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Antimicrobial Activity and Spectrum of LY146032, a Lipopeptide Antibiotic, Including Susceptibility Testing Recommendations

RONALD N. JONES* AND ARTHUR L. BARRY
Clinical Microbiology Institute, Tualatin, Oregon 97062

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LY146032, a new lipopeptide, was found to have a spectrum of gram-positive antimicrobial activity that includes activity against staphylococci (methicillin susceptible and resistant), beta-hemolytic *Streptococcus* spp., pneumococci, viridans group *Streptococcus* spp., anaerobic gram-positive cocci, *Clostridium* spp., and enterococci. The new lipopeptide was generally bactericidal and showed more rapid killing of *Listeria* spp. (MIC, 1 to 2 μg/ml) and staphylococci than either vancomycin or teicoplanin. The 30-μg disk was preferred to the 15-μg disk on the basis of the preliminary interpretive criteria for susceptibility which indicated zone diameters of ≥16 mm for susceptible strains (MIC, ≤2.0 μg/ml) and ≤12 mm for resistant strains (MIC, ≥8.0 μg/ml). These criteria are valid pending the testing of additional gram-positive strains which have LY146032 MICs of ≥8 μg/ml.

LY146032 is a novel member of a new cyclic lipopeptide antibiotic group that is produced from *Streptomyces roseosporus*. Drugs in this group contain a lipid moiety which is unlike those of glycopeptide antimicrobial agents. Like vancomycin and teicoplanin, LY146032 possesses an active spectrum against gram-positive organisms, especially *Staphylococcus* spp., enterococci, *Streptococcus pyogenes*, *Streptococcus agalactiae*, viridans group streptococci, pneumococci, and serogroup G *Streptococcus* spp. (1–4, 6, 10). *Listeria monocytogenes* strains have moderate susceptibility to LY146032, and gram-negative organisms appear resistant (2). The activity of LY146032 requires calcium concentrations in the test medium for maximal bactericidal action on peptidoglycan synthesis (2). Synergistic antimicrobial activity has also been reported between LY146032 and aminoglycosides by using time-kill-curve methods (2).

In this in vitro evaluation, we present the results of studies as follows: (i) comparisons of the activity of LY146032 with that of vancomycin, teicoplanin, coumermycin, ampicillin, and imipenem; (ii) effect of divalent cation content of the test medium (9) on LY146032 antimicrobial potency; (iii) effects of varying inoculum concentrations on LY146032 MICs and MBCs; (iv) comparisons of the time-kill rates of LY146032 and vancomycin, both alone and in the presence of gentamicin; and (v) disk diffusion test interpretive criteria.

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LY146032 and vancomycin were supplied by Eli Lilly & Co., Indianapolis, Ind. Other comparative drugs were obtained from their respective manufacturers. LY146032 disks were prepared by Eli Lilly & Co. at 15- and 30-μg concentrations. Control vancomycin (30-μg) disks were purchased from Difco Laboratories, Detroit, Mich.

Recent isolates, typical of current clinical strains, were collected from five geographically diverse microbiology laboratories. The 334 isolates, supplemented with strains having methicillin resistance as determined by standardized methods (8, 9), included *Staphylococcus aureus* isolates (50 methicillin resistant), 51 coagulase-negative *Staphylococcus* isolates (25 methicillin resistant), 104 *Streptococcus* isolates from 12 species or serogroups, 2 *Enterococcus avium* isolates, 5 *Enterococcus durans* isolates, 16 *Enterococcus faecalis* isolates, 6 *Enterococcus faecium* isolates, 10 *L. monocytogenes* isolates, 14 isolates from members of the family *Enterobacteriaceae* (12 species), and 6 other gram-negative bacilli from 4 species (Table 1). In addition, 20 strict anaerobes were tested. These organisms included *Bacteroides fragilis* (eight strains), *Clostridium perfringens* (four strains), *Clostridium sporogenes* (two strains), *Peptostreptococcus anaerobius* (three strains), and *Peptococcus asaccharolyticus* (three strains).

Broth microdilution test panels (Prepared Media Laboratory, Tualatin, Oreg.) were prepared, and tests were performed by the method of the National Committee for Clinical Laboratory Standards (NCCLS) (9). Anaerobic organisms were tested in Wilkins-Chalgren broth (WCB) by the microdilution method described by Jones et al. (5). Agar diffusion disk tests were also performed by NCCLS procedures (8). Interpretive zone standards were selected by correlating zone diameters with MICs by using both regression analysis (method of least-squares) and the error-rate-bounding method of Metzler and DeHaan (7). LY146032 MICs were determined in Mueller-Hinton broth (MHB) supplemented with calcium and magnesium (9). A lot of WCB without agar was also used to determine LY146032 MICs for both the anaerobic bacteria and the facultative strains (5).

The MBC was defined as the lowest concentration producing a ≥99.9% reduction in the CFU from the initial 5×10^5 CFU/ml inoculum concentration at 24 h. The CFU per milliliter determinations were done by the method of Pearson et al. (11), by replicate subculturing of 10-μl inoculum volumes to drug-free agar plates. MBCs were determined for 25 strains at 24 h only, and time-kill curves were done for 15 isolates at 0, 4, and 24 h of incubation. The effects on LY146032, vancomycin, and teicoplanin MICs of altering the inoculum concentrations were determined by using inoculum sizes of approximately 10^4, 5×10^5, and 10^7 CFU/ml (see Table 3).

Comparative antimicrobial activity. The in vitro broth microdilution test results with gram-positive bacteria are

* Corresponding author.

TABLE 1. Comparative in vitro antimicrobial activity of LY146032 and five other drugs

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)			% Susceptible[a]
		Range	Geometric mean	90%	
Staphylococcus spp.	LY146032	0.06–0.25	0.16	0.25	100
Methicillin susceptible (76)[b]	Coumermycin	≤0.015–0.12	≤0.015	≤0.015	100
	Imipenem	≤0.008–0.12	0.02	0.03	100
	Teicoplanin	0.06–4.0	0.44	0.5	100
	Vancomycin	0.5–2.0	0.96	1.0	100
Methicillin resistant (75)[c]	LY146032	0.06–0.5	0.23	0.5	100
	Coumermycin	≤0.015–0.12	≤0.015	≤0.015	100
	Imipenem	0.06–>8.0	3.8	>8.0	80
	Teicoplanin	0.06–16	1.5	4.0	93
	Vancomycin	0.5–4.0	1.2	2.0	100
Streptococcus spp. (104)[d]	LY146032	≤0.015–1.0	0.16	0.25	100
	Ampicillin	0.12–>4	0.30	0.25	97
	Coumermycin	≤0.015–8.0	0.67	1.0	62
	Imipenem	≤0.008–0.5	0.03	0.03	100
	Teicoplanin	≤0.015–0.5	0.11	0.25	100
	Vancomycin	≤0.015–1.0	0.38	0.5	100
Enterococci (29)[e]	LY146032	0.06–2.0	0.71	1.0	100
	Ampicillin	0.12–>4.0	1.6	4.0	93
	Coumermycin	0.25–32	2.9	8.0	34
	Imipenem	0.06–>8.0	2.3	4.0	93
	Teicoplanin	0.06–0.5	0.15	0.5	100
	Vancomycin	0.25–2.0	1.3	2.0	100
Listeria monocytogenes (10)	LY146032	1.0–2.0	1.6	2.0	100
	Ampicillin	0.25–1.0	0.63	1.0	100
	Coumermycin	≤0.015–0.06	0.03	0.03	100
	Imipenem	0.06–0.12	0.11	0.12	100
	Teicoplanin	0.12–0.25	0.16	0.25	100
	Vancomycin	1.0	1.0	1.0	100

[a] Interpretive criteria for susceptibility with available drugs from the NCCLS (8,9). Susceptibility for the other compounds was defined as follows: LY146032, ≤2.0 μg/ml; coumermycin, ≤0.5 μg/ml; and teicoplanin, ≤4.0 μg/ml.
[b] Includes 50 strains of *S. aureus* (22 penicillinase producers) and 26 coagulase-negative *Staphylococcus* spp. (12 penicillinase producers).
[c] Includes 50 strains of *S. aureus* and 25 isolates of coagluase-negative staphylococci.
[d] Includes *S. agalactiae* (21 strains), *S. anginosus* (3 strains), *S. mitis* (1 strain), *S. mutans* (3 strains), *S. pneumoniae* (20 strains), *S. pyogenes* (22 strains), *Streptococcus* spp. serogroup C (12 strains) and serogroup G (10 strains), *S. sanguis* I and II (7 strains), *S. salivarius* (4 strains), and *S. uberis* (1 strain).
[e] Includes *Enterococcus avium* (2 strains), *E. durans* (5 strains), *E. faecalis* (16 strains), and *E. faecium* (6 strains).

shown in Table 1. An additional 20 facultative gram-negative bacilli were tested, and the LY146032, vancomycin, and teicoplanin MICs were all >32 μg/ml, i.e., the bacilli were categorically resistant (data not shown). With the 75 methicillin-resistant strains, LY146032 had only a slightly higher MIC for 90% of the strains tested (MIC_{90}), MIC range, and geometric mean MIC. All staphylococcal isolates were susceptible to LY146032, vancomycin, and coumermycin. Imipenem MICs for the methicillin-resistant strains were 190-fold higher (mean MIC, 3.8 μg/ml) than those for the methicillin-susceptible isolates. Teicoplanin inhibited 93.1% of methicillin-resistant *Staphylococcus* strains at ≤4.0 μg/ml; the MICs for all of the remaining isolates were 8.0 or 16 μg/ml. The highest LY146032 MICs were for the *E. faecium* (MIC range, 0.5 to 2.0 μg/ml) and *E. faecalis* (MIC range, 0.5 to 1.0 μg/ml) isolates. *L. monocytogenes* was inhibited by very low concentrations (MIC_{90}, 0.03 μg/ml) of coumermycin. LY146032 was also tested against 20 strict anaerobes (data not shown), with the following results for MIC for 50% of strains tested (MIC_{50}): *B. fragilis*, >32 μg/ml; *C. perfringens*, 4.0 μg/ml; *C. sporogenes*, 8.0 μg/ml; and anaerobic gram-positive cocci, 0.12 μg/ml.

Effect of medium on activity. LY146032 MICs were determined in three broth media (Table 2). The LY146032 MICs with cation-supplemented Mueller-Hinton broth (CSMHB) (9) were 16- to 64-fold lower than those produced in unsupplemented MHB or in the cation-deficient anaerobe testing broth, WCB. Comparisons of LY146032 MICs in different media by regression statistics produced results as follows: CSMHB versus MHB, $r = 0.81$ and $y = -0.06 + 0.60x$; CSMHB versus WCB, $r = 0.77$ and $y = 0.46 + 0.57x$; and MHB versus WCB, $r = 0.92$ and $y = 1.44 + 0.89x$. These

TABLE 2. Effect of medium on MIC_{50} of LY146032

Organism (no. of isolates)	LY146032 MIC_{50} (μg/ml) in the following medium[a]:		
	CSMHB	MHB	WCB
Clostridium spp. (6)	0.25	8.0	4.0
Enterococci (29)	0.5	32	16
Listeria monocytogenes (10)	2.0	>32	>32
Staphylococcus spp.			
Methicillin susceptible (76)	0.12	4.0	4.0
Methicillin resistant (75)	0.25	4.0	4.0
Streptococcus spp. (104)	0.12	2.0	2.0

[a] CSMHB, Cation-supplemented MHB (8); MHB, unsupplemented MHB; WCB, WCB without significant divalent cation supplement (5).

TABLE 3. Effect of inoculum concentration on in vitro activities, including bactericidal action, of LY146032, vancomycin, and teicoplanin

Organism (no of isolates)	Test and inoculum concn (CFU/ml) for:		LY146032		Vancomycin		Teicoplanin	
	MIC	MBC	MIC[a]	MBC[b]	MIC	MBC	MIC	MBC
Streptococcus spp. (5)[c]	1×10^7		0.12		0.50		0.12	
	5×10^5		0.07		0.44		0.09	
	1×10^4		0.05		0.44		0.05	
		5×10^5		0.25		>32		9.2
Enterococcus spp. (5)[d]	1×10^7		3.48		2.3		0.66	
	5×10^5		0.57		2.0		0.25	
	1×10^4		0.57		1.3		0.16	
		5×10^5		6.96		>32		>32
Listeria monocytogenes (5)	1×10^7		1.20		1.0		0.29	
	5×10^5		1.15		1.0		0.22	
	1×10^4		0.66		0.87		0.19	
		5×10^5		3.03		16		>32
Staphylococcus spp.								
Methicillin susceptible (5)[e]	1×10^7		2.64		3.48		0.87	
	5×10^5		0.44		1.32		0.14	
	1×10^4		0.14		1.0		0.11	
		5×10^5		1.0		32		24.2
Methicillin resistant (5)[f]	1×10^7		2.64		3.03		2.30	
	5×10^5		0.44		1.52		0.25	
	1×10^4		0.12		1.0		0.16	
		5×10^5		0.76		>32		24.2

[a] Determined with CSMHB (9). MICs, expressed in micrograms per milliliter, are geometric means.
[b] MBCs, expressed in micrograms per milliliter, are geometric means.
[c] Includes two *S. agalactiae*, one *S. pyogenes*, and two viridans group strains.
[d] Includes three *E. faecalis* and two *E. faecium* isolates.
[e] Includes three *S. aureus* and 2 coaglulase-negative isolates.
[f] Includes three *S. aureus* and two coagulase-negative isolates.

data imply an LY146032 MIC which is an average of 64-fold lower with CSMHB.

Inoculum studies and MBCs. By geometric mean comparisons, LY146032 was bactericidal for all genera except the enterococci (Table 3). The LY146032 MBCs were at least 10-fold greater than the mean MICs. Increasing inoculum concentrations generally had little effect on the MICs of the drug when inocula of 10^4 and 5×10^5 CFU/ml were compared. However, the LY146032 MICs increased greater than fivefold with the 10^7 CFU/ml inoculum concentration for the enterococci and *Staphylococcus* strains.

Time-kill studies. LY146032 was rapidly bactericidal (inoculum reduction of ≥ 2 $\log_{10}$ CFU at 4 h) for 13 of 15 strains and bactericidal (reduction of ≥ 3 $\log_{10}$ at 24 h) against all tested isolates (Table 4). This killing activity was generally enhanced by the addition of gentamicin at therapeutic levels (4 μg/ml). In contrast, vancomycin alone at therapeutic concentrations (8.0 μg/ml) demonstrated slower bactericidal action.

Disk diffusion tests. Scattergrams for the 15- and 30-μg-disk zone diameters and LY146032 MICs determined in CSMHB are shown in Fig. 1. All LY146032 MICs for gram-positive organisms were ≤2.0 μg/ml, and the zones were ≥13 or ≥16 mm for the 15- and 30-μg disks, respectively. For all gram-negative strains tested with LY146032, MICs were >32 μg/ml, and no zones were produced around the disks. Regression statistics were poor because of the widely separated populations of bacteria which are considered to be either within (susceptible) or outside (resistant) the spectrum of LY146032. Similarly, error-rate bounding could assure only low, minor, and false-resistant errors without absolutely minimizing the false-susceptible result, e.g., rare resistant gram-positive isolates. Organisms for which MICs are in the indeterminate or resistant ranges have not been widely identified by the CSMHB microdilution method. Absolute spectrum agreement (all 313 strains) was observed between LY146032 and vancomycin for the 293 gram-positive organisms and for the 20 selected gram-negative strains. A 98.4% interpretive agreement was seen for LY146032 and teicoplanin with interpretive variations from coagulase-negative, methicillin-resistant staphylococci categorized as susceptible to LY146032 and resistant or indeterminate to teicoplanin.

The NCCLS susceptibility tests confirmed that the LY146032 spectrum includes nearly all clinically important gram-positive organisms (2–4, 6). The organisms for which the LY146032 MICs were closest to the proposed susceptibility breakpoint of ≤2.0 μg/ml were *E. faecium* (MIC_{90}, 2.0 μg/ml), *E. faecalis* (MIC_{90}, 1.0 μg/ml), and *L. monocytogenes* (MIC_{90}, 2.0 μg/ml). The results for antimicrobial activity and spectrum were most similar to results previously found for glycopeptides, vancomycin, and teicoplanin (1–4, 6, 10). In this study, the clinically popular antistaphylococcal agent vancomycin was observed to have slower bactericidal activity against the staphylococci, regardless of methicillin susceptibility. LY146032 activity was greatly reduced (16- to 64-fold) by the absence of physiologic concentrations of divalent cations in the test media (2). Since the NCCLS procedures recommend the presence of these concentrations, we believe that LY146032 activity will be accurately assessed in most clinical laboratories (9). When LY146032 is tested against anaerobes in a WCB medium, supplemental

TABLE 4. Bactericidal activity with therapeutic concentrations of LY146032 and vancomycin (8.0 μg/ml) alone and in combination with gentamicin (4.0 μg/ml) tested in CSMHB

Species and initial inoculum (CFU/ml)	Log_{10} decrease or increase (+) in viable cells (CFU/ml) after 4 and 24 h of incubation at 35°C with:									
	LY146032		LY146032 + gentamicin		Vancomycin		Vancomycin + gentamicin		Gentamicin	
	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h	4 h	24 h
Enterococcus faecalis										
8×10^6	5.2	5.9	5.9	>5.9[a]	0.3	0.9	4.8	5.3	+0.4	+1.4
8×10^6	5.3	5.9	5.6	5.9	0.2	0.4	3.4	3.5	+1.5	+1.9
1×10^7	4.5	5.8	5.9	>6.1	0.5	0.8	4.1	>7.1	+1.5	+1.4
Enterococcus faecium										
9×10^6	3.5	5.3	4.9	>5.9	0.3	1.4	1.4	2.9	+0.8	+1.2
9×10^6	3.7	1.4	4.4	>5.9	0.2	0.4	2.6	2.3	+2.0	+1.1
Streptococcus pyogenes (3×10^6)	4.7	>5.4	2.6	>5.4	0.5	3.2	2.0	>5.4	1.4	>5.4
Streptococcus agalactiae (5×10^6)	5.1	>5.7	5.2	>5.7	0.6	1.9	2.8	>5.7	+0.6	+1.7
Viridans group streptococci (1×10^6)	2.0	>5.1	2.7	>5.1	0.1	1.3	1.7	>5.7	1.3	3.3
Listeria monocytogenes										
1×10^7	2.2	5.3	4.3	>6.1	0.1	0.8	2.8	6.1	2.7	4.8
2×10^7	1.6	4.8	3.7	>6.3	0.1	0.7	3.0	6.3	2.7	4.4
Staphylococcus aureus (methicillin susceptible)										
5×10^6	1.5	5.0	2.0	5.2	0.3	2.7	2.2	5.7	1.9	3.6
8×10^6	4.8	>5.9	>5.9	>5.9	0.4	3.0	5.1	>5.9	5.3	>5.9
2×10^7	4.6	6.2	5.7	>6.2	0.7	3.0	4.6	6.2	3.9	4.2
Staphylococcus aureus (methicillin resistant)										
1×10^7	>6.0	>6.0	>6.0	>6.0	1.2	3.6	5.5	>6.0	5.1	>6.0
2×10^7	4.2	>6.2	4.3	>6.2	0.4	2.7	0.5	3.0	+0.7	+1.1

[a] Less than 10 CFU/ml (no growth when 100 μl of undiluted culture was plated).

calcium should be added to produce accurate MICs for the gram-positive anaerobic species.

Selection of in vitro susceptibility test breakpoints for the glycopeptide drugs has been difficult because of the rarity of truly resistant gram-positive strains (1). Therefore, we suggest LY146032 MIC and zone diameter breakpoints for all gram-positive species that should be considered susceptible and which also represent a conservative application of early human pharmacokinetic results (H. R. Black, G. L. Brier, J. D. Wolny, and E. H. Nyhart, Jr., ICAAC, abstr. no. 894, 1986). The preliminary criteria for LY146032 tested by NCCLS standardized methods favor the use of a 30-μg disk (the same as for vancomycin and teicoplanin). The criteria indicate zone diameters of ≥16 mm (MIC correlate, ≤2.0 μg/ml) for susceptible isolates and ≤12 mm (MIC correlate, ≥8.0 μg/ml) for resistant isolates (8, 9). As gram-positive strains with resistant MICs emerge, these tentative LY146032 interpretive criteria may require revision.

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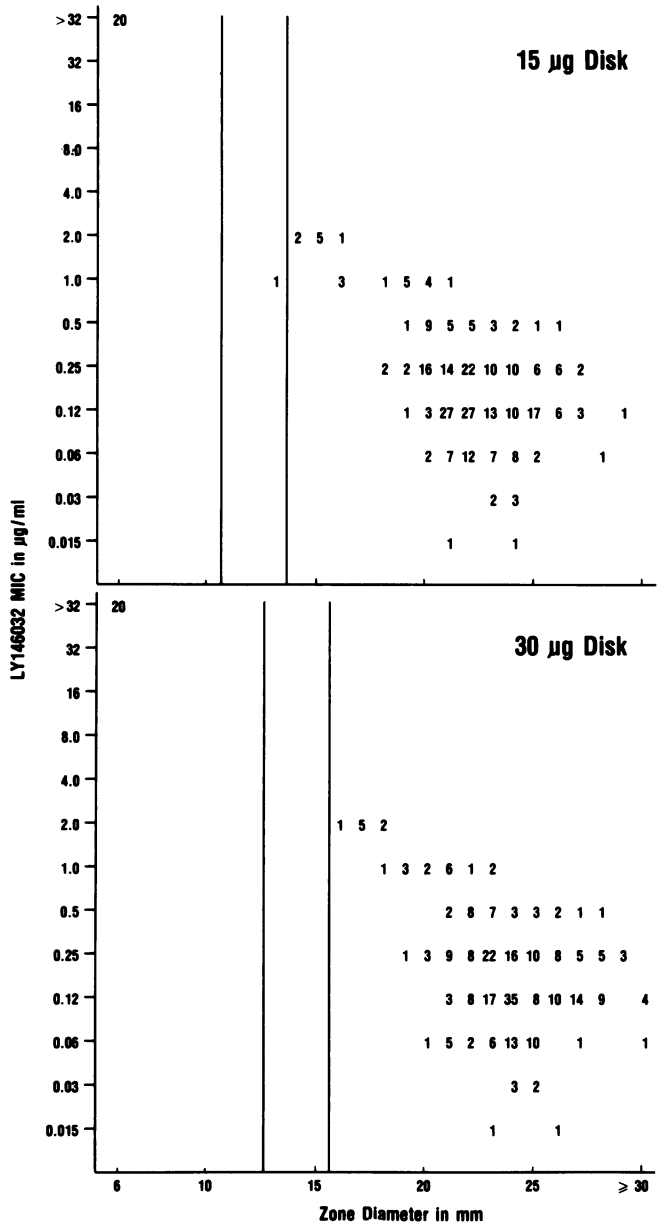

FIG. 1. Scattergram showing correlation between LY146032 MICs and zone diameters around 15- and 30-µg disks. The numbers of datum points (organisms) at each location for the 292 facultative strains tested are shown. Vertical lines are proposed zone diameter interpretive guidelines. The 20 strains for which the MICs were >32 µg/ml were LY146032-resistant, gram-negative bacilli.

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