Potential Utility of a Peptide Deformylase Inhibitor, LBM415, Against Oxazolidinone-Resistant or Streptogramin-Resistant Gram-Positive Isolates

P918
ECCMID 2004

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ABSTRACT

Background: New and novel antimicrobials have been introduced into infectious disease practice in the last decade to address emerging resistances (R) among gram-positive cocci. R to oxazolidinones, streptogramin combinations and various glycopeptides require expanded development of agents with alternative modes of action. In this investigation, LBM415, a new peptide deformylase inhibitor, was tested against clinical isolates having documented R to linezolid (LZD) or quinupristin/dalfopristin (Q/D) using reference susceptibility test methods. ***Methods:*** A total of 45 organisms were collected from our recent (2001–2002) stock culture collection that were originally isolated at surveillance sites in the United States, Canada, Brazil and Europe. These organisms included *E. faecalis* (LZD-R, 3 strains; Q/D-R was intrinsic), *E. faecium* (LZD-R, 10 strains; Q/D-R, 6 strains), *S. aureus* (LZD-R, 5 strains; Q/D-R, 10 strains), CoNS (LZD-R, 1 strain; Q/D-R, 9 strains) and *S. oralis* (LZD-R, 1 strain). The mechanisms of R for all LZD-R strains (MIC, ≥ 8 mg/L) was confirmed by gene sequencing with the detection of a G2576U mutation. PCR tests for *vatD* and *vatE* were negative. QC of the reference LBM415 MIC results was performed using acceptable MIC ranges reported by Anderegg et al. The proposed or tentative susceptible breakpoint for LBM415 was ≤ 8 mg/L based on pharmacokinetic/pharmacodynamic characteristics. ***Results***: Among the 13 LZD-R strains, 3 were *E. faecalis* and 10 were *E. faecium*. Also 10 enterococci were R to vancomycin (VANCO) and all *E. faecalis* strains had the intrinsic Q/D R (MIC, 8 mg/L). These enterococci had LBM415 MIC results between 0.5 and 4 mg/L (MIC_{90}, 4 mg/L). The 6 Q/D-R *E. faecium* were susceptible to VANCO and were inhibited by 1 or 2 mg/L of LBM415. LBM415 was highly active against LZD-R *S. aureus* (MICs, 0.25 - 0.5 mg/L), *S. epidermidis* (MIC, 2 mg/L) and the viridans group streptococcus (MIC, 0.5 mg/L). Q/D and vancomycin were active against these LZD-R organisms. The Q/D-R staphylococci (19) were inhibited by LBM415 (MIC range, 0.12 - 2 mg/L), LZD (1 or 2 mg/L) and VANCO (1 or 2 mg/L). ***Conclusions***: These results indicate that LBM415 among the candidate peptide deformylase inhibitors, demonstrated excellent activity (all MICs at ≤ 4 mg/L) against emerging gram-positive clinical isolates that have become R to oxazolidinones or streptogramin combinations.

INTRODUCTION

- Numerous new and novel antimicrobial agents have been introduced into infectious disease practice in the last decade to address emerging resistances among gram-positive cocci. Resistances to oxazolidinones, streptogramin combinations and various glycopeptides require expanded development of agents with alternative targets or modes of action.
- Peptide deformylase, an enzyme required for prokaryote protein synthesis, has been suggested as a potential target for inhibitors such as the potent hydroxamic acid derivatives. The concept has been validated and several candidate agents have been screened.
- During initial development, resistance mechanisms were also described among multiple gram-positive organisms including *Staphylococcus aureus*.
- In this investigation, LBM415, a new peptide deformylase inhibitor, was tested against a collection of recent clinical isolates having documented resistances to linezolid or quinupristin/ dalfopristin (Q/D) using reference susceptibility test methods.

MATERIALS AND METHODS

BACTERIAL STRAINS. A total of 45 organisms were collected from the recent stock culture collection (2001–2002) of JMI Laboratories (North Liberty, Iowa, USA) that were originally isolated at surveillance sites in the United States, Canada, Brazil and Europe.

- These organisms included *Enterococcus faecalis* (linezolid-resistant, 3 strains; Q/D resistance was intrinsic), *E. faecium* (linezolid-resistant, 10 strains; Q/D-resistant, 6 strains), *S. aureus* (linezolid-resistant, 5 strains; Q/D-resistant, 10 strains), coagulase-negative staphylococci (linezolid-resistant, 1 strain; Q/D-resistant, 9 strains) and *Streptococcus oralis* (linezolid-resistant, 1 strain).
- Definitions of resistance were those published by the National Committee for Clinical Laboratory Standards (NCCLS).

SUSCEPTIBILITY TESTING. All susceptibility tests were performed by NCCLS M7-A6 methods with 2–5% lysed horse blood supplement for the fastidious streptococci. Cation-adjusted Mueller-Hinton broth was used for all other tested species.

- The mechanisms of resistance for all linezolid-resistant strains (MIC ≥ 8 mg/L) was confirmed by gene sequencing of the ribosomal target and the detection of a G2576U mutation.
- All Q/D-resistant strains were phenotypically confirmed by disk diffusion and Etest (AB BIODISK, Solna, Sweden) to have a MIC at ≥ 4 mg/L.
- PCR tests for *vatD* and *vatE* were negative.

QUALITY CONTROL (QC) of the LBM415 MIC results was performed using acceptable MIC ranges reported by Anderegg *et al*. for QC strains *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619 and *E. faecalis* ATCC 29212.

- All QC results for LBM415 and comparison agents used to categorize resistant isolates (linezolid, Q/D, vancomycin) were within NCCLS published limits. Trays were manufactured by TREK Diagnostics (Cleveland, Ohio, USA) to specified NCCLS standards.
- The proposed or tentative susceptible breakpoint for LBM415 to be applied by clinical trial laboratories was ≤ 8 mg/L based on pharmacokinetic/pharmacodynamic characteristics of this compound and similar peptide deformylase inhibitors [Craig WA, Andes D. 41st ICAAC, 2001; Chicago, IL; #F-355].

RESULTS

- Among the 13 linezolid-resistant strains (Table 1), 3 were *E. faecalis* and 10 were *E. faecium*. Also 10 enterococci were resistant to vancomycin and all *E. faecalis* strains had the characteristically intrinsic Q/D resistance (MIC, 8 mg/L). These enterococci had LBM415 MIC results between 0.5 and 4 mg/L (MIC_{90}, 4 mg/L). The 6 Q/D-resistant *E. faecium* isolates were susceptible to vancomycin (MICs, 1– 4 mg/L) and also inhibited by 1 or 2 mg/L of LBM415.
- LBM415 was highly active against linezolid-resistant *S. aureus* (MICs, 0.25–0.5 mg/L), *S. epidermidis* (MIC, 2 mg/L) and the viridans group streptococcus isolate (MIC, 0.5 mg/L), ie, 7 strains overall (Table 2). Q/D and vancomycin were also active against these oxazolidinone-resistant organisms. The Q/D-resistant staphylococci (19 strains) were susceptible to LBM415 (MIC range, 0.12–2 mg/L), linezolid (MICs, 1 or 2 mg/L) and vancomycin (MICs, 1 or 2 mg/L).

CONCLUSIONS

- LBM415, among the new candidate peptide deformylase inhibitors, demonstrates excellent activity (all MICs at ≤ 4 mg/L) against emerging gram-positive clinical isolates that have become resistant to oxazolidinones (linezolid and AZD-2563) or streptogramin combinations.
- Since numerous members of this new peptide deformylase (PDF) inhibitor class may be advanced into clinical trials, close surveillance should be initiated for 'PDF inhibitor-resistant' isolates, as could be predicted from early molecular studies regarding this class.
- Continued development of agents in the peptide deformylase inhibitor class seems prudent and further synthetic modifications may further enhance potency and other microbiologic features, particularly against important gram-negative species.

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TABLE 1. Activity of LBM415 tested against 19 isolates of linezolid or quinupristin/ dalfopristin-resistant Enterococcus spp

Organism (no. tested)	MIC (mg/L) LBM415	Linezolid	Quinupristin/ Dalfopristin	Vancomycin
LINEZOLID-RESISTANT STRAINS (13)[a]				
E. faecalis				
15-5341	4	8	8	16
04-2V	4	16	8	>32
21-6943A	2	8	8	>32
E. faecium				
17-982A	1	16	0.5	0.5
24-1575A	1	16	1	1
15-4011A	0.5	8	1	16
11-4103A	2	8	0.5	>32
17-14203A	1	16	1	>32
84-7093A	2	16	1	>32
Chicago-1	1	8	0.25	>32
Chicago-2	4	8	1	>32
Chicago-4	4	8	1	>32
Chicago-5	1	32	0.5	0.5
STREPTOGRAMIN-RESISTANT STRAINS (6)				
E. faecium				
2-12989A	2	2	4	1
38-6823A	2	2	8	4
38-11656A	2	2	8	1
42-13111A	1	2	8	4
65-1208A	1	1	>8	2
103-13202A	1	1	4	2
MIC range (mg/L)	0.5-4	8-32[b]	4->8[b]	0.5->32
Median (mg/L)	1	8[b]	8[b]	16

a. All linezolid-resistant isolates had a documented G2576U mutation.
b. Resistant subsets only.

TABLE 2. Activity of LBM415 tested against 26 isolates of linezolid- or quinupristin/dalfopristin-resistant staphylococci or streptococci.

Organism (no. tested)	MIC (mg/L) LBM415	Linezolid	Quinupristin/ Dalfopristin	Vancomycin
LINEZOLID-RESISTANT STRAINS (7)[a]				
Staphylococcus aureus				
106-12591A	0.5	32	0.5	1
BZ-2	0.25	16	0.5	1
BZ-3	0.5	16	0.5	2
PF-3839	0.5	>32	0.25	2
PF-3840	0.25	>32	0.25	2
Staphylococcus epidermidis				
82-1645A	2	32	0.25	2
Streptococcus oralis				
27-2832A	0.5	32	0.5	0.5
STREPTOGRAMIN-RESISTANT STRAINS (19)				
S. aureus				
61-4725C	1	1	8	1
61-7949C	0.25	1	>8	2
61-10880A	1	1	8	1
90-2728C	0.5	1	8	1
90-11371A	0.5	2	4	1
91-2220C	1	1	>8	2
91-2811C	1	1	8	2
300-12053A	1	2	>8	1
301-4244A	1	1	8	2
301-10721A	0.5	2	4	1
Coagulase-negative staphylococci				
48-11901A	0.12	2	4	1
53-8021A	1	1	4	2
57-4260A	0.25	2	8	1
63-5921A	1	1	>8	1
78-5445A	2	1	4	2
78-7937A	2	1	8	2
78-13608A	2	1	>8	2
90-10840A	0.5	1	>8	1
300-15319A	2	1	4	2
MIC range (mg/L)	0.12-2	16->32[b]	4->8[b]	0.5-2
Median (mg/L)	0.5	32[b]	8[b]	1, 2

a. All linezolid-resistant isolates had a documented G2576U mutation.
b. Resistant subsets only.