F1-1357e

Antimicrobial Activity of Four Oxazolidinone Investigational Compounds Tested against a Collection of Linezolid-non-susceptible Strains with Genetically Defined Resistance Mechanisms

HS SADER, DJ BIEDENBACH, RE MENDES, DJ FARRELL, RN JONES
JMI Laboratories, North Liberty, Iowa, USA

ICAAC 2011
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
ph.319.665.3370, fax 319.665.3371
helio-sader@jmilabs.com

ABSTRACT

Background: The potency of 4 novel oxazolidinone (OXZ) compounds were compared to that of linezolid when tested by reference methods against a collection of linezolid (linezolid)-non-susceptible (NS) Gram-positive cocci (GPC) having genetically defined mechanisms of OXZ resistance (R).

Methods: 103 linezolid-NS and 25 linezolid-S GPC, obtained through the SENTRY Antimicrobial Surveillance Program, were tested for S by CLSI broth microdilution methods (M07-A8) against 4 novel OXZ (LCB-676/647/229/648) and linezolid. Strains were screened for mutations in the 23S rRNA, L3 and L4 encoding genes by PCR and DNA sequencing, and for the presence of the *cfr* gene.

Results: The linezolid-NS *S. aureus* strains were either 23S rRNA G2576T mutants or *cfr* positive, while 42 coagulase-negative staphylococci (CoNS) isolates showed a broad variety of linezolid R mechanisms, including 23S rRNA, L3 and L4 mutations and *cfr*, alone or in combination. The LCB compounds were eight- to 32-fold more active than linezolid, and LCB-648 was slightly (two-fold) more active than the others 3 LCB compounds when tested against staphylococci. All linezolid-NS enterococci contained G2576T mutations and the LCB compounds exhibited similar activities against these organisms, which were eight- to 32-fold greater than that of linezolid. When compared to previous results published for radezolid (RDZ) and torezolid (TZL) on a subset of 20 strains (4 species), LCB-229, LCB-648 and RDZ ($MIC_{50/90}$, 0.5/1 μg/ml for all 3 drugs) were two-, two-, four- and 32-fold more active than LCB-676, LCB-647 ($MIC_{50/90}$,1/2 μg/ml for both drugs), TZL ($MIC_{50/90}$,2/8 μg/ml) and linezolid ($MIC_{50/90}$,16/32 μg/ml), respectively.

Conclusions: These investigational OXZ compounds demonstrated enhanced in vitro activity against genetically characterized linezolid-NS as well as linezolid-S GPC strains.

Organism (no. of isolates)	MIC_{50}/MIC_{90} (μg/ml)				
	LCB-676	LCB-647	LCB-229	LCB-648	Linezolid
S. aureus (25)[a]	0.5/2	0.5/1	0.5/1	0.25/0.5	4/8
Linezolid-S (6)	0.5/-	0.25/-	0.25/-	0.25/-	1/-
Linezolid-NS (19)[a]	0.5/2	0.5/1	0.5/1	0.5/0.5	4/16
CoNS (48)[b]	2/>64	2/4	2/4	1/4	32/128
Linezolid-S (6)	0.12/-	0.12/-	0.12/-	0.12/-	0.5/-
Linezolid-NS (42)[b]	2/>64	2/4	2/4	1/4	32/128
E. faecalis (17)	0.5/1	0.5/2	0.25/1	0.25/1	4/32
Linezolid-S (6)	0.12/-	0.25/-	0.12/-	0.12/-	1/-
Linezolid-NS (11)	0.5/1	0.5/2	0.5/1	0.5/1	8/32
E. faecium (38)	0.5/1	0.5/1	0.5/1	0.25/0.5	8/16
Linezolid-S (7)	0.12/-	0.12/-	0.06/-	0.06/-	1/-
Linezolid-NS (31)	0.5/1	1/1	0.5/1	0.5/0.5	8/16

a. Includes four *cfr* positive strains.
b. Includes seven *cfr* positive strains.

INTRODUCTION

Linezolid, currently the only member of the oxazolidinone class approved by the United States (USA) Food and Drug Administration (FDA) for clinical use, has demonstrated broad activity against many clinically important Gram-positive pathogens including multidrug-resistant (MDR) subsets of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS), and vancomycin-resistant enterococci (VRE).

Although rare (<1% in large surveillance studies), resistance to linezolid can occur by alterations to the 50S bacterial ribosome (domain V of 23S rRNA), the proteins L3 or L4, or by the expression of *cfr*, an acquired methyltransferase gene. In the present study, we evaluated the antimicrobial activities of four novel oxazolidinone compounds when tested against a collection of linezolid-non-susceptible Gram-positive cocci having genetically defined mechanisms of oxazolidinone resistance.

MATERIALS AND METHODS

Organism collection: 103 linezolid-non-susceptible and 25 linezolid-susceptible Gram-positive cocci were utilized in this investigation. The strains were obtained through the SENTRY Antimicrobial Surveillance Program and all strains with decreased susceptibility to linezolid were screened for mutations in the 23S rRNA, L3 and L4 encoding genes by PCR and DNA sequencing, and for the presence of the *cfr* gene.

Antimicrobial agents: The organisms were tested against four novel oxazolidinone compounds (LCB-676, LCB-647, LCB-229 and LCB-648; see chemical structure at poster F1-1357b) developed by LegoChem Biosciences, Inc. (Korea), linezolid, daptomycin, tigecycline and vancomycin. In addition, MIC results obtained with the novel oxazolidinone compounds were compared to previous results published for radezolid [Farrell et al., 2011] and torezolid [Jones et al., 2009] on a subset of 20 common strains (4 species).

Susceptibility test methods: All isolates were tested for susceptibility by reference broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009) recommendations using fresh-frozen panels manufactured by JMI Laboratories. The quality assurance of the minimum inhibitory concentration (MIC) values was performed by concurrent testing of CLSI-recommended (M100-S21, 2011) control strains, including: *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. Categorical interpretation of comparator MIC values was performed according to CLSI (M100-S21, 2011) criteria.

RESULTS

- The linezolid-non-susceptible *S. aureus* strains were either 23S rRNA G2576T mutants or *cfr* positive, while 42 coagulase-negative staphylococci (CoNS) isolates showed a broad variety of linezolid resistant mechanisms, including 23S rRNA, L3 and L4 mutations and *cfr*, alone or in combination.
- The LCB compounds were eight- to 32-fold more active than linezolid, and LCB-648 was slightly (two-fold) more active than the others three LCB compounds when tested against staphylococci (Table 1).
- All linezolid-non-susceptible enterococci contained G2576T mutations and the LCB compounds exhibited similar activities against these organisms, which were eight- to 32-fold greater than that of linezolid (Table 1).
- When compared to previous results published for radezolid and torezolid on a subset of 20 strains (4 species), LCB-229, LCB-648 and radezolid ($MIC_{50/90}$, 0.5/1 μg/ml for all three compounds) were two-, two-, four- and 32-fold more active than LCB-676, LCB-647 ($MIC_{50/90}$, 1/2 μg/ml for both drugs), torezolid ($MIC_{50/90}$, 2/8 μg/ml) and linezolid ($MIC_{50/90}$, 16/32 μg/ml), respectively (Table 2).

Table 2. MIC distributions for seven oxazolidinone compounds when testing 20 linezolid non-susceptible strains (4 species)[a].

Oxazolidinone	No. of strains at MIC (μg/ml) of:									$MIC_{50/90}$
	0.25	0.5	1	2	4	8	16	32	>32	
LCB-676	-	7	6	5	-	-	-	-	2	1/2
LCB-647	1	4	8	5	2	-	-	-	-	1/2
LCB-229	2	8	4	5	1	-	-	-	-	0.5/2
LCB-648	4	9	4	2	1	-	-	-	-	0.5/2
Linezolid	-	-	-	-	3	6	5	4	2	16/32
Torezolid	-	2	4	6	4	2	2	-	-	2/8
Radezolid	6	4	6	3	1	-	-	-	-	0.5/2

a. Includes *S. aureus* (three strains), CoNS (seven), *E. faecalis* (five) and *E. faecium* (five). Data combined from Farrell et al. (2011), Jones et al. (2009), and this study.

Table 1. Antimicrobial activity of novel oxazolidinones in comparison to linezolid and antimicrobial agents from other classes.

Organism/Antimicrobial	MIC_{50}	MIC_{90}	MIC range	% S[a]	%R[a]	Organism/Antimicrobial	MIC_{50}	MIC_{90}	MIC range	% S[a]	%R[a]
S. aureus (25)						*E. faecalis* (17)					
LCB-676	0.5	2	0.25-2	-	-	LCB-676	0.5	1	0.12-2	-	-
LCB-647	0.5	1	0.25-2	-	-	LCB-647	0.5	2	0.12-2	-	-
LCB-229	0.5	1	0.25-2	-	-	LCB-229	0.25	1	0.12-2	-	-
LCB-648	0.25	0.5	0.12-1	-	-	LCB-648	0.25	1	0.12-2	-	-
Linezolid	4	8	1-16	76.0	24.0	Linezolid	4	32	1-64	35.3	47.1
Daptomycin	0.5	1	0.5-2	96.0	-	Daptomycin	1	2	1-2	100.0	-
Tigecycline	0.12	0.25	0.03-0.5	100.0	-	Tigecycline	0.06	0.12	0.015-0.12	100.0	-
Oxacillin	>2	>2	≤0.25->2	16.0	84.0	Vancomycin	2	>16	1->16	58.8	41.2
Vancomycin	1	2	0.5-2	100.0	0.0	*E. faecium* (38)					
CoNS (48)						LCB-676	0.5	1	≤0.03-2	-	-
LCB-676	2	>64	0.12->64	-	-	LCB-647	0.5	1	0.12-2	-	-
LCB-647	2	4	0.12-16	-	-	LCB-229	0.5	1	0.06-2	-	-
LCB-229	2	4	0.12-8	-	-	LCB-648	0.25	0.5	0.06-1	-	-
LCB-648	1	4	0.06-16	-	-	Linezolid	8	16	0.5-32	18.4	52.6
Linezolid	32	128	0.5->128	12.5	87.5	Daptomycin	2	2	1-8	97.4	-
Daptomycin	0.5	1	0.25-2	97.9	-	Tigecycline	0.03	0.25	0.015-2	94.7	-
Tigecycline	0.12	0.12	0.03-0.25	100.0	-	Vancomycin	>16	>16	0.25->16	29.0	71.0
Oxacillin	>2	>2	≤0.25->2	10.4	89.6						
Vancomycin	2	2	0.5-4	100.0	0.0						

a. Percentages susceptible (%S) and resistant (%R) according to the CLSI breakpoints when available [CLSI, 2011].

CONCLUSIONS

- These investigational oxazolidinone compounds (LCB-676, LCB-647, LCB-229 and LCB-648) demonstrated enhanced in vitro activity against genetically characterized linezolid-non-susceptible as well as linezolid-susceptible Gram-positive strains.
- In general, LCB-648 was slightly more active (two-fold) than the other investigation compounds and 16- to 32-fold more active than linezolid.
- The potent in vitro activities demonstrated by these new investigational oxazolidinones against contemporary linezolid non-susceptible Gram-positive pathogens support further development.

ACKNOWLEDGEMENTS

This study was sponsored by LegoChem Biosciences, Inc., Daejeon, Korea.

REFERENCES

1. Biedenbach DJ, Farrell DJ, Mendes RE, Ross JE, Jones RN (2010). Stability of linezolid activity in an era of mobile oxazolidinone resistance determinants: Results from the 2009 Zyvox(R) Annual Appraisal of Potency and Spectrum program. *Diagn Microbiol Infect Dis* 68: 459-467.
2. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition.* Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2011). *M100-S21. Performance standards for antimicrobial susceptibility testing: 21st informational supplement.* Wayne, PA: CLSI.
4. Farrell DJ, Sader HS, Mendes RE, Jones RN (2011). Potency of radezolid (RX-1741) and torezolid (DA-7157) tested against a collection of linezolid-non-susceptible strains with genetically defined resistance mechanisms. *21st ECCMID, May 7-10, 2011,* Milan, Italy.
5. Jones RN, Moet GJ, Sader HS, Mendes RE, Castanheira M (2009). TR-700 in vitro activity against and resistance mutation frequencies among Gram-positive pathogens. *J Antimicrob Chemother* 63: 716-720.
6. Locke JB, Finn J, Hilgers M, Morales G, Rahawi S, G CK, Picazo JJ, Im W, Shaw KJ, Stein JL (2010). Structure-activity relationships of diverse oxazolidinones for linezolid-resistant *Staphylococcus aureus* strains possessing the cfr methyltransferase gene or ribosomal mutations. *Antimicrob Agents Chemother* 54: 5337-5343.
7. Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B (2006). The *cfr* rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob Agents Chemother* 50: 2500-2505.
8. Mendes RE, Deshpande LM, Castanheira M, DiPersio J, Saubolle MA, Jones RN (2008). First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. *Antimicrob Agents Chemother* 52: 2244-2246.