patients with CDI. The other isolates were obtained from stool or infection–containing organisms presumed to be of intestinal origin, identified by standard methods (12) and occasionally by 16S rRNA gene sequence analysis (20), and stored in 20% skim milk at −70°C.

MIC values were determined by the agar dilution method according to CLSI procedures (4). Vancomycin and metronidazole laboratory standard powders were obtained from Sigma (St. Louis, MO), and CB-183,315 was provided by Cubist Pharmaceuticals, Inc. (Lexington, MA). All assay media for testing CB-183,315 were supplemented with a final concentration of 50 µg/liter calcium, where Ca²⁺ concentrations were confirmed by Laboratory Specialists, Inc. (Westlake, OH).

The Escherichia coli, Klebsiella pneumoniae, and Enterobacter spp. strains were tested using Mueller–Hinton agar and incubated at 37°C in ambient air, except for strains with metronidazole, which were incubated in the anaerobic chamber (4).

Quality control strains included Clostridium difficile ATCC 700057, Bacteroides fragilis ATCC 25285, Staphylococcus aureus ATCC 29213, and E. coli ATCC 25922 and were used each day of testing with the relevant set of test organisms.

Time-killed studies were carried out on one REA type BI strain (NAP1, ribotype 027) and one REA type Y strain (NAP4, ribotype 014) (3). CB-183,315 and vancomycin were prepared at 2, 4, and 8 times their MICs for each strain in supplemented Brucella broth. A drug-free growth control tube was included. Tubes were inoculated with ~10⁶ CFU/ml, placed on a shaker, and assayed at 0, 2, 4, 8, and 24 h of incubation at 37°C.

Table 1 shows the ranges, MIC₅₀, and MIC₉₀ for the major groups of organisms. The CB-183,315 MIC range for C. difficile was 0.06 to 2.0 µg/ml, with a MIC₉₀ of 0.5 µg/ml; other Gram-positive strains were inhibited by 0.03 to 16 µg/ml of CB-183,315. The MIC₉₀ of CB-183,315 for the Bacteroides fragilis group, Prevotella spp., Gram-negative cocci (Veillonella spp. and Acidaminococcus spp.), E. coli, Enterobacter spp., and Klebsiella spp. were all greater than 8,192 µg/ml. The MIC₉₀ were 8,192 µg/ml and 2,048 µg/ml for fusobacteria and Porphyromonas spp., respectively. In contrast, vancomycin had equally high MIC₉₀ of >8,192 µg/ml against only the fusobacteria and the Gram-negative cocci; the B. fragilis group was inhibited by vancomycin at a MIC₉₀ of 128 µg/ml (range, 32 to 256 µg/ml), Porphyromonas spp. were inhibited by vancomycin at a MIC₉₀ of 4 µg/ml, and Prevotella spp. were inhibited at a MIC₉₀ of 256 µg/ml of vancomycin.
TABLE 1 | In vitro activity of CB-183,315, vancomycin, and metronidazole against intestinal organisms

| Group (phylogenetic cluster) or species | No. of isolates | Indicated MIC values (µg/ml) of each antibiotic | CB-183,315 | Vancomycin | Metronidazole |
|----------------------------------------|----------------|-----------------------------------------------|------------|-------------|---------------|
|                         | Range | MIC₅₀ | MIC₉₀ | Range | MIC₅₀ | MIC₉₀ | Range | MIC₅₀ | MIC₉₀ |
| Actinomyces spp. | 15   | 0.125–8.0 | 1.0 | 8.0 | 0.25–1.0 | 0.5 | 1.0 | NT | |
| Bifidobacterium spp. | 14   | 0.06–2.0 | 0.5 | 2.0 | 0.25–2.0 | 0.5 | 1.0 | NT | |
| Eggerthella lenta | 17   | 1.0–16.0 | 4.0 | 8.0 | 1.0–2.0 | 2.0 | 2.0 | NT | |
| Eubacterium limosum | 13   | 0.06–1.0 | 0.25 | 0.5 | 1.0–2.0 | 2.0 | 2.0 | NT | |
| Eubacterium group (other)b | 35   | 0.06–4.0 | 0.25 | 2.0 | 0.25–32 | 1.0 | 2.0 | NT | |
| Lactobacillus spp., VANc | 20   | 0.5–2.0 | 1.0 | 2.0 | >32–>32 | >32 | >32 | NT | |
| Lactobacillus spp., VNAd | 17   | 0.125–16.0 | 1.0 | 4.0 | 0.25–4.0 | 1.0 | 2.0 | NT | |
| Propionibacterium spp. | 15   | 0.5–2.0 | 0.5 | 2.0 | 0.5–2.0 | 0.5 | 1.0 | NT | |
| Clostridium clutridiforme (XIVa) | 20   | 1.0–16.0 | 8.0 | 16.0 | 0.5–1.0 | 1.0 | 1.0 | NT | |
| Clostridium difficile (XI) | 556  | 0.06–2.0 | 0.5 | 0.5 | 0.25–4.0 | 1.0 | 2.0 | NT | |
| Clostridium innocuum (XV) | 22   | 1.0–4.0 | 2.0 | 4.0 | 8.0–16.0 | 8.0 | 16.0 | NT | |
| Clostridium perfringens (I) | 20   | 0.23–1.0 | 0.5 | 1.0 | 0.5–1.0 | 0.5 | 1.0 | NT | |
| Clostridium ramosum (XVIII) | 20   | 2.0–8.0 | 4.0 | 8.0 | 2–8 | 4 | 8 | NT | |
| Clostridium species (other)c | 39   | 0.125–16.0 | 1.0 | 16.0 | 0.25–32 | 1 | 2 | NT | |
| Anaerobic Gram-positive cocci | 49   | ≤0.03–4.0 | 0.25 | 0.5 | 0.06–1 | 0.25 | 0.5 | NT | |
| Bacteroides fragilis group | 21   | 8,192–>8,192 | 8,192 | >8,192 | 32–256 | 64 | 128 | 0.25–2 | 1 | 2 |
| Fusobacterium spp.b | 20   | 128–>8,192 | 512 | 8,192 | 128–>8,192 | 512 | >8,192 | 0.06–1 | 0.25 | 0.5 |
| Porphyromonas spp.b | 21   | 64–8,192 | 1,024 | 2,048 | 1–32 | 4 | 4 | 0.06–1 | 0.25 | 1 |
| Prevotella spp.e | 20   | 8,192–>8,192 | 8,192 | >8,192 | 32–512 | 128 | 256 | 0.25–4 | 1 | 4 |
| Gram-negative cocci | 21   | 8,192–>8,192 | >8,192 | >8,192 | 256–>8,192 | 2,048 | >8,192 | 0.06–8.2 | 2 | 4 |
| E. coli | 18   | 4,096–>8,192 | >8,192 | >8,192 | 64–256 | 128 | 256 | 128–1,024 | 256 | 512 |
| Enterobacter spp. | 18   | >8,192–>8,192 | >8,192 | >8,192 | 256–1,024 | 512 | 1,024 | 32–1,024 | 512 | 1,024 |
| Klebsiella | 20   | >8,192–>8,192 | >8,192 | >8,192 | 256–1,024 | 1,024 | 1,024 | 128–1,024 | 512 | 1,024 |

MICs ranged from 64 to 1,024 µg/ml for E. coli and 256 to 1,024 µg/ml for Enterobacter and Klebsiella species. Metronidazole was active against the Gram-negative anaerobes at 0.06 to 8 µg/ml but showed poor activity against the Enterobacteriaceae, with MICs ranging from 32 to 1,024 µg/ml.

The time-kill studies showed that CB-183,315 at 4 and 8 times the MIC resulted in a 3-log₁₀ reduction in the colony count for both REA types tested after 24 h of incubation. Vancomycin produced similar results.

CB-183,315 reaches a concentration of 6,494 ± 3,104 µg/g of feces after a 1-g twice-a-day (b.i.d.) dose at day 5 of a 14-day course (Cubist Pharmaceuticals, unpublished data), a concentration which is lower than the high MICs (>8,192 µg/ml) demonstrated for Bacteroides and other groups of organisms of the intestinal anaerobes; thus, CB-183,315 is likely to spare many of these important members of the normal microbiota. In contrast, vancomycin at a dose of 250 mg four times a day (q.i.d.) results in fecal levels that are generally above 2,000 µg/ml (11). Vancomycin thus has the potential for killing or inhibiting much of the normal aerobic and anaerobic fecal microbiotas. Moreover, oral vancomycin was shown to decrease or suppress Bacteroides in volunteers while increasing the occurrence of vancomycin-resistant enterococci (8, 13). In contrast, fidaxomycin, a narrow-spectrum macrocyclic, displayed less impact on the Bacteroides populations in patients treated for CDI (13), an observation which has been postulated as one reason for the associated lower relapse rate.

Using temporal temperature gradient electroooosmosis and fluorescent in situ hybridization and flow cytometry, Tannock...
et al. (19) compared the impacts of fidaxomicin and vancomycin on seven phylogenetic groups, including the clostridial clusters IV and XIVa, the Bacteroides-Prevotella group, Bifidobacterium spp., Atopobium spp., enterobacteria, and the Enterococaceae-Lactobacillaceae group. Minimal changes were seen in these groups for the fidaxomicin-treated patients, while the vancomycin-treated patients showed a marked decrease in Clostridium clusters IV and XIVa, Bacteroides spp., and Bifidobacterium spp. and an increase in the Enterococcus-Lactobacillus and enterobacteria groups. The MICs of CB-183,315 against clostridia were similar to those reported for fidaxomicin (9), showing decreased activity against several species in Clostridium clusters XIVa, XVI, and XVIII, although unlike fidaxomicin, decreased activity of CB-183,315 was also present against several species in cluster I (5, 9, 18). The MICₘₐₓ for the Clostridium clustriiforme group (cluster XIVa) was 16 μg/ml; for Clostridium innocuum (cluster XVI), it was 4 μg/ml; and for Clostridium ramosum (Cluster XVIII), it was 8 μg/ml; and for one strain of Clostridium sphenoides (cluster XIVa), it was 16 μg/ml. These findings suggest that CB-183,315 shows a lack of impact similar to that of fidaxomicin on the compositions of the major groups of bowel microbiiota.

With its excellent activity against C. difficile and its narrower spectrum and lack of activity against other colonic aerobic and anaerobic microbiiota and with the potential for fewer relapses, CB-183,315 is a promising new drug treatment for CDI.

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