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In Vitro Activity of LY146032, a New Lipopeptide Antibiotic, against Gram-Positive Cocci

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The activity of LY146032, a new lipopeptide antibiotic, was compared in vitro with those of vancomycin, oxacillin, and ampicillin against 261 staphylococcal and 154 streptococcal isolates. The MICs of LY146032 and vancomycin were similar, but the bactericidal activity and killing kinetics of LY146032 were more pronounced.

LY146032 is a new lipopeptide antibiotic with a spectrum of activity that is limited to gram-positive organisms. It is the semisynthetic *N*-decanoyl derivative of A21978C_1 a cyclic polypeptide antibiotic containing a fatty acid side chain.

The original substance A21978C_1, from which LY146032 was derived, has been shown to inhibit the peptidoglycan synthesis in *Staphylococcus aureus* and *Streptococcus faecalis*; and the activity of the drug apparently depends on the calcium content of the medium (1).

It has also been reported that LY146032 blocks the incorporation of radiolabeled amino acids into peptidoglycan of gram-positive organisms (N. Allen, W. Alborn, Jr., J. Hobbs, Jr., and H. Percifield, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother. abstr. no. 1081, 1984).

In this study I evaluate the in vitro activity of LY146032 against staphylococci, including oxacillin-resistant isolates, and against a variety of streptococci, in comparison with vancomycin, oxacillin, and ampicillin. The antibiotics were gifts from the manufacturers. LY146032 and vancomycin were obtained from Eli Lilly & Co., Indianapolis, Ind.; oxacillin and ampicillin were obtained from Beecham Laboratories, Betchworth, England.

The in vitro activity of LY146032 was tested by the agar dilution method in Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) in comparison with vancomycin and oxacillin against staphylococci and with ampicillin against streptococci. Mueller-Hinton agar was supplemented with 5% lysed horse blood to support the growth of streptococci. The antibiotic dilutions (final concentrations, between 32 and 0.03 mg/liter) were prepared fresh daily.

The strains were recent isolates from clinical specimens: *Staphylococcus aureus* (186 isolates), *Staphylococcus epidermidis* (75 isolates), *Streptococcus faecalis* (40 isolates), *Streptococcus pneumoniae* (20 isolates), *Streptococcus pyogenes* (20 isolates), *Streptococcus agalactiae* (20 isolates), and viridans group streptococci (*S. sanguis*, 17 isolates; *S. milleri*, 12 isolates; *S. mitis*, 10 isolates; *S. salivarius*, 10 isolates; *S. mutans*, 5 isolates). The strains were cultured overnight in Trypticase soy broth (BBL) and properly diluted in fresh broth to give a final inoculum of between 10^4 and 10^5 CFU per spot. Spots were applied with a multipoint inoculator. The MIC was determined as the lowest concentration which did not allow visible growth after incubation for 24 h at 36°C for streptococci and at 30°C for staphylococci.

Table 1 shows the comparative in vitro activity (range of MICs, MICs for 50% of strains tested [MIC_{50}], MICs for 90% of strains tested [MIC_{90}], geometric mean MIC) of LY146032 and other antibiotics. The activity of LY146032 against *Staphylococcus aureus* and *Staphylococcus epidermidis* was similar, regardless of their susceptibility or resistance to penicillin and oxacillin. LY146032 was comparable in activity to vancomycin against *Staphylococcus aureus* but was twice as active as vancomycin against *Staphylococcus epidermidis*. LY146032 was also similar in activity to vancomycin against *Streptococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and viridans group streptococci

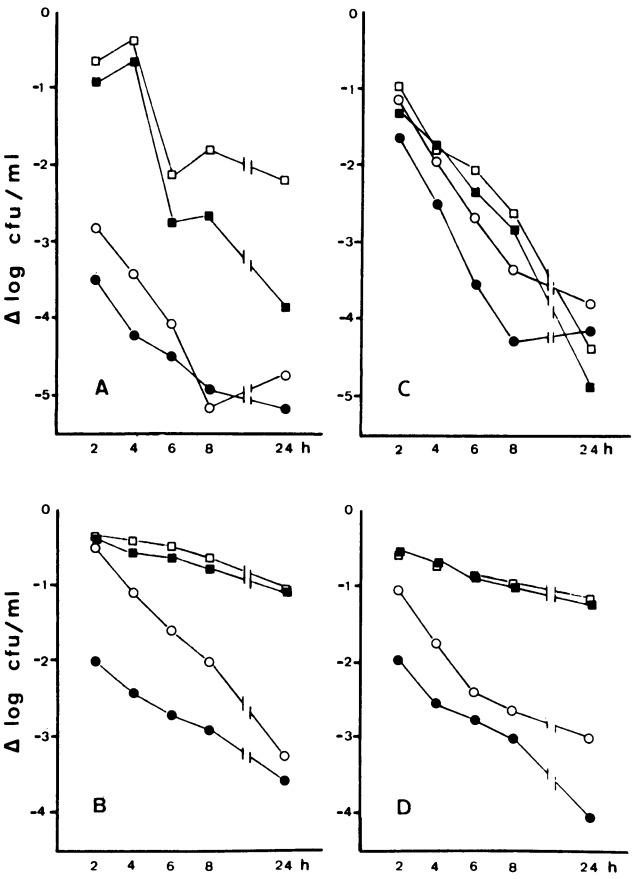

FIG. 1. Reduction of viable counts after increasing contact time with concentrations corresponding to 4× MICs or 16× MICs of LY146032 (○ or ●, respectively) and of vancomycin (□ or ■, respectively). Penicillin-susceptible *Staphylococcus aureus* (A), oxacillin-resistant *Staphylococcus aureus* (B), *Staphylococcus epidermidis* (C), and *Streptococcus faecalis* (D) were tested.

TABLE 1. In vitro activity of LY-146032 against staphylococci and streptococci

Organism and antimicrobial agent (no. of isolates)	MIC (mg/liter)			
	Range	50%	90%	Mean
Staphylococcus aureus, penicillin susceptible (25)				
LY-146032	0.25–0.5	0.5	0.5	0.44
Vancomycin	0.5–1	0.5	1	0.64
Oxacillin	0.12–0.5	0.25	0.25	0.21
Staphylococcus aureus, penicillin resistant (50)				
LY-146032	0.25–0.5	0.5	0.5	0.36
Vancomycin	0.5–1	0.5	1	0.57
Oxacillin	0.06–1	0.25	0.5	0.26
Staphylococcus aureus, oxacillin resistant (111)				
LY-146032	0.12–1	0.5	0.5	0.43
Vancomycin	0.25–2	0.5	1	0.56
Oxacillin	4–>32	>32		>32
Staphylococcus epidermidis, oxacillin susceptible (50)				
LY-146032	0.12–0.5	0.25	0.5	0.28
Vancomycin	0.12–1	0.5	1	0.61
Oxacillin	0.06–1	0.12	1	0.20
Staphylococcus epidermidis, oxacillin resistant (25)				
LY-146032	0.12–0.5	0.25	0.5	0.31
Vancomycin	0.25–1	1	1	0.82
Oxacillin	2–>32	>32		25.63
Streptococcus pneumoniae (20)				
LY-146032	0.12–0.5	0.12	0.12	0.13
Vancomycin	0.25–0.5	0.25	0.25	0.26
Ampicillin	0.03–0.25	0.06	0.06	0.04
Streptococcus pyogenes (20)				
LY-146032	0.12–2	0.25	0.5	0.22
Vancomycin	0.12–1	0.25	0.25	0.23
Ampicillin	0.03–0.25	0.12	0.12	0.04
Streptococcus agalactiae (20)				
LY-146032	0.12–2	0.5	1	0.55
Vancomycin	0.12–0.5	0.25	0.5	0.31
Ampicillin	0.06–0.12	0.12	0.12	0.09
Streptococcus faecalis (40)				
LY-146032	0.25–4	1	2	0.87
Vancomycin	0.5–2	1	2	0.93
Ampicillin	0.5–1	1	1	0.73
"Viridans streptococcus" (54)				
LY-146032	0.12–2	0.5	1	0.42
Vancomycin	0.25–1	0.25	0.5	0.28
Ampicillin	0.03–4	0.12	1	0.15

but was more active against *Streptococcus pneumoniae*. However, both compounds were substantially less active than ampicillin against susceptible nonenterococcal streptococci. Penicillin-resistant viridans group streptococci were inhibited by LY146032 at the same concentrations as the penicillin-susceptible isolates.

The MBCs of LY146032 and vancomycin against one strain each of penicillin-susceptible *Staphylococcus aureus*, oxacillin-resistant *Staphylococcus aureus*, oxacillin-resistant *Staphylococcus epidermidis*, and *Streptococcus faecalis* were assayed in Mueller-Hinton broth (MHB) and in MHB supplemented with $CaCl_2$ and $MgCl_2$ (MHB-CMS) to achieve final concentrations of 50 mg of Ca^{2+} per liter and 25 mg of Mg^{2+} per liter. The inoculum effect was tested with an inoculum of between approximately 10^5 and 10^7 CFU/ml in 1-ml test tubes. Viable counts were determined for all tubes without visible growth; 100 μl of the low inoculum or 10 μl of the high inoculum was transferred in pour plates. The MBC was defined as the lowest concentration that reduced the original inoculum by at least 3 log 10 units.

TABLE 2. MIC AND MBC of LY146032 and vancomycin in MHB and MHB-CMS

Species and antimicrobial agent	CFU/ml	Concn (mg/liter) in:			
		MHB-CMS		MHB	
		MIC	MBC	MIC	MBC
Staphylococcus aureus, penicillin susceptible					
LY 146032	10^5	0.25	1	8	16
	10^7	1	8		
Vancomycin	10^5	1	1	1	2
	10^7	2	16		
Staphylococcus aureus, oxacillin resistant					
LY 146032	10^5	0.5	1	8	16
	10^7	0.5	2		
Vancomycin	10^5	1	8	1	8
	10^7	1	64		
Staphylococcus epidermidis, oxacillin resistant					
LY 146032	10^5	0.25	1	4	8
	10^7	1	4		
Vancomycin	10^5	1	4	1	2
	10^7	2	8		
Streptococcus faecalis					
LY 146032	10^5	1	4	8	32
	10^7	2	16		
Vancomycin	10^5	1	16	1	16
	10^7	1	>128		

The MICs and MBCs of LY146032 and vancomycin against representative isolates of *Staphylococcus aureus* and *Streptococcus faecalis* in MHB and MHB-CMS are shown in Table 2. MICs and MBCs of LY146032 were very close. There was a moderate effect of inoculum size on the MICs and MBCs of LY146032 and on the MIC of vancomycin, but a larger effect on the MBCs of vancomycin against *Streptococcus faecalis* and the oxacillin-resistant *Staphylococcus aureus* isolate. The substantially higher MICs and MBCs of LY146032 in unsupplemented MHB, in contrast to those of vancomycin, confirm that Ca^{2+}, Mg^{2+}, or both are required for the activity of this compound.

The killing kinetics of LY146032 and vancomycin were studied on the same organisms with an inoculum of approximately 10^6 CFU/ml in MHB-CMS. Overnight cultures in Trypticase soy broth were properly diluted in prewarmed MHB-CMS (37°C), and the antibiotics were added after preincubation for 30 min to reach logarithmic growth. Viable counts were made at time zero and after 2, 4, 6, 8, and 24 h of contact with concentrations corresponding to 4× MICs and to 16× MICs of each antibiotic against the different isolates.

The killing kinetics of LY146032 and vancomycin are shown in Fig. 1. The bactericidal effect of LY146032 was more pronounced and appeared earlier than that of vanco-

mycin; a difference of at least 3 log 10 units in viable counts was reached after 4 to 8 h against *Staphylococcus epidermidis* and the penicillin-susceptible *Staphylococcus aureus* isolate with both concentrations. Killing of *Streptococcus faecalis* and the oxacillin-resistant *Staphylococcus aureus* isolate by LY146032 was somewhat slower, but killing of these organisms could not be achieved at all by vancomycin.

These in vitro data on a large number of gram-positive cocci isolated from clinical specimens indicate that LY146032 is at least similar in activity to vancomycin. The MBCs and the killing kinetics on a limited number of strains, however, suggest that the bactericidal activity of LY146032 is superior to that of vancomycin, both in the kinetics and in the endpoint, and that it is less influenced by the inoculum size. Therefore, LY146032 deserves further pharmacokinetic and clinical evaluation.

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