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Antistaphylococcal Activity of LBM415, a New Peptide Deformylase Inhibitor, Compared with Those of Other Agents

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The MICs of LBM415, a new peptide diformylase inhibitor, were ≤0.06 to 4.0 μg/ml for 258 isolates of *Staphylococcus aureus* and coagulase-negative staphylococci. LBM415 MICs were similar irrespective of whether the strains were methicillin susceptible or resistant. All strains were also susceptible to vancomycin, linezolid, ranbezolid, daptomycin, oritavancin, and quinupristin-dalfopristin. LBM415 at the MIC was bacteriostatic after 24 h.

The emergence of staphylococci intermediate and resistant to methicillin, quinolones, and, recently, glycopeptides, as well as the propensity of these organisms to spread nosocomially and in the community and to cause serious systemic infections in immunocompromised hosts, necessitates other therapeutic modalities (5, 11, 18, 23, 24).

LBM415 (previously known as NVP-PDF713) is a new peptide deformylase inhibitor with excellent activities against gram-positive organisms (1, 2, 9, 13, 14). The present study examined (i) the activity of LBM415 compared with those of four fluoroquinolones (ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin), three glycopeptides (vancomycin, teicoplanin, and oritavancin), two oxazolidinones (linezolid and ranbezolid), a lipopeptide (daptomycin), and a combination of two streptogramins (quinupristin and dalfopristin) against 258 staphylococci by MIC testing and (ii) the activities of the compounds listed above against 12 staphylococci by time-kill assay. Because of problems with the preparation of frozen microdilution trays with tigecycline, a broad-spectrum tetracycline (G. A. Pankuch, personal communication), experienced in the past, this compound was tested only by the time-kill method.

For microdilution MIC determinations, the staphylococci tested comprised 62 methicillin-resistant *Staphylococcus aureus* isolates, 69 methicillin-susceptible *S. aureus* isolates, 60 methicillin-resistant coagulase-negative staphylococci, and 67 methicillin-susceptible coagulase-negative staphylococci. For the purposes of the present study, no species identification of the coagulase-negative staphylococci was attempted. We also included a recently recovered vancomycin-resistant *S. aureus* (VRSA) isolate (5) in these studies.

LBM415 powder was obtained from Novartis Research Laboratories, Hanover, N.J. Other antimicrobials were obtained from their respective manufacturers. The standardized microdilution method was performed (17) with commercially prepared microtiter trays (TREK, Inc., Cleveland, Ohio) containing cation-adjusted Mueller-Hinton broth, to which calcium was added for the testing of daptomycin (17). Vancomycin MICs were read after a full 24 h of incubation (17). A separate macrodilution MIC methodology (5) was used to test the activity of LBM415 against the VRSA strain.

For time-kill studies, tubes containing 5 ml of cation-adjusted Mueller-Hinton broth (BBL Microbiology Systems) with doubling antibiotic concentrations were inoculated with 5×10^5 to 5×10^6 CFU/ml, and the tubes were incubated at 35°C in a shaking water bath. The methods were based on those described previously (12, 19), with calcium added for the testing of daptomycin (12, 19).

Time-kill assays were performed by determining the number of strains which yielded changes in the $\log_{10}$ CFU per milliliter of −1, −2, and −3 at 0, 3, 6, 12, and 24 h compared with the counts at time zero. The antimicrobials were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥3 $\log_{10}$ CFU/ml (99.9%) at each of the time periods and bacteriostatic if the inoculum was reduced by 0 to <3 $\log_{10}$ CFU/ml. The problem of antibiotic carryover was addressed by dilution, as described previously (12, 19).

The MICs of the drugs tested for the staphylococci are listed in Tables 1 and 2. The LBM415 MICs at which 50% of isolates are inhibited (MIC_{50}s) and MIC_{90}s were 1.0 and 2.0 μg/ml, respectively, for *S. aureus* and 1.0 and 2.0 μg/ml, respectively, for coagulase-negative staphylococci. Linezolid MICs, especially those for the coagulase-negative strains, were often 1 or more dilutions higher than ranbezolid MICs. Vancomycin

TABLE 1. MICs for *S. aureus* strains

Drug	MIC (μg/ml)					
	Methicillin-resistant strains (*n* = 62)			Methicillin-susceptible strains (*n* = 69)		
	Range	50%	90%	Range	50%	90%
LBM415	0.25–4.0	2.0	4.0	0.125–4.0	1.0	2.0
Ciprofloxacin	0.25–>16.0	>16.0	>16.0	≤0.06–>16.0	0.25	>16.0
Levofloxacin	0.125–>16.0	8.0	>16.0	≤0.06–>16.0	0.125	8.0
Gatifloxacin	0.06–>8.0	4.0	8.0	≤0.016–8.0	0.06	4.0
Moxifloxacin	0.03–>8.0	2.0	4.0	≤0.016–4.0	0.03	2.0
Vancomycin	0.25–2.0	1.0	1.0	0.25–1.0	0.5	1.0
Teicoplanin	0.125–4.0	0.5	1.0	0.125–2.0	0.5	1.0
Oritavancin	0.25–4.0	2.0	2.0	0.25–4.0	2.0	2.0
Linezolid	1.0–4.0	2.0	4.0	1.0–4.0	2.0	4.0
Ranbezolid	0.5–4.0	2.0	4.0	≤0.06–2.0	1.0	2.0
Daptomycin	0.125–2.0	0.5	0.5	0.125–1.0	0.5	0.5
Quinupristin-dalfopristin	0.125–1.0	0.5	0.5	≤0.06–0.5	0.25	0.5

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TABLE 2. MICs for coagulase-negative staphylococci

Drug	MIC (μg/ml)					
	Methicillin-resistant strains (n = 60)			Methicillin-susceptible strains (n = 67)		
	Range	50%	90%	Range	50%	90%
LBM415	≤0.06–2.0	1.0	2.0	0.125–4.0	1.0	2.0
Ciprofloxacin	≤0.06–>16.0	8.0	>16.0	≤0.06–>16.0	0.125	4.0
Levofloxacin	≤0.06–>16.0	4.0	8.0	≤0.06–16.0	0.125	4.0
Gatifloxacin	0.03–4.0	1.0	2.0	0.03–4.0	0.06	1.0
Moxifloxacin	≤0.016–8.0	1.0	2.0	≤0.016–2.0	0.03	0.5
Vancomycin	0.5–2.0	2.0	2.0	0.25–2.0	1.0	2.0
Teicoplanin	0.5–>16.0	4.0	8.0	0.125–16.0	1.0	8.0
Oritavancin	0.5–4.0	2.0	2.0	0.25–4.0	2.0	2.0
Linezolid	1.0–2.0	1.0	2.0	1.0–4.0	1.0	2.0
Ranbezolid	≤0.06–4.0	0.25	1.0	≤0.06–1.0	0.125	0.5
Daptomycin	0.25–1.0	0.5	0.5	0.125–1.0	0.5	0.5
Quinupristin-dalfopristin	0.125–1.0	0.125	0.5	≤0.06–1.0	0.125	0.5

MICs were low for all strains, but teicoplanin was much less active against the coagulase-negative strains. Quinupristin-dalfopristin was equally active against all strains. Ranbezolid MICs were lower for the coagulase-negative strains than for the *S. aureus* strains, and teicoplanin was relatively inactive against coagulase-negative staphylococci; both of these findings are noteworthy. Oxazolidinone MICs were not influenced by the methicillin susceptibilities of the staphylococcal strains. Quinolone MICs were generally higher for the methicillin-resistant strains. The LBM415 MIC for the VRSA strain was 0.5 μg/ml.

The MICs for the strains tested by the time-kill assay are listed in Table 3, and the killing kinetics are presented in Table 4. The results of the time-kill assays showed that after 24 h LBM415 at the MIC was bacteriostatic against all strains tested. Similar bacteriostatic activities were observed for linezolid, ranbezolid, tigecycline, and quinupristin-dalfopristin. After 24 h, the quinolones at two times the MIC were bactericidal against four to eight strains. After 24 h at two times the MICs, vancomycin was bactericidal against nine strains and teicoplanin was bactericidal against six strains. By contrast, daptomycin and oritavancin showed rapid bactericidal activity, with 99.9% killing of 11 to 12 strains at two times the MIC after 24 h and significant activity as early as 3 h.

LBM415 is a new peptide deformylase inhibitor (1, 2, 9, 13, 14) with excellent activities against clinically significant gram-positive strains, including multiresistant organisms, such as linezolid-resistant staphylococci (MIC range, 0.25 to 2 μg/ml), *Enterococcus faecalis* (MIC range, 2 to 4 μg/ml) and *Enterococcus faecium*, including quinupristin-dalfopristin-resistant strains (MIC range, 0.5 to 4 μg/ml) (14). In another recent study of 1,837 recently isolated gram-positive strains, LBM415 MICs were ≤4 μg/ml for all strains except six enterococci (13). LBM415 MICs ranged from 0.5 to 4.0 μg/ml (MIC_{50}, 0.5 μg/ml; MIC_{90}, 1.0 μg/ml) and 1 and 4 μg/ml (MIC_{50}, 1 μg/ml; MIC_{90}, 2 μg/ml) for 875 *S. aureus* isolates and 381 coagulase-negative staphylococci, respectively (13). The results of the present study confirm the low MICs of LBM415 for staphylococci obtained by Jones and coworkers (13, 14). Jones and Rhomberg (15) have also recently reported MIC results for NVP-PDF386 (VCR4887), a related peptide deformylase inhibitor. The range of MICs of NVP-PDF386 for 104 *S. aureus* strains was 0.06 to 2 μg/ml, with an MIC_{50} of 0.5 μg/ml and an MIC_{90} of 1.0 μg/ml. NVP-PDF386 inhibited all 49 coagulase-

TABLE 3. MICs for strains tested by time-kill assays

Drug	MIC (μg/ml)[a]											
	SA8	SA9	SA12	SA13	SA16	SA19	CN3	CN6	CN20	CN5	CN11	CN18
LBM415	1	0.25	0.25	1	1	0.5	2	2	0.5	2	2	1
Ciprofloxacin	0.5	1	0.5	>32	>32	32	0.25	0.25	0.25	0.25	>32	16
Levofloxacin	0.25	0.25	0.25	32	32	16	0.25	0.25	0.25	0.25	32	8
Gatifloxacin	0.125	0.125	0.125	4	16	4	0.125	0.125	0.125	0.125	4	2
Moxifloxacin	0.06	0.03	0.06	4	8	2	0.125	0.06	0.06	0.06	8	2
Vancomycin	1	1	1	1	2	1	2	2	2	4	2	4
Teicoplanin	0.5	0.5	0.5	0.5	1	1	4	8	4	4	1	16
Oritavancin	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	1	0.5	0.5
Linezolid	2	2	4	2	4	2	2	2	2	2	4	1
Ranbezolid	2	2	4	2	4	4	0.125	0.125	0.25	0.125	2	0.125
Daptomycin	0.5	0.5	1	2	1	1	0.5	0.25	0.125	0.25	0.5	0.5
Tigecycline	0.5	0.5	0.5	0.5	1	0.5	0.25	0.25	1	1	0.5	0.5
Quinupristin-dalfopristin	0.25	0.5	0.5	0.5	0.5	1	0.125	0.125	0.125	0.25	1	0.25

[a] Strains SA8, SA9, and SA12, methicillin-susceptible *S. aureus*; strains SA13, SA16, and SA19, methicillin-resistant *S. aureus*; strains CN3, CN6, and CN20 methicillin-susceptible coagulase-negative staphylococci; strains CN5, CN11, and CN18 methicillin-resistant coagulase-negative staphylococci.

TABLE 4. Time-kill analyses

Drug and multiple of MIC	No. of strains with the indicated level of killing[a] at the following times:											
	3 h			6 h			12 h			24 h		
	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
LBM415												
4	0	0	0	1	1	0	2	1	0	1	1	0
2	0	0	0	1	1	0	2	1	0	0	0	0
1	0	0	0	1	1	0	1	1	0	0	0	0
Ciprofloxacin[b]												
4	5	2	0	7	4	3	7	5	4	7	7	5
2	5	2	0	7	3	2	7	5	3	7	7	4
1	3	1	0	6	2	2	6	2	2	6	2	2
Levofloxacin[c]												
4	7	2	1	8	6	1	9	9	5	9	9	7
2	5	2	0	8	5	1	9	8	5	9	8	7
1	4	1	0	8	3	1	9	4	1	9	7	3
Gatifloxacin												
4	11	4	1	12	10	5	12	12	8	12	12	11
2	10	2	0	12	9	4	12	12	7	12	12	8
1	6	2	0	10	5	3	11	9	2	10	7	3
Moxifloxacin												
4	12	7	1	12	9	5	12	11	9	12	12	10
2	10	5	0	11	9	3	12	9	7	12	9	7
1	8	2	0	9	4	1	10	7	2	8	3	2
Vancomycin												
4	4	0	0	10	3	1	12	10	4	12	10	9
2	3	0	0	10	2	0	12	9	3	12	10	9
1	3	0	0	9	1	0	11	4	3	8	5	3
Teicoplanin												
4	2	0	0	10	0	0	11	8	1	12	11	7
2	2	0	0	8	0	0	10	6	0	12	11	6
1	0	0	0	4	0	0	10	4	0	9	6	3
Oritavancin												
4	12	12	9	12	12	12	12	12	12	12	12	12
2	12	12	7	12	12	12	12	11	10	12	12	12
1	12	10	2	12	12	8	11	9	9	8	6	6
Linezolid												
4	0	0	0	0	0	0	0	0	0	4	0	0
2	0	0	0	0	0	0	0	0	0	1	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
Ranbezolid												
4	1	1	0	2	1	0	5	0	0	11	2	0
2	0	0	0	1	0	0	3	0	0	10	1	0
1	0	0	0	1	0	0	1	0	0	2	0	0
Daptomycin												
4	12	9	7	12	12	10	12	12	12	12	12	12
2	11	9	5	12	11	9	12	12	10	12	12	11
1	9	6	0	12	9	6	12	11	5	11	10	8
Tigecycline												
4	0	0	0	0	0	0	3	1	1	6	0	0
2	0	0	0	0	0	0	2	1	0	6	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
Quinupristin-dalfopristin												
4	2	0	0	6	0	0	7	2	0	9	4	2
2	0	0	0	5	0	0	7	2	0	8	3	1
1	0	0	0	4	0	0	5	1	0	5	1	0

[a] −1, 90% killing; −2, 99% killing; −3, 99.9% killing.
[b] Five strains were not tested with ciprofloxacin due to resistance (Table 3).
[c] Three strains were not tested with levofloxacin due to resistance (Table 3).

negative staphylococcal strains tested at an MIC of 2 μg/ml, with an MIC range of ≤0.25 to 2 μg/ml. NVP-PDF713 was bacteriostatic against all strains tested. The latter property leads to trailing end points, which makes agar dilution testing of the MICs of this compound for staphylococci unreliable (P. C. Appelbaum, unpublished data).

The MIC and time-kill assay results for the other compounds whose activities were tested against staphylococci were similar to those described previously (3, 4, 6–8, 10, 11, 16, 20–22), with oritavancin and daptomycin having the most rapid bactericidal activities, followed by vancomycin and teicoplanin; tigecycline, linezolid, ranbezolid, and quinupristin-dalfopristin were mainly bacteriostatic. The bacteriostatic activity of quinupristin-dalfopristin was due to the constitutive expression of *erm*(B) genes in these strains (data not shown).

The results of this study indicate a potential role for LBM415 for the treatment of staphylococcal infections. However, interpretation of these in vitro results must be complemented by toxicity and pharmacokinetic-pharmacodynamic studies before the drug can be recommended for clinical testing.

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