Activity of a Novel Cyclic Lipopeptide, CB-183,315, against Resistant *Clostridium difficile* and Other Gram-Positive Aerobic and Anaerobic Intestinal Pathogens

D. R. Snyderman,^{a,b} N. V. Jacobus,^a and L. A. McDermott^a

Tufts Medical Center^a and Tufts University School of Medicine,^b Boston, Massachusetts, USA

We evaluated the activity of CB-183,315 against *Clostridium difficile*, including strains that are resistant to fluoroquinolones and metronidazole and with elevated MICs to vancomycin as well as other Gram-positive intestinal pathogens. The MICs of CB-183,315 against all *C. difficile* isolates were ≤ 1 $\mu\text{g/ml}$. CB-183,315 had greater activity than vancomycin and metronidazole against *C. difficile* isolates and was more active than the comparators against vancomycin-resistant enterococcus (VRE). CB-183,315 also had excellent activity against methicillin-resistant *Staphylococcus aureus* (MRSA), other *Clostridium* spp., and *Peptostreptococcus* spp.

In the past decade the incidence and severity of *Clostridium difficile*-associated diarrhea (CDAD) have increased significantly. Outbreaks caused by a highly virulent and epidemic strain (BI/NAP1/027) have emerged in Canada, the United States, Europe, and Asia (4, 10, 12, 13, 17, 21).

There has been little incentive to perform susceptibility testing on *C. difficile* isolates since these isolates have been thought to be uniformly susceptible to metronidazole and vancomycin, the primary antimicrobials used in treatment. Recently, isolates with elevated MICs to vancomycin and metronidazole have been reported (1, 16, 19, 20). Knowing that the rate of response to *C. difficile* treatment is less than optimal, and with knowledge that resistance to the primary agents may be occurring, there is a need to develop new agents for the treatment of *Clostridium difficile* infections (CDI) (2, 9, 11, 17).

CB-183,315 is a novel, cyclic lipopeptide analogue of daptomycin with very good activity against anaerobic-Gram-positive bacteria, including *C. difficile*, and limited activity against Gram-negative pathogens (3, 20). A recent phase 2 clinical trial evaluating the safety and effectiveness of CB-183,315, which was administered orally in doses of 125 and 250 mg twice a day, concluded that either dose was safe and well tolerated. The authors also concluded that CB-183,315 sustained better cure rates than vancomycin in the treatment of CDI (18).

We undertook this study to determine the activity of CB-183,315 against isolates of *C. difficile* that are resistant to metronidazole and highly resistant to fluoroquinolones and clindamycin and with elevated MICs to vancomycin. Other intestinal pathogens, such as *Enterococcus* spp. (vancomycin-susceptible *Enterococcus* [VSE] and vancomycin-resistant *Enterococcus* [VRE]), *Staphylococcus aureus* (methicillin-susceptible *S. aureus* [MSSA] and methicillin-resistant *S. aureus* [MRSA]), *Peptostreptococcus* spp., and *Clostridium* spp., were also included.

The antimicrobials were obtained from their respective manufacturers or distributors. Standard powders were diluted according to their specifications. Resistance percentages were calculated using breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI). For agents without recommendations for anaerobic bacteria, such as vancomycin, minocycline, and linezolid, the CLSI breakpoint for resistance against aerobes caus-

ing systemic infections was used (5, 6, 7). CB-183,315 is an investigational compound; therefore, resistance breakpoints were not calculated.

The MICs of the antibiotics against 369 isolates were determined by agar dilution by following CLSI M11-A7 and CLSI M7-A8 recommendations (5, 6). ATCC reference control strains were included in each test as indicated by CLSI. The Ca^{2+} concentration in the media (brucella and Mueller-Hinton agars) was adjusted to 50 mg/liter as recommended by the manufacturer. Moxifloxacin was used to determine susceptibility to the fluoroquinolones.

Table 1 shows the susceptibilities of 55 *C. difficile* isolates to CB-183,315 compared with those of the other agents. It should be noted that many multidrug-resistant isolates were included in this study; 38.2% of the isolates were resistant to moxifloxacin and 18.2% of the isolates were resistant to clindamycin. In addition, 23.6% showed elevated MICs to metronidazole ($\text{MIC} \geq 8$ $\mu\text{g/ml}$) and 21.8% had MICs for vancomycin of ≥ 4 $\mu\text{g/ml}$. In order to underscore the activity of CB-183,315, the results are shown in subsets of specific susceptibilities.

Among the agents, CB-183,315 was the most active against all *C. difficile* isolates, with a MIC_{90} of ≤ 0.25 $\mu\text{g/ml}$. All *C. difficile* isolates were inhibited at concentrations of ≤ 1 $\mu\text{g/ml}$. CB-183,315 was more active than the two agents that are routinely used for treatment of CDI, namely, vancomycin and metronidazole. CB-183,315 exceeded the potency of vancomycin by 4-fold and that of metronidazole by greater than 16-fold.

The activity of CB-183,315 to *C. difficile* was identical for moxifloxacin-resistant isolates and moxifloxacin-susceptible isolates. Activity was maintained for those isolates that had elevated MICs

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Address correspondence to D. R. Snyderman, dsnyderman@tuftsmedicalcenter.org.

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TABLE 1 Activities of CB-183,315 and comparative agents against *Clostridium difficile* isolates^a

Species (no. of isolates)	Antibiotic	MIC (μg/ml)			
		Range	50%	90%	% resistant ^b
<i>Clostridium difficile</i> (55)	CB-183,315	≤0.125–1	≤0.125	0.25	NA
	Vancomycin	≤0.5–4	2	4	0
	Piperacillin-tazobactam	≤0.5–16	8	16	0
	Amoxicillin-clavulanic acid	≤0.03–1	0.5	1	0
	Minocycline	≤0.5–4	≤0.5	1	0
	Moxifloxacin	≤0.5–16	2	16	38.2
	Meropenem	≤0.125–2	1	2	0
	Metronidazole	≤0.125–32	4	16	3.6
	Linezolid	≤0.5–2	1	2	0
	Clindamycin	≤0.5–>16	2	>16	27.3
<i>C. difficile</i> , quinolone resistant (21)	CB-183,315	≤0.125	≤0.125	≤0.125	NA
	Vancomycin	1–4	2	4	0
	Piperacillin-tazobactam	≤0.5–16	8	16	0
	Amoxicillin-clavulanic acid	≤0.03–1	1	1	0
	Minocycline	≤0.5–1	≤0.5	1	0
	Moxifloxacin	8–>16	16	>16	100
	Meropenem	≤0.125–2	2	2	0
	Metronidazole	1–32	4	16	4.8
	Linezolid	≤0.5–2	1	2	0
	Clindamycin	1–>16	16	>16	52.4
<i>C. difficile</i> , quinolone susceptible (34)	CB-183,315	≤0.125–1	≤0.125	0.5	NA
	Vancomycin	≤0.5–4	2	4	0
	Piperacillin-tazobactam	≤0.5–16	8	16	0
	Amoxicillin-clavulanic acid	≤0.03–1	0.5	1	0
	Minocycline	≤0.5–4	≤0.5	1	0
	Moxifloxacin	≤0.5–4	2	2	0
	Meropenem	≤0.125–2	1	2	0
	Metronidazole	≤0.125–32	2	16	2.9
	Linezolid	≤0.5–2	1	1	0
	Clindamycin	≤0.5–>16	1	8	11.8
<i>C. difficile</i> , with a metronidazole MIC of ≥8 μg/ml (13)	CB-183,315	≤0.125–1	≤0.125	0.5	NA
	Vancomycin	1–4	2	4	0
	Piperacillin-tazobactam	2–16	8	16	0
	Amoxicillin-clavulanic acid	0.125–1	1	1	0
	Minocycline	≤0.5–1	≤0.5	1	0
	Moxifloxacin	≤0.5–16	2	16	46.2
	Meropenem	≤0.125–2	2	2	0
	Metronidazole	8–32	16	32	15.4
	Linezolid	≤0.5–2	1	2	0
	Clindamycin	≤0.5–>16	2	>16	38.5
<i>C. difficile</i> , with a metronidazole MIC of ≤4 μg/ml (42)	CB-183,315	≤0.125–0.5	≤0.125	0.25	NA
	Vancomycin	≤0.5–4	2	4	0
	Piperacillin-tazobactam	≤0.5–16	8	16	0
	Amoxicillin-clavulanic acid	≤0.03–1	0.5	1	0
	Minocycline	≤0.5–4	≤0.5	1	0
	Moxifloxacin	≤0.5–32	2	16	35.7
	Meropenem	≤0.125–2	1	2	0
	Metronidazole	≤0.125–4	2	4	0
	Linezolid	≤0.5–2	1	1	0
	Clindamycin	≤0.5–>16	2	>16	23.8
<i>C. difficile</i> , with a vancomycin MIC of ≤2 μg/ml (43)	CB-183,315	≤0.125–1	≤0.125	0.25	NA
	Vancomycin	≤0.5–2	2	2	0
	Piperacillin-tazobactam	≤0.5–16	8	16	0
	Amoxicillin-clavulanic acid	≤0.03–1	0.5	1	0
	Minocycline	≤0.5–4	≤0.5	1	0
	Moxifloxacin	≤0.5–32	2	16	32.6
	Meropenem	≤0.125–2	1	2	0
	Metronidazole	≤0.125–32	4	16	4.7
	Linezolid	≤0.5–2	1	2	0
	Clindamycin	≤0.5–32	2	32	20.9
<i>C. difficile</i> , with a vancomycin MIC of ≥4 μg/ml (12)	CB-183,315	≤0.125	≤0.125	0.125	NA
	Vancomycin	4	4	4	0
	Piperacillin-tazobactam	4–16	8	8	0
	Amoxicillin-clavulanic acid	0.5–1	0.5	1	0
	Minocycline	≤0.5–1	≤0.5	≤0.5	0
	Moxifloxacin	2–32	16	16	58.3
	Meropenem	1–2	2	2	0
	Metronidazole	2–16	4	16	0
	Linezolid	≤0.5–2	≤0.5	2	0
	Clindamycin	1–32	2	32	50

^a MICs were determined by agar dilution with the Ca²⁺ agar concentration adjusted to 50 mg/ml. Isolates were classified as susceptible (or resistant) to moxifloxacin, metronidazole, and vancomycin.

^b Resistance breakpoints are those recommended by CLSI. Resistance breakpoints for CB-183,315 have not been established, thus not available (NA). CLSI has not issued breakpoint recommendations for the following agents: vancomycin, minocycline, and linezolid; thus, the breakpoints for aerobes were used.

Table 2 Activities of CB-183,315 and comparative agents against other Gram-positive intestinal isolates^a

Species (no. of isolates)	Antibiotic	MIC (μg/ml)			
		MIC range	50%	90%	% resistant ^b
<i>Enterococcus</i> (60)	CB-183,315	≤0.125–2	1	2	NA
	Vancomycin	≤0.5–>64	2	>64	35
	Ampicillin	0.5–>32	1	>32	30
	Minocycline	≤0.125–16	8	16	18.3
	Levofloxacin	0.25–>8	4	16	53.3
	Erythromycin	0.25–>16	>16	>16	63.3
	Metronidazole	>32	>32	>32	100
	Linezolid	1–4	2	2	0
VRE (21)	CB-183,315	≤0.125–2	1	2	NA
	Vancomycin	>64	>64	>64	100
	Ampicillin	0.5–>32	>32	>32	76.9
	Minocycline	≤0.125–16	8	16	14.3
	Levofloxacin	>8	>8	>8	100
	Erythromycin	>8	>8	>8	100
	Metronidazole	>32	>32	>32	100
	Linezolid	1–4	1	2	0
VSE (39)	CB-183,315	≤0.125–2	0.5	2	NA
	Vancomycin	≤0.5–4	2	4	0
	Ampicillin	0.5–>32	1	2	5.1
	Minocycline	≤0.125–16	4	8	20.5
	Levofloxacin	0.25–>8	2	>8	28.2
	Erythromycin	>8	>8	>8	100
	Metronidazole	>32	>32	>32	100
	Linezolid	1–2	2	2	0
MSSA (12)	CB-183,315	0.25–1	0.5	0.5	NA
	Oxacillin	0.25–2	0.5	0.5	0
	Vancomycin	1–2	1	2	0
	Ampicillin	1–>4	2	>4	100
	Amoxicillin-clavulanic acid	0.125–2	0.5	1	0
	Meropenem	≤0.125	≤0.125	≤0.125	0
	Minocycline	≤0.5	≤0.5	≤0.5	0
	Levofloxacin	0.5–>8	4	4	66.6
	Erythromycin	2–>16	>16	>16	66.6
	Linezolid	1–2	2	2	0
	Cefuroxime	0.25–1	0.5	1	0
	Trimethoprim-sulfamethoxazole	≤0.5	≤0.5	≤0.5	0
MRSA (12)	CB-183,315	0.25–1	0.5	1	NA
	Oxacillin	4–>8	>8	>8	100
	Vancomycin	2	2	2	0
	Ampicillin	>4	>4	>4	100
	Amoxicillin-clavulanic acid	2–>16	8	>16	58.3
	Meropenem	0.5–>16	2	8	8.3
	Minocycline	0.5–16	0.5	8	8.3
	Levofloxacin	0.5–>8	>8	>8	91.6
	Erythromycin	>16	>16	>16	100
	Linezolid	1–2	1	2	0
	Cefuroxime	2–>64	>64	>64	75.0
	Trimethoprim-sulfamethoxazole	≤0.5–>16	≤0.5	16	16.6
<i>Clostridium</i> (33) ^c	CB-183,315	≤0.125–4	1	2	NA
	Vancomycin	1–16	1	4	0
	Piperacillin-tazobactam	≤0.5–8	<0.5	8	0
	Amoxicillin-clavulanic acid	0.06–2	0.125	1	0
	Minocycline	≤0.5–16	≤0.5	16	15.2
	Moxifloxacin	≤0.5–>8	1	8	12.1
	Meropenem	≤0.125–2	≤0.125	2	0

(Continued on following page)

TABLE 2 (Continued)

Species (no. of isolates)	Antibiotic	MIC ($\mu\text{g/ml}$)			
		MIC range	50%	90%	% resistant ^b
<i>Peptostreptococcus</i> (45) ^d	Metronidazole	0.25–32	4	8	3
	Linezolid	1–4	2	2	0
	Clindamycin	≤ 0.5 –>16	1	4	9.1
	CB-183,315	≤ 0.125 –2	≤ 0.125	0.5	NA
	Vancomycin	≤ 0.5 –2	≤ 0.5	1	0
	Piperacillin-tazobactam	≤ 0.5 –16	≤ 0.5	≤ 0.5	0
	Amoxicillin-clavulanic acid	≤ 0.03 –1	0.125	0.5	0
	Minocycline	≤ 0.5 –8	≤ 0.5	8	0
	Moxifloxacin	≤ 0.5 –32	≤ 0.5	8	13.3
	Meropenem	≤ 0.125 –0.5	≤ 0.125	0.25	0
Miscellaneous Gram-positive bacilli (6) ^e	Metronidazole	≤ 0.125 –>32	1	4	4.4
	Linezolid	≤ 0.5 –8	1	1	2.2
	Clindamycin	≤ 0.5 –>16	1	>16	20
	CB-183,315	1–16			
	Vancomycin	≤ 0.125 –>64			
	Piperacillin-tazobactam	≤ 0.5 –4			
	Amoxicillin-clavulanic acid	0.25–2			
	Minocycline	≤ 0.5 –8			
	Moxifloxacin	≤ 0.5 –2			
	Meropenem	≤ 0.125 –8			
<i>Bacteroides fragilis</i> group (86)	Metronidazole	>32			
	Linezolid	≤ 0.5 –4			
	Clindamycin	≤ 0.5 –2			
	CB-183,315	>16	>16	>16	
	Piperacillin-tazobactam	≤ 0.5 –32	2	16	0
	Amoxicillin-clavulanic acid	0.25–16	1	4	1.2
	Minocycline	≤ 0.5 –64	4	16	12.8
	Moxifloxacin	≤ 0.5 –>16	2	16	22.1
	Meropenem	≤ 0.125 –>16	0.25	1	1.2
	Metronidazole	≤ 1 –2	<1	<1	0
	Linezolid	≤ 1 –16	2	8	1.2
	Clindamycin	≤ 0.5 –>128	1	>128	31.4

^a MICs determined by agar dilution with a Ca^{2+} concentration for CB-183,315 were adjusted to 50 mg/liter and confirmed by Laboratory Specialist Inc.

^b Resistance breakpoints are those recommended by CLSI. Resistance breakpoints for CB-183,315 have not been established, thus not available (NA). CLSI has not issued breakpoint recommendations for the following agents: vancomycin, minocycline, and linezolid; thus, the breakpoints for aerobes were used.

^c Includes 10 *C. perfringens*, 5 *C. cadaveris*, 2 *C. innocuum*, 2 *C. bifermentans*, 2 *C. histolyticum*, 1 *C. subterminale*, 1 *C. clostridioforme*, 1 *C. ramosum*, 1 *C. septicum*, and 6 *Clostridium* species isolates.

^d Includes 10 *Finegoldia magna* (*Peptostreptococcus magnus*), 5 *Peptoniphilus asaccharolyticus*, 3 *Peptostreptococcus micros*, 1 *Peptostreptococcus anaerobius*, 1 *Peptostreptococcus tetradius*, and 25 *Peptostreptococcus* isolates (not identified to the species level).

^e Includes 1 *Actinomyces* sp., 2 *Bifidobacterium* sp., 1 *Lactobacillus casei*, and 2 *Lactobacillus* sp. isolates. The number of isolates was too small to determine other statistics.

to metronidazole as well as those with decreased susceptibility to vancomycin.

The activities of CB-183,315 and the comparative agents against other Gram-positive isolates are illustrated in Table 2. All enterococcal isolates were inhibited by CB-183,315 at concentrations of $\leq 2 \mu\text{g/ml}$. All *S. aureus* isolates (MRSA and MSSA) were inhibited at CB-183,315 concentrations of $\leq 1 \mu\text{g/ml}$.

The MIC range of CB-183,315 against 33 *Clostridium* sp. was 0.125 to 4 $\mu\text{g/ml}$. Seventy percent of *Clostridium* sp., were inhibited by $\leq 1 \mu\text{g/ml}$ of CB-183,315. Two strains, one *Clostridium clostridioforme* and a *Clostridium* sp., showed MICs of 4 $\mu\text{g/ml}$. CB-183,315 inhibited 93% of *Peptostreptococcus* spp. at 0.5 $\mu\text{g/ml}$. Among 45 *Peptostreptococcus* spp., 2 isolates showed CB-183,315 MICs of 1 $\mu\text{g/ml}$ and another isolate showed a MIC of 2 $\mu\text{g/ml}$. Eighty-six *Bacteroides fragilis* group isolates demonstrated MICs of >16 $\mu\text{g/ml}$, thus con-

firmed the limited activity of CB-183,315 against anaerobic Gram-negative bacteria.

CB-183,315 is structurally related to daptomycin and appears to share a mechanism of action, i.e., membrane depolarization and loss of cell viability (14). *In vitro* studies by Citron et al. demonstrated bactericidal activity (3). In addition, development of resistance to CB-183,315 is a rare event as shown by *in vitro* serial passage (14). Besides these *in vitro* findings, a hamster model of CDAD demonstrated potent efficacy, similar to that of vancomycin, against initial onset of the disease (15). To date, the results of a clinical trial, published in abstract form, demonstrated that CB-183,315 was efficacious and well tolerated for the treatment of CDI in adults (18).

Our results and those of Citron et al. (3) confirm the excellent activity of CB-183,315 against *C. difficile* and its enhanced activity

against *C. difficile* resistant to fluoroquinolones, clindamycin, and metronidazole and with elevated MICs to vancomycin. These data, taken together with its reported bactericidal activity, rare resistance development, and effectiveness in an animal model and in clinical trials, constitute an excellent preclinical profile, placing CB-183,315 as a prominent agent for clinical development in the treatment of CDI, particularly in infections caused by resistant strains (3, 14, 15, 18).

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