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Activity of Cadazolid Against Gram-positive Clinical Isolates, Including Linezolid-resistant Subsets with Defined Resistance Mechanisms

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ICAAC 2013

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ABSTRACT

Background: Cadazolid (formerly ACT-179811) is a new antibiotic currently in development for the treatment of *Clostridium difficile*-associated diarrhea. The activity of cadazolid was assessed against aerobic Gram-positive bacteria, including molecularly characterized strains with elevated linezolid MIC values (≥4 μg/mL).

Methods: A total of 124 staphylococcal and enterococcal isolates (78 linezolid-nonsusceptible and 46 wildtype strains) were included. Strains had mutations in 23S rRNA (G2576T), and alterations in L3 and L4 (*S. epidermidis*). Cfr-producing isolates (21) and isogenic strains were also included. Identification was performed by Vitek® 2 and confirmed by 16S rRNA. Susceptibility testing was performed by broth microdilution (CLSI, M07-A9 and M100-S23).

Results: Cadazolid showed similar modal MIC and MIC_{50} values when tested against wildtype and linezolid-nonsusceptible enterococci. This drug was 16- to 32-fold more potent than linezolid ($MIC_{50/90}$, 8/16-32 μg/mL) when tested against linezolid-nonsusceptible enterococci. Cadazolid had modal MIC values for wildtype, *cfr*- and G2576T-harboring *S. aureus* of 0.25, 0.25 and 0.5 μg/mL, respectively; and cadazolid was 16- to 32-fold more potent than linezolid ($MIC_{50/90}$, 8/16 μg/mL) when tested against linezolid-nonsusceptible *S. aureus*. When tested against non-wildtype (elevated MIC values for linezolid) *S. epidermidis*, cadazolid ($MIC_{50/90}$, 1/2 μg/mL) was 32- to 128-fold more active than linezolid ($MIC_{50/90}$, 32/256 μg/mL). Cadazolid ($MIC_{50/90}$, 0.25/0.5 μg/mL) was 8- to 32-fold more potent than moxifloxacin ($MIC_{50/90}$, 2/16 μg/mL) against all *S. aureus*. Cadazolid ($MIC_{50/90}$, 0.5/2 μg/mL) was 4- to 16-fold more active than moxifloxacin ($MIC_{50/90}$, 2/32 μg/mL) against all *S. epidermidis*. Presence of *cfr* did not affect the cadazolid MIC results, while linezolid MIC values were 4-fold higher when compared with those obtained from isogenic strains.

Conclusions: Cadazolid was highly active against staphylococcal and enterococcal isolates with characterized linezolid resistance mechanisms and inhibited all strains at ≤2 μg/mL.

INTRODUCTION

Cadazolid (formerly ACT-179811) is a novel antimicrobial agent currently in development for the treatment of *Clostridium difficile*-associated diarrhea and has recently completed Phase II clinical trials. Cadazolid has shown potent *in vitro* activity against *C. difficile* and has an antibacterial spectrum against Gram-positive bacteria, including *Staphylococcus aureus* and enterococci, while activity against Gram-negative bacteria is limited. Cadazolid is an oxazolidinone-type antibacterial with a quinolone moiety and acts primarily by inhibition of bacterial protein synthesis.

Linezolid was the first member of the oxazolidinone class to be introduced into clinical practice (2000). The prevalence of linezolid resistance among Gram-positive organisms remains very low among surveillance clinical isolates, while the resistance mechanisms detected have been mostly comprised of mutations in the domain V of 23S rRNA; but alterations in the ribosomal proteins L3 and L4 have also been associated with decreased susceptibility. Moreover, a more recently recognized resistance mechanism, *cfr*, has been detected. *cfr* encodes a methyltransferase that catalyzes the post-transcriptional methylation of nucleotide A2503 in the 23S rRNA causing decreased susceptibility to phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A ($PhLOPS_A$) compounds.

The objective of this investigation was to assess the activity of cadazolid when tested against aerobic staphylococcal and enterococcal bacteria, including molecularly characterized isolates with elevated linezolid MIC values (≥4 μg/mL). Activity against such strains may be of importance in order to prevent their propagation during cadazolid treatment of *C. difficile*-associated diarrhea.

MATERIALS AND METHODS

Bacterial strains. A total of 124 staphylococcal and enterococcal isolates (46 wildtype and 78 strains displaying elevated MIC results for linezolid [≥4 μg/mL]) were included in this investigation. Strains had mutations in 23S rRNA (G2576T), and alterations in L3 and L4 (*Staphylococcus epidermidis*). Cfr-producing isolates (21) and isogenic strains were also included. Bacterial identification was performed by Vitek® 2 (bioMérieux; Hazelwood, Missouri) and confirmed by 16S rRNA sequencing.

Characterization of linezolid resistance mechanisms. Isolates displaying elevated MIC results for linezolid included herein were screened for the presence of *cfr*, and mutations in the 23S rRNA and ribosomal proteins (L3 and L4) by PCR and sequencing. Amplicons were sequenced on both strands. Ribosomal proteins obtained were compared to those from wildtype linezolid-susceptible *Enterococcus faecalis* ATCC 29212 and *S. aureus* NTCC 4220 using the Lasergene® software package (DNAStar; Madison, Wisconsin).

Antimicrobial susceptibility testing. Cadazolid and comparator agents were tested for susceptibility by broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (M07-A9, 2012). Validation of the MIC values was performed by concurrent testing of CLSI-recommended quality control (QC) reference strains (*S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212). All QC results were within published acceptable ranges. MIC interpretations were based on the CLSI M100-S23 (2013) and European Committee on Antimicrobial Susceptibility Testing (EUCAST; 2013) breakpoint criteria, as available. In addition, the inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event.

RESULTS

- Cadazolid ($MIC_{50/90}$, 0.12-0.25/0.25 μg/mL) demonstrated potent activity when tested against selected wildtype clinical isolates of *E. faecalis* and *Enterococcus faecium*. G2576T enterococcal mutants had MIC values ($MIC_{50/90}$, 0.25-0.5/0.5-1 μg/mL) two- to four-fold higher than the respective susceptible counterparts (**Tables 1** and **2**).
- Cadazolid ($MIC_{50/90}$, 0.25-0.5/0.5-1 μg/mL) was 16- to 32-fold more potent than linezolid ($MIC_{50/90}$, 8/16 - 32 μg/mL) when tested against linezolid-nonsusceptible *E. faecalis* and *E. faecium* clinical isolates (**Table 2**).
- Moreover, cadazolid ($MIC_{50/90}$, 0.25-0.5/0.5-1 μg/mL) was at least 32-fold more active than moxifloxacin when tested against linezolid-nonsusceptible *E. faecalis* ($MIC_{50/90}$, 8/16 μg/mL) and *E. faecium* ($MIC_{50/90}$, 32/>32 μg/mL) clinical isolates (**Table 2**).
- Wildtype and non-wildtype (linezolid MIC values of ≥4 μg/mL) *S. aureus* were inhibited by cadazolid at ≤0.25 and ≤0.5 μg/mL, respectively (**Table 1**). *cfr*-carrying (MIC_{50} and modal MIC values of 0.25 μg/mL) and 23S rRNA mutant (MIC_{50} and modal MIC values of 0.5 μg/mL) *S. aureus* strains displayed cadazolid MIC_{50} and modal MIC values within two-fold dilutions. Furthermore, equivalent cadazolid MIC_{90} results were observed for non-wildtype *S. aureus*, regardless of resistance mechanisms.
- Non-wildtype *S. aureus* showed cadazolid MIC results ($MIC_{50/90}$, 0.5/0.5 μg/mL) 16- to 32-fold lower than linezolid ($MIC_{50/90}$, 8/16 μg/mL) and four- to 32-fold lower than moxifloxacin ($MIC_{50/90}$, 2/16 μg/mL; 11.5% susceptible; **Table 2**).
- When tested against non-wildtype (linezolid MIC values of ≥4 μg/mL) *S. epidermidis* possessing multiple mechanisms of resistance, cadazolid ($MIC_{50/90}$, 1/2 μg/mL) demonstrated MIC_{50} and MIC_{90} results eight- to 16-fold higher than against the wildtype strains ($MIC_{50/90}$, 0.12/0.12 μg/mL); all isolates were inhibited at ≤2 μg/mL (**Table 1**).
- A direct comparison between cadazolid and linezolid showed that the former compound was 32- to 128-fold more potent than linezolid when tested against *S. epidermidis* non-wildtype strains (**Table 2**).
- When tested against non-wildtype *S. epidermidis* isolates, cadazolid ($MIC_{50/90}$, 1/2 μg/mL) was four- to 16-fold more potent than moxifloxacin ($MIC_{50/90}$, 4/32 μg/mL; 9.1% susceptible; **Table 2**).
- A four-fold increase in the linezolid MIC results was observed when tested against Cfr-producing *S. aureus* and *S. epidermidis* compared with their respective isogenic strains (**Table 3**), while presence of *cfr* increased cadazolid MIC results by no more than two-fold.

Table 3. Antimicrobial activity of cadazolid and linezolid when tested against *cfr*-carrying staphylococcal and respective isogenic strains.

Strain	Genotype	MIC (μg/mL) Cadazolid	MIC (μg/mL) Linezolid
S. aureus			
RN4220	Wildtype	0.12	1
RN4220[Transformant]	*cfr*	0.25	4
S. epidermidis			
8177J[Cured]	L3 (F_{147}L/A_{152}R)	1	8
8177J	*cfr* and L3 (F_{147}L/A_{152}R)	1	32

Strain 8177J[Cured] originated from *S. epidermidis* 8177J, which had the *cfr*-carrying plasmid cured.

Table 1. Antimicrobial activity and MIC distribution for cadazolid tested against wildtype Gram-positive pathogens and those possessing molecularly characterized linezolid resistance mechanisms.

Organism/genotype (No tested)	MIC (μg/mL) 50%	MIC (μg/mL) 90%	No. (cumulative %) of isolates inhibited at each cadazolid MIC (μg/mL) of: [a] 0.015	0.03	0.06	0.12	0.25	0.5	1	2
E. faecalis (21)										
Wildtype (11)	0.12	0.25	0(0.0)	0(0.0)	0(0.0)	8(72.7)	3(100.0)			
Non-wildtype[b] (10)	0.25	0.5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	7(70.0)	2(90.0)	1(100.0)	
E. faecium (32)										
Wildtype (12)	0.25	0.25	0(0.0)	0(0.0)	0(0.0)	2(16.7)	9(91.7)	1(100.0)		
Non-wildtype (20)	0.5	1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(15.0)	13(80.0)	3(95.0)	1(100.0)
S. aureus (38)										
Wildtype (12)	0.25	0.25	0(0.0)	0(0.0)	0(0.0)	4(33.3)	8(100.0)			
Non-wildtype (26)	0.5	0.5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	10(38.5)	16(100.0)		
cfr (12)	0.25	0.5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	9(75.0)	3(100.0)		
G2576T mutants (14)	0.5	0.5	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(7.4)	13(100.0)		
S. epidermidis (33)										
Wildtype (11)	0.12	0.12	0(0.0)	0(0.0)	1(9.1)	9(90.9)	1(100.0)			
Non-wildtype (22)	1	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(13.6)	4(31.8)	8(68.2)	7(100.0)

a. Modal MIC results are underlined.
b. Isolates displaying elevated MIC results for linezolid (≥4 μg/mL).

Table 2. Activity of cadazolid and comparator antimicrobial agents when tested against staphylococci and enterococci displaying elevated MIC results (≥4 μg/mL) for linezolid.

Organism (no. tested)/ antimicrobial agent	MIC (μg/mL) 50%	MIC (μg/mL) 90%	Range	% susceptible / %resistant[a] CLSI	% susceptible / %resistant[a] EUCAST
E. faecalis (11)[b]					
Cadazolid	0.25	0.5	0.25 – 1	- / -	- / -
Linezolid	8	16	4 – 64	0.0 / 60.0	40.0 / 60.0
Vancomycin	2	>256	1 – >256	60.0 / 40.0	60.0 / 40.0
Daptomycin	1	1	0.5 – 2	100.0 / -	- / -
Erythromycin	>128	>128	0.25 – >128	10.0 / 90.0	- / -
Clindamycin	>64	>64	32 – >64	- / -	- / -
Quinupristin/dalfopristin	8	8	4 – 32	0.0 / 100.0	0.0 / 80.0
Moxifloxacin	8	16	0.25 – 16	- / -	- / -
E. faecium (20)[b]					
Cadazolid	0.5	1	0.25 – 2	- / -	- / -
Linezolid	8	32	4 – 64	0.0 / 85.0	15.0 / 85.0
Vancomycin	128	256	0.5 – >256	35.0 / 60.0	35.0 / 65.0
Daptomycin	1	2	1 – 2	100.0 / -	- / -
Erythromycin	>128	>128	0.25 – >128	10.0 / 65.0	- / -
Clindamycin	>64	>64	0.25 – >64	- / -	- / -
Quinupristin/dalfopristin	0.5	2	0.5 – 4	85.0 / 5.0	85.0 / 0.0
Moxifloxacin	32	>32	16 – >32	- / -	- / -
S. aureus (26)[c]					
Cadazolid	0.5	0.5	0.25 – 0.5	- / -	- / -
Linezolid	8	16	4 – 16	26.9 / 73.1	26.9 / 73.1
Oxacillin	>2	>2	≤0.25 – >2	11.5 / 88.5	11.5 / 88.5
Vancomycin	1	1	0.5 – 2	100.0 / 0.0	100.0 / 0.0
Daptomycin	0.5	0.5	0.25 – 1	100.0 / -	100.0 / 0.0
Erythromycin	128	>128	0.25 – >128	15.4 / 84.6	15.4 / 84.6
Clindamycin	>64	>64	0.12 – >64	30.8 / 69.2	19.2 / 69.2
Quinupristin/dalfopristin	1	4	0.25 – 4	57.7 / 11.5	57.7 / 11.5
Moxifloxacin	2	16	0.03 – >32	11.5 / 69.2	11.5 / 69.2
S. epidermidis (22)[d]					
Cadazolid	1	2	0.25 – 2	- / -	- / -
Linezolid	32	256	4 – >256	9.1 / 90.9	9.1 / 90.9
Oxacillin	>2	>2	>2	0.0 / 100.0	0.0 / 100.0
Vancomycin	2	2	1 – 2	100.0 / 0.0	100.0 / 0.0
Daptomycin	0.5	0.5	0.25 – 1	100.0 / -	100.0 / 0.0
Erythromycin	4	>128	0.25 – >128	22.7 / 50.0	27.3 / 68.2
Clindamycin	2	>64	0.25 – >64	31.8 / 50.0	18.2 / 68.2
Quinupristin/dalfopristin	0.5	2	0.25 – 8	86.4 / 9.1	86.4 / 9.1
Moxifloxacin	4	32	0.5 – >32	9.1 / 90.9	9.1 / 90.9

a. Criteria as published by the CLSI [2013] and EUCAST [2013], when available.
b. All strains possess 23S rRNA (G2576T) only.
c. Includes 12 *cfr*-carrying and 14 23S rRNA (G2576T) mutant strains.
d. Isolates possess multiple mutations in 23S rRNA (G2576T) and/or alterations in L3 and/or L4 and/or *cfr*.

CONCLUSIONS

- The *in vitro* activity of cadazolid was marginally affected (two- to four-fold) when tested against *E. faecalis* and *E. faecium* strains having mutated 23S rRNA alleles compared with wildtype isolates. In comparison, linezolid exhibited MIC results against linezolid-nonsusceptible enterococci eight- to 16-fold higher than the wildtype isolates.
- Overall, cadazolid tested against Cfr-producing *S. aureus* and wildtype strains demonstrated comparable potencies (MIC_{90}, 0.5 and 0.25 μg/mL, respectively). Moreover, similar potency (MIC_{90}, 0.5 μg/mL) was obtained for cadazolid when tested against 23S rRNA mutants of *S. aureus*.
- When tested against all *S. aureus* and *S. epidermidis* strains, cadazolid was eight- to 32- and four- to 16-fold more potent than moxifloxacin, respectively. In addition, cadazolid retained activity when tested against moxifloxacin-nonsusceptible *S. aureus* ($MIC_{50/90}$, 0.25/0.5 μg/mL) and *S. epidermidis* ($MIC_{50/90}$, 0.5/2 μg/mL) strains.
- Cadazolid was highly active against staphylococcal and enterococcal isolates with characterized linezolid resistance mechanisms and inhibited all strains at ≤2 μg/mL. In addition, it retained potent activity against strains nonsusceptible to fluoroquinolones (moxifloxacin). Therefore, cadazolid is not expected to promote the propagation of such strains during treatment of *C. difficile*-associated diarrhea.

ACKNOWLEDGEMENT

This study at JMI Laboratories was supported by an Education/Research grant from Actelion Pharmaceuticals Ltd (Allschwil, Switzerland). JMI Laboratories also received compensation fees for services with regards to abstract/poster preparation, which was funded by Actelion Pharmaceuticals Ltd.

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