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In Vitro Activity of a New Cyclic Lipopeptide Antibiotic, LY146032, against Gram-Positive Clinical Bacteria

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The in vitro activity of LY146032, a novel cyclic lipopeptide antibiotic, was tested against different gram-positive clinical isolates. The activity of LY146032 was clearly higher than that of vancomycin against all isolates tested. However, in some instances rifampin and imipenem showed higher activity than did LY146032.

LY146032, a new semisynthetic cyclic lipopeptide antibiotic originally isolated from *Streptomyces roseosporus* (M. Debono, M. Barnhart, C. B. Carrell, J. A. Hoffman, and R. L. Hamill, Program Abstr. 20th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 68, 1980) has been found to be active against a broad range of gram-positive bacteria (4; G. M. Eliopoulos, G. M. Caputo, E. Reiszner, R. C. Moellering, Jr., 25th ICAAC, abstr. no. 796, 1985).

Because of the increasing rate of severe infections due particularly to enterococci, *Clostridium difficile*, and aminoglycoside-resistant and slime-producing coagulase-negative staphylococci, there is a need for new highly active compounds against these gram-positive isolates (1, 3, 5). The present report describes the in vitro activity of LY146032 and seven other antimicrobial agents against 228 gram-positive clinical isolates.

The bacterial strains tested, except *Streptococcus faecalis* and *C. difficile*, were blood culture isolates from the Turku University Central Hospital, Turku, Finland, collected in 1985 and 1986. Enterococci were isolated from urine samples from the Turku University Central Hospital and the Turku City Hospital, taken mostly from long-term patients during the same period. *C. difficile* isolates were collected from stool specimens at different hospitals in the Turku area, including both hospitals mentioned above. Strains were identified by routine methods including the API Staph procedure (Analytab Products, Plainview, N.Y.) and gas chromatography for *C. difficile* (7). Penicillinase production by staphylococci was tested by the clover leaf method and the rapid chromogenic cephalosporin method (7). Methicillin resistance was determined by a microdilution test; all methicillin-resistant *Staphylococcus epidermidis* strains required for inhibition an oxacillin MIC of ≥16 μg/ml. These results were in agreement with results obtained by the routine disk diffusion method.

The antimicrobial agents used were kindly provided by the following sources: LY146032 and vancomycin, Lilly Research Centre, Ltd., Windlesham, England; ampicillin, erythromycin, and rifampin, Laakefarmos Co., Turku, Finland; imipenem, Suomen MSD, Espoo, Finland; piperacillin, Lederle Co., Espoo, Finland; metronidazole, Sigma Chemical Co., St. Louis, Mo.; clindamycin, The Upjohn Co., Helsinki, Finland.

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MICs were determined by the microtiter technique with Mueller-Hinton broth supplemented with 50 mg of Ca^{2+} per liter and 25 mg of Mg^{2+} per liter (4, 6, 9). Doubling dilutions of each antimicrobial agent were prepared immediately before susceptibility tests. The final inoculum used was ca. 5×10^5 CFU/ml. Plates were examined for growth after 18 to 20 h of incubation at 37°C. Susceptibility tests for *C. difficile* were performed with Wilkins-Chalgren broth, and plates were examined also after 48 h of anaerobic incubation (9). *Staphylococcus aureus* ATCC 25923, *S. faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *C. difficile* ATCC 9689, ATCC 17857, and ATCC 17858 were used as controls. The MICs for the control strains were within the expected ranges. The aminoglycoside resistance patterns of the *S. epidermidis* strains were determined by the MICs of nine different aminoglycoside derivatives (gift from G. Miller, Schering Corp., Bloomfield, N.J.) as described previously (8). All aminoglycoside-resistant strains were resistant either to tobramycin and gentamicin [mediated by the phosphorylating and acetylating enzyme APH(2″)-AAC(6′)] or to tobramycin and amikacin [mediated by the adenylating enzyme ANT(4′)]. With only one exception, all aminoglycoside-resistant *S. epidermidis* strains were also methicillin resistant. Slime production was determined by a tube test described by Christensen et al. (2). Slime-producing *S. epidermidis* strains were all penicillin resistant, and half of them were also methicillin resistant; however, aminoglycoside resistance did not occur among these strains.

The MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of the strains were inhibited are shown in Table 1. The MIC_{90} of LY146032 for most strains tested ranged from 0.125 to 1.0 μg/ml; the only exception was slime-producing *S. epidermidis*, for which the MIC_{90} was 2.0 μg/ml. Against enterococci, the MICs of LY146032 were comparable to those of ampicillin, ranging from 0.125 to 2.0 μg/ml. There was no clear difference in the MICs of any of the agents for penicillinase-positive and -negative *S. aureus* strains, other than those of piperacillin and ampicillin. However, methicillin-resistant *S. epidermidis* strains were clearly more resistant to antimicrobial agents other than LY146032, vancomycin, and rifampin than were methicillin-susceptible strains. *S. epidermidis* strains with enzyme-mediated aminoglycoside resistance mechanisms [ANT(4′) or APH(2″)-AAC(6′)] were more resistant to all agents tested than were strains which are only methicillin resistant; the MIC_{90}s of rifampin and LY146032, which were the only active agents against these isolates, were 0.125 and 1.0 μg/ml, respectively. The same phenomenon was also found among slime-producing strains of *S.*

TABLE 1. In vitro activity of LY146032 compared with that of other antibiotics against gram-positive organisms from clinical sources

Strain (no. studied)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (30), penicillinase negative	LY146032	0.06–1.0	0.25	0.5
	Vancomycin	0.5–4.0	1.0	2.0
	Rifampin	≤0.016–0.06	≤0.016	0.03
	Imipenem	≤0.06	≤0.06	≤0.06
	Piperacillin	≤0.25–1.0	≤0.25	0.5
	Ampicillin	≤0.06–0.5	≤0.06	0.125
	Erythromycin	≤0.06–>64	0.25	1.0
	Clindamycin	≤0.016–0.5	0.125	0.25
Staphylococcus aureus (32), penicillinase positive	LY146032	0.06–0.5	0.125	0.25
	Vancomycin	0.5–2.0	1.0	1.0
	Rifampin	≤0.016–0.06	≤0.016	≤0.016
	Imipenem	≤0.06	≤0.06	≤0.06
	Erythromycin	≤0.06–>64	0.25	0.5
	Clindamycin	≤0.016–0.125	0.06	0.125
Staphylococcus epidermidis (30), methicillin susceptible, penicillinase positive	LY146032	0.016–0.5	0.125	0.25
	Vancomycin	0.06–2.0	1.0	2.0
	Rifampin	≤0.016–0.06	≤0.016	≤0.016
	Imipenem	0.06–8.0	0.06	0.25
	Erythromycin	≤0.06–>64	≤0.06	>64
	Clindamycin	≤0.016–>16	0.03	0.125
Staphylococcus epidermidis (20), methicillin resistant	LY146032	≤0.008–0.5	0.25	0.25
	Vancomycin	0.25–8.0	1.0	4.0
	Rifampin	≤0.016	≤0.016	≤0.016
	Imipenem	0.06–4.0	2.0	4.0
	Erythromycin	0.25–>64	>64	>64
	Clindamycin	0.03–>16	>16	>16
Staphylococcus epidermidis (23), aminoglycoside resistant [APH(2″)–AAC(6′) or ANT(4′)]	LY146032	0.03–8.0	0.5	1.0
	Vancomycin	0.03–16	4.0	16
	Rifampin	≤0.016–>16	≤0.016	0.125
	Imipenem	≤0.06–64	0.5	32
	Erythromycin	0.125–>64	>64	>64
	Clindamycin	≤0.016–>16	>16	>16
Staphylococcus epidermidis (32), slime producing	LY146032	0.5–4.0	1.0	2.0
	Vancomycin	1.0–8.0	4.0	8.0
	Rifampin	≤0.016–>16	0.06	>16
	Imipenem	≤0.06–64	16	32
	Erythromycin	0.25–>64	0.5	>64
	Clindamycin	≤0.016–>16	0.125	>16
Streptococcus faecalis (31)	LY146032	0.125–2.0	0.5	1.0
	Vancomycin	0.5–4.0	2.0	4.0
	Rifampin	2.0–>16	4.0	>16
	Imipenem	≤0.06–4.0	0.5	1.0
	Piperacillin	1.0–16	2.0	4.0
	Ampicillin	0.25–2.0	0.5	1.0
	Erythromycin	0.125–>64	4.0	>64
	Clindamycin	4.0–>16	>16	>16
Clostridium difficile (30)	LY146032	0.03–0.5	0.125	0.25
	Vancomycin	0.06–2.0	0.25	1.0
	Rifampin	≤0.016	≤0.016	≤0.016
	Imipenem	≤0.03–4.0	1.0	2.0
	Metronidazole	≤0.016–0.06	≤0.016	0.03
	Ampicillin	≤0.06–16	2.0	8.0
	Erythromycin	≤0.06–>64	1.0	>64
	Clindamycin	0.06–>16	0.25	>16

epidermidis: the MIC_{90} of only LY146032 was less than 2.0 μg/ml. Why the MICs for aminoglycoside-resistant and slime-producing strains of *S. epidermidis* were higher is not clear. *C. difficile* isolates were all highly susceptible to LY146032, vancomycin, rifampin, and metronidazole.

This study showed that LY146032, a novel cyclic lipopeptide antibiotic, is active against several clinically important

isolates which have been shown to be potential pathogens in severely ill hospital patients. On the basis of these in vitro results, LY146032 is a promising new antimicrobial agent against these pathogens.

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