ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1986, p. 532–535
0066-4804/86/100532-04$02.00/0

Vol. 30, No. 4

In Vitro and In Vivo Activity of LY 146032, a New Cyclic Lipopeptide Antibiotic

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Received 10 March 1986/Accepted 26 June 1986

The in vitro activity of LY 146032, a cyclic lipopeptide antibiotic belonging to the class of agents designated A21978C, was compared with those of vancomycin, cefpirome, cefotaxime, and clindamycin against selected gram-positive bacteria. The new drug inhibited all staphylococcal isolates, including methicillin-resistant strains, at concentrations of ≤1.0 μg/ml. The activity of LY 146032 was comparable to that of vancomycin against most streptococci, but the latter demonstrated greater potency against *Streptococcus faecium* and penicillin-resistant strains of pneumococci and viridans group streptococci. LY 146032 was markedly less active than vancomycin against *Listeria monocytogenes* (MICs for 90% of strains tested, 16 and 1.0 μg/ml, respectively). The activity of LY 146032 was enhanced as the concentration of calcium in the test medium was increased. MBCs were within eightfold of the MIC for each of 12 strains tested. In a rat model of enterococcal endocarditis, the administration of LY 146032 resulted in increased survival and a reduction in the bacterial titer within cardiac vegetations compared with untreated control animals.

LY 146032 is a recently developed antibiotic belonging to the complex of acid lipopeptide antibiotics which has been designated A21978C (M. DeBono, B. J. Abbott, V. M. Krupinski, R. M. Molloy, D. R. Berry, F. T. Counter, L. C. Howard, J. L. Ott, and R. L. Hamill, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1077, 1984). It is prepared by the addition of an *n*-decanoyl side chain to the A21978C core peptide obtained after the removal of other fatty acid acyl groups found on the native compounds. Preliminary data indicate that the new drug, like the prototypal member of this group, A21978C$_1$, a C$_{11}$-branched fatty acid derivative (1), is active against gram-positive bacteria (F. T. Counter, P. J. Baker, L. D. Boeck, M. DeBono, P. W. Ensminger, R. L. Hamill, V. M. Krupinski, R. M. Molloy, and J. L. Ott, 24th ICAAC, abstr. no. 1078, 1984).

This study examined the in vitro activity of LY 146032 against gram-positive bacteria compared with those of vancomycin, cefpirome, cefotaxime, and clindamycin. Finding the new drug to demonstrate significant in vitro activity against enterococci, we also tested the therapeutic efficacy of LY 146032 in a rat model of enterococcal endocarditis.

(This work was presented in part at the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy [G. M. Eliopoulos, G. M. Caputo, E. Reiszner, and R. C. Moellering, Jr., 25th ICAAC, abstr. no. 796, 1985; S. Willey, G. M. Eliopoulos, and R. C. Moellering, Jr., 25th ICAAC, abstr. no. 797, 1985].)

MATERIALS AND METHODS

Organisms. The bacterial strains used in this study were collected as previously described (1, 3). They were clinical isolates collected at Massachusetts General Hospital and New England Deaconess Hospital, Boston, Mass., with the exception of the penicillin-resistant pneumococci and viridans group streptococci, which were obtained from South Africa.

Antimicrobial agents. Standard antimicrobial reference powders and their sources were as follows: LY 146032 and vancomycin, Eli Lilly & Co., Indianapolis, Ind.; cefpirome (HR-810) and cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; and clindamycin, The Upjohn Co., Kalamazoo, Mich.

Susceptibility studies. Antibiotic susceptibility was determined by an agar dilution technique (8) using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.). This medium was supplemented with 5% defibrinated sheep blood when nonenterococcal streptococci and diphtheroids were tested. For most strains, inocula of ca. 10^4 CFU were prepared by appropriate dilutions of overnight broth cultures of organisms in fresh Mueller-Hinton broth (BBL) and were applied to plates with a 32-prong inoculating device. The plates were examined for growth after 18 to 20 h of incubation at 37°C. When diphtheroids were tested, inocula were prepared from overnight cultures in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) with 5% rabbit serum (Whittaker M.A. Bioproducts, Walkersville, Md.). The inoculum was then used to stamp blood agar plates, which were read after 48 h of incubation at 37°C. Four selected strains of three species were also tested by a macrobroth dilution method (8) in Dextrose Phosphate Broth (GIBCO Diagnostics, Madison, Wis.) with or without supplementation with specific concentrations of calcium chloride. Final inocula of 5×10^5 to 1×10^6 CFU/ml were prepared from overnight cultures (total volume, 2 ml). MICs were determined by visual inspection at 20 h of incubation, at which time the tubes were shaken in a vortex mixer. Samples (0.01 ml) from clear tubes were transferred to antibiotic-free plates at 24 h of incubation. MBCs, defined by a ≥99.9% reduction in CFU relative to the inoculum, were determined by the method of Pearson et al. (4).

Animal studies. Aortic valve endocarditis was established in male Sprague-Dawley rats by a modification of the method of Santoro and Levison (7). The rats were put under anes-

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TABLE 1. Comparative in vitro activity of LY 146032 against gram-positive bacteria

Organism(s) (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50	90
Staphylococcus aureus (methicillin susceptible) (44)	LY 146032	0.125–0.5	0.25	0.5
	Vancomycin	0.5–2	1.0	1.0
	Cefpirome	0.5–2	1.0	1.0
	Cefotaxime	1.0–4	2.0	4.0
	Clindamycin	≤0.06–0.125	0.125	0.125
Staphylococcus aureus (methicillin resistant) (15)	LY 146032		0.5	0.5
	Vancomycin	0.5–1.0	1.0	1.0
	Cefpirome	4–64	32	64
	Cefotaxime	32–>128	>128	>128
	Clindamycin		>128	>128
Staphylococcus epidermidis (30)	LY 146032	0.25–1.0	0.5	1.0
	Vancomycin	0.5–4	2.0	4.0
	Cefpirome	0.125–16	4.0	8.0
	Clindamycin	≤0.06–>128	0.125	>128
Streptococcus faecalis (58)	LY 146032	0.25–2	1.0	2.0
	Vancomycin	0.5–2	2.0	2.0
	Cefpirome	0.5–64	8.0	32
	Cefotaxime	≤0.06–>128	>128	>128
	Clindamycin	0.125–>128	32	>128
Streptococcus faecium (10)	LY 146032	4–8	4.0	8.0
	Vancomycin	0.5–2	0.5	2.0
	Cefpirome		>128	>128
	Cefotaxime	32–>128	>128	>128
	Clindamycin	0.125–>128	1.0	>128
Streptococcus avium (10)	LY 146032	0.25–4	1.0	2.0
	Vancomycin	0.5–1	0.5	0.5
	Cefpirome	2–8	4.0	4.0
	Cefotaxime	4–8	4.0	4.0
	Clindamycin	1.0–2	1.0	2.0
Streptococci, groups A, C, and G (20)	LY 146032	≤0.06–0.5	≤0.06	0.125
	Vancomycin	0.25–0.5	0.5	0.5
	Cefpirome	≤0.06–0.125	≤0.06	≤0.06
	Cefotaxime	≤0.06–0.25	≤0.06	≤0.06
	Clindamycin	≤0.06–0.125	≤0.06	≤0.06
Streptococcus agalactiae (10)	LY 146032	0.125–0.25	0.25	0.25
	Vancomycin	0.25–0.5	0.5	0.5
	Cefpirome	0.125–0.25	0.125	0.125
	Cefotaxime	≤0.06–0.25	≤0.06	≤0.06
	Clindamycin	≤0.06–0.125	≤0.06	≤0.06
Viridans group streptococci (penicillin susceptible) (23)	LY 146032	≤0.06–0.5	0.25	0.5
	Vancomycin	≤0.06–0.5	0.5	0.5
	Cefpirome		≤0.06	≤0.06
	Cefotaxime	≤0.06–0.125	≤0.06	0.125
	Clindamycin		≤0.06	≤0.06
Viridans group streptococci (penicillin resistant) (11)	LY 146032	0.5–2	1.0	2.0
	Vancomycin	0.25–0.5	0.5	0.5
	Cefpirome	0.5–8	1.0	8.0
	Cefotaxime	1.0–8	2.0	4.0
	Clindamycin	≤0.06–64	0.125	64
Streptococcus pneumoniae (penicillin resistant) (10)	LY 146032	0.5–2	0.5	2.0
	Vancomycin	0.25–1.0	0.25	0.5
	Cefpirome	≤0.06–8	1.0	8.0
	Cefotaxime	≤0.06–4	1.0	4.0
	Clindamycin	≤0.06–16	≤0.06	16
Listeria monocytogenes (10)	LY 146032	8–32	8.0	16
	Vancomycin	0.5–1.0	1.0	1.0
	Cefpirome	4–128	64	128
	Cefotaxime	2–>128	128	>128
	Clindamycin	2–4	4.0	4.0
Diphtheroids (26)	LY 146032	0.25–4	2.0	4.0
	Vancomycin	0.5–1.0	1.0	1.0

thesia with ether and pentobarbital, and a polyethylene catheter was introduced through an incision into the right carotid artery and advanced to the aortic valve. Thirty minutes later, a saline suspension of ca. 10^7 CFU of *Streptococcus faecalis* 1310 (MIC/MBC: LY 146032, 8/32 μg/ml; vancomycin, 1/128 μg/ml) was injected through the cannula, which was then heat sealed and sutured in place. Twenty-four hours later, blood cultures were obtained and central venous access was established by the method of Maiz et al. (2). By this technique, a Silastin catheter (Dow Corning Corp., Midland, Mich.) was introduced into the superior vena cava via the left internal jugular vein and then tunneled subcutaneously to an exit site in the interscapular region. The animals with negative blood cultures at this stage were excluded from the study.

Antibiotics were administered by continuous infusion with a Sage 352 syringe pump (Orion Research, Inc., Cambridge, Mass.), beginning within 4 h of line insertion. LY 146032 or vancomycin was given at doses of 100 or 50 mg/kg (body weight) per day, respectively, to achieve mean concentrations in serum of 4 to 8 times the MIC of each drug. A series of untreated animals served as controls once preliminary studies indicated that the infusion of saline alone did not influence the course of endocarditis. Antibiotic levels in serum were determined by microbiological assay (5) using a test strain of "*Bacillus globigii*" for vancomycin and "*Sarcina luteus*" for LY 146032. The latter assay detected serum levels of ≥1 μg/ml and was reproducible to ±7% in the range of concentrations achieved in vivo.

After 5 days of antibiotic therapy, blood cultures were obtained and the animals were sacrificed. Vegetations were excised, weighed, and homogenized in a tissue grinder with sterile saline for subsequent colony counts. Statistical analysis was performed by using the χ^2 test with Yates' correction or Student's *t* test for discrete or continuous variables, respectively.

RESULTS

Susceptibility studies. The results of in vitro agar dilution susceptibility studies are shown in Table 1. LY 146032 was slightly more active than vancomycin against both methicillin-susceptible and -resistant strains of *Staphylococcus aureus*. Only clindamycin was more active against the former group. The new drug was also the most active agent tested against coagulase-negative staphylococci. Vancomycin demonstrated greater activity than LY 146032 against *Streptococcus faecium*, *Streptococcus avium*, and penicillin-resistant strains of viridans group streptococci and pneumococci. The cephems and clindamycin were more active than either LY 146032 or vancomycin against other streptococci. Five strains of *Streptococcus bovis* were inhibited by the new drug at a concentration of 2 μg/ml. *Listeria monocytogenes* was notably less susceptible to LY 146032 than to vancomycin. With the exception of *S. faecium* and *L. monocytogenes*, all strains tested were inhibited by LY 146032 at concentrations of ≤4 μg/ml.

TABLE 2. Effect of calcium supplementation of growth medium on the activity of LY 146032 against gram-positive bacteria

Organism[a]	MIC (μg/ml) in:		MBC (μg/ml) in:	
	DPB[b]	DPB + calcium (50 mg/liter)	DPB	DPB + calcuim (50 mg/liter)
Staphylococcus aureus	64	2	64	16
	64	2	64	16
	64	2	64	8
	64	2	64	4
Streptococcus faecalis	64	8	64	8
	64	16	64	16
	64	8	64	16
	64	8	64	16
Streptococcus pyogenes	4	0.25	16	2
	4	0.25	16	1.0
	4	0.5	16	2
	4	0.5	8	1.0

[a] Data shown for four strains of each species.
[b] DPB, Dextrose Phosphate Broth.

TABLE 3. Effectiveness of LY 146032 and vancomycin in experimental enterococcal endocarditis in rats

Regimen	No. of rats surviving/ no. treated	No. of surviving rats with sterile		Bacterial titer in vegetations (CFU/g [mean ± SD])
		Blood	Vegetations	
Control	15/28	3	0	8.02 ± 1.7
LY 146032	17/22	14	2	4.68 ± 2.0
Vancomycin	19/22	14	2	5.84 ± 1.6

The in vitro activity of LY 146032 against representative strains of several bacterial species was enhanced when broth medium (normal calcium concentration, <4 mg/liter [1]) was supplemented with calcium chloride to physiologic concentrations of the cation (Table 2). The activity of the drug against an *S. faecalis* strain increased in a continuous fashion with increasing concentrations of calcium to the highest level tested, with MICs ranging from 128 μg/ml at calcium concentrations of ≤6.25 mg/liter to 4 μg/ml when calcium was added at 100 mg/liter. Of note is the fact that the broth dilution MICs shown in Table 2 (for calcium-supplemented media) were higher than the corresponding agar dilution MICs. Detailed studies with four isolates indicated that broth dilution MICs obtained with an inoculum of 10^6 CFU/ml were two- to fourfold higher than those obtained with an inoculum of 10^4 CFU/ml. Inherent technical differences between the two techniques (8) may also have contributed to the higher values derived in the tube dilution study. It was also apparent that LY 146032 was bactericidal at concentrations ≤8 times the MIC against each of the isolates tested, including all four strains of *S. faecalis* (Table 2). Limited time-kill studies performed in calcium-supplemented Mueller-Hinton broth against two strains each of *S. faecalis* and *S. aureus* revealed reductions of >3 $\log_{10}$ CFU/ml after 4 h of incubation in the presence of LY 146032 at 8 μg/ml (data not shown).

Animal studies. Approximately 46% of untreated control animals died of infection within 5 days. None of the survivors had sterile cardiac vegetations (Table 3). Animals treated with either LY 146032 or vancomycin had increased survival ($P < 0.05$) and sterilization of blood cultures ($P < 0.01$) compared with control animals. At the doses administered, LY 146032 and vancomycin achieved mean concentrations (± standard deviation) in serum of 35 ± 11 and 7 ± 3 μg/ml, respectively. Both LY 146032 and vancomycin significantly reduced bacterial counts within vegetations ($P < 0.001$ and $P < 0.01$, respectively). Although LY 146032 therapy resulted in a ca. 10-fold greater reduction in bacterial

counts than vancomycin, this difference was not statistically significant.

DISCUSSION

The in vitro activity of LY 146032 against the gram-positive bacteria tested was found to be generally comparable to that of the previously described lipopeptide, A21978C_1, as reported in an earlier study (1). Although the new analog was somewhat less active than A21978C_1 against *S. faecalis* (MIC for 90% of strains tested [MIC_{90}], 2 and 1 μg/ml, respectively) and *S. faecium* (MIC_{90}, 8 and 2 μg/ml, respectively), these results may be offset by the superior therapeutic index of LY 146032 in initial animal studies (M. DeBono et al., 24th ICAAC, abstr. no. 1077, 1984). The new lipopeptide antibiotic was more active than vancomycin against staphylococci, including methicillin-resistant strains, and some streptococci; however, vancomycin exhibited greater potency against penicillin-resistant pneumococci and viridans group streptococci, as well as *L. monocytogenes* and some enterococci.

As seen with A21978C_1, the in vitro activity of LY 146032 demonstrated a striking dependence on calcium concentration in the growth medium (1). The mechanism by which this occurs has not been established. Like A21978C_1, the antibacterial properties of the new lipopeptide appear to result, at least in part, from its ability to inhibit cell wall peptidoglycan synthesis (N. Allen, W. Alborn, Jr., J. Hobbs, Jr., and H. Percifield, 24th ICAAC, abstr. no. 1081, 1984).

The observation that LY 146032 was bactericidal at concentrations near the MIC against all five enterococci tested is of interest, given the requirement that serious enterococcal infections be treated with combinations of a cell-wall-active agent and an aminoglycoside (6). Our animal model of enterococcal endocarditis confirmed the significant activity of LY 146032 under physiologic conditions, which resulted in a 3-$\log_{10}$ reduction in bacterial titers within cardiac vegetations (in comparison with controls) after only 5 days of therapy. Preliminary studies by others using a different model indicated that more prolonged courses of the drug are highly effective in clearing enterococci from cardiac vegetations in rats (A. M. F. Hansen, D. H. Holmes, D. A. Preston, and R. S. Pekarek, 24th ICAAC, abstr. no. 1079, 1984). However, it is unclear based on these data whether LY 146032 as a single agent would prove any more effective than ampicillin alone in the therapy of enterococcal endocarditis in humans (C. Thauvin, G. M. Eliopoulos, S. Willey, and R. C. Moellering, Jr., 24th ICAAC, abstr. no. 338, 1984). Our animal studies were intended only to validate in vivo the activity of LY 146032 compared with that of vancomycin against enterococci. For this reason, target levels in serum of both drugs were chosen based on multiples of MICs and were maintained by continuous infusion. Neither the mode of administration nor the actual levels attained is necessarily suitable in clinical use.

These results confirm the activity of LY 146032, a member of the newly developed class of cyclic lipopeptide antibiotics, against a variety of gram-positive bacteria, many of which are resistant to available antimicrobial agents.

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