ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1996, p. 2661–2663 Vol. 40, No. 11
0066-4804/96/$04.00+0

Comparative Activities of LY 333328, a New Glycopeptide, against Penicillin-Susceptible and -Resistant Pneumococci

ELIZABETH FASOLA,[1] SHEILA K. SPANGLER,[2] LOIS M. EDNIE,[2] MICHAEL R. JACOBS,[1] SARALEE BAJAKSOUZIAN,[1] AND PETER C. APPELBAUM[2]*

Department of Pathology (Clinical Microbiology), Case Western Reserve University, Cleveland, Ohio 44106,[1] and Department of Pathology, Hershey Medical Center, Hershey, Pennsylvania 17033[2]

Received 16 May 1996/Returned for modification 26 July 1996/Accepted 12 August 1996

Microdilution MIC testing was used to test the susceptibility of 202 pneumococci to LY 333328 and six other agents. LY 333328 was the most active glycopeptide (MIC at which 90% of the pneumococci were inhibited [MIC_{90}], 0.008 μg/ml), followed by teicoplanin (MIC_{90}, 0.06 μg/ml) and vancomycin (MIC_{90}, 0.5 μg/ml). Rifampin resistance was seen in some penicillin-resistant strains. The MICs of imipenem and ceftriaxone rose with those of penicillin. Time-kill testing confirmed the excellent antipneumococcal activity of LY 333328.

The incidence of pneumococci resistant to penicillin and other agents has increased worldwide at an alarming rate (1, 2, 4). The problem is exacerbated by the tendency of these strains to spread from country to country and from continent to continent (1, 7).

There is an urgent need for additional compounds for treatment of serious systemic infections caused by pneumococci with reduced penicillin susceptibility (5, 7). This is especially the case for meningitis (15). Of the currently available glycopeptides, vancomycin does not penetrate reliably into the cerebrospinal fluid, and treatment failures with this compound have been encountered despite low MICs (18). The antipneumococcal MICs of teicoplanin are lower than those of vancomycin (6, 16, 17). However, the drug is not currently available in the United States.

LY 333328 is a new semisynthetic glycopeptide active against vancomycin-resistant enterococci and other gram-positive organisms (3, 10, 12, 13). This study examined the antipneumococcal activities of LY 333328, vancomycin, teicoplanin, penicillin G, ceftriaxone, rifampin, and imipenem by microdilution MIC and time-kill testing.

In MIC experiments, 51 penicillin-susceptible (MICs, ≤0.06 μg/ml), 75 penicillin-intermediate (MICs, 0.125 to 1.0 μg/ml), and 76 penicillin-resistant (MICs, ≥2.0 μg/ml) pneumococci were tested. All susceptible and some intermediate and resistant strains were recent U.S. isolates. Other intermediate and resistant strains were from South Africa, France, Spain, Central and eastern Europe, and Korea. For time-kill testing, three susceptible, three intermediate, and three resistant strains were tested. LY 333328 was obtained from Eli Lilly & Co., Indianapolis, Ind., and other compounds were obtained from their respective manufacturers.

MICs were determined by microdilution, as recommended by the National Committee for Clinical Laboratory Standards, by using cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) with 5% lysed defibrinated horse blood (11). Trays were incubated overnight in ambient air at 37°C.

For time-kill studies, tubes containing 5 ml of cation-adjusted Mueller-Hinton broth (Difco) and 5% lysed horse blood with doubling antibiotic concentrations were inoculated with 5×10^5 to 5×10^6 CFU/ml and were incubated at 35°C in a shaking water bath. The bacterial inoculum was prepared by diluting a 16-h broth (medium described above) culture in the same medium (14).

Viability counts of antibiotic-containing suspensions were performed at 0, 2, 4, 6, 12, and 24 h. Time-kill assay results were analyzed by determining the number of strains yielding $\Delta\log_{10}$ CFU per milliliter values of −1, −2, and −3 with respect to counts at time zero for all seven compounds at all five time periods. Antimicrobial agents were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥3 $\log_{10}$ CFU/ml (99.9%) at each time period and were considered bacteriostatic if the inoculum was reduced by 0 to 3 $\log_{10}$ CFU/ml. Bacterial carryover was addressed as reported previously (14).

Results of MIC tests of 202 strains are presented in Table 1. LY 333328 had the lowest MICs of all three glycopeptides tested, with MICs at which 50% of the strains were inhibited (MIC_{50}s) against penicillin-susceptible, -intermediate, and -resistant strains of ≤0.002, ≤0.002, and 0.008 μg/ml, respectively, and MIC_{90}s of 0.008, 0.008, and 0.03 μg/ml, respectively. Vancomycin had a MIC_{50} of 0.25 μg/ml and a MIC_{90} of 0.5 μg/ml for all three groups, and teicoplanin had a MIC_{50} of 0.03 μg/ml and a MIC_{90} of 0.06 μg/ml for all three groups. Rifampin was very active against penicillin-susceptible and -intermediate strains (MIC_{50}s, 0.015 and ≤0.008 μg/ml, respectively, and MIC_{90}s of 0.03 and 0.015 μg/ml, respectively); some penicillin-intermediate and -resistant strains were rifampin resistant (MICs, ≥4.0 μg/ml). The MICs of ceftriaxone and imipenem rose with those of penicillin G, but all pneumococci were inhibited at MICs of ≤4.0 and ≤1.0 μg/ml, respectively.

The MICs for strains included in time-kill testing are presented in Table 2. As can be seen, the MICs of LY 333328 (0.001 to 0.016 μg/ml) were several dilutions lower than those of vancomycin (0.25 to 0.5 μg/ml) and teicoplanin (0.03 to 0.25 μg/ml). Ceftriaxone and imipenem MICs rose with those of penicillin G, but all strains were inhibited at MICs of ≤2.0 and ≤0.5 μg/ml, respectively. Three of the nine strains were resistant to rifampin (i.e., MICs were ≥16.0 μg/ml; for the remainder the MICs were 0.008 to 0.06 μg/ml).

Broth MICs were all within one dilution of bacteriostatic levels at 24 h. All compounds were bactericidal at the MIC after 24 h. LY 333328 was bactericidal against all strains after 12 h at eight times the MIC and yielded 90% killing of all

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953.

TABLE 1. Broth microdilution MICs for 202 pneumococcal strains

Compound and strain characteristic[a]	MIC (μg/ml)[b]		
	Range	50%	90%
Penicillin G			
Penicillin S	≤0.008–0.06	0.015	0.06
Penicillin I	0.125–1.0	0.25	1.0
Penicillin R	2.0–8.0	2.0	4.0
LY 333328			
Penicillin S	≤0.002–0.06	≤0.002	0.008
Penicillin I	≤0.002–0.06	≤0.002	0.008
Penicillin R	≤0.002–0.125	0.008	0.03
Vancomycin			
Penicillin S	0.06–0.5	0.25	0.5
Penicillin I	0.125–0.5	0.25	0.5
Penicillin R	0.25–0.5	0.25	0.5
Teicoplanin			
Penicillin S	0.015–0.125	0.03	0.06
Penicillin I	0.008–0.06	0.03	0.06
Penicillin R	0.015–0.125	0.03	0.06
Ceftriaxone			
Penicillin S	≤0.008–0.125	≤0.008	0.06
Penicillin I	0.03–0.5	0.125	0.5
Penicillin R	0.25–4.0	0.5	2.0
Rifampin			
Penicillin S	≤0.008–0.03	0.015	0.03
Penicillin I	≤0.008–8.0	≤0.008	0.015
Penicillin R	≤0.008–>8.0	0.015	>8.0
Imipenem			
Penicillin S	≤0.004–0.03	≤0.004	0.03
Penicillin I	0.008–0.25	0.03	0.125
Penicillin R	0.06–1.0	0.25	0.5

[a] S, susceptible; I, intermediate; R, resistant.
[b] 50% and 90%, MICs at which 50 and 90% of the strains were inhibited, respectively.

strains after 6 h at eight times the MIC. LY 333328 was uniformly bactericidal at ≤0.125 μg/ml after 12 h. By comparison, vancomycin was bactericidal against eight of the nine strains after 12 h at twice the MIC and yielded 90% killing of all strains after 6 h at twice the MIC. Teicoplanin was bactericidal against six of the nine strains after 12 h at eight times the MIC and yielded 99% killing of all strains after 6 h at eight times the MIC. Ceftriaxone was uniformly bactericidal after 12 h at eight times the MIC and yielded 90% killing of all strains after 6 h at four times the MIC, while rifampin was bactericidal for six of the nine strains after 12 h at twice the MIC, yielding 90% killing of all strains after 6 h at four times the MIC. Imipenem was bactericidal for eight of the nine strains at twice the MIC after 12 h and showed 90% killing of all strains after 6 h at the MIC. Time-kill test results for the three glycopeptides against a penicillin-resistant strain are depicted in Fig. 1.

LY 333328 is a new semisynthetic glycopeptide active against vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*, with MICs against VanA and VanB strains of 0.03 to 2.0 and 0.016 to 1.0 μg/ml, respectively. LY 333328 is also very active against methicillin-susceptible *Staphylococcus aureus*, teicoplanin-susceptible and -resistant coagulase-negative staphylococci, and *Streptococcus pyogenes* (12, 13). One preliminary report has described MICs of ≤0.008 μg/ml against *S. pneumoniae* (12). This compound is also active against vancomycin-resistant enterococci in a mouse model (3) and has the additional advantage of having a terminal half-life in rats of 9.8 h, which is 13.5 times greater than that for vancomycin (10).

Results of the present study confirm the excellent antipneumococcal activity of LY 333328. All strains tested were inhibited at a MIC of ≤0.125 μg/ml, and results of time-kill studies showed uniform bactericidal activity after 12 h at ≤0.125 μg/ml. MIC and time-kill results showed superior antipneumococcal activity compared with those of vancomycin and teicoplanin (6, 16, 17). The superior in vitro activity of teicoplanin compared with that of vancomycin has been described before (6). MICs of imipenem rose with those of penicillin G and were similar to those reported previously (17).

The latest recommendation for treatment of meningitis caused by penicillin-intermediate and -resistant pneumococci is high-dose cefotaxime or ceftriaxone alone or in combination with vancomycin (15). Treatment schedules are based upon anecdotal case report studies, however (8), and no prospective studies on which to base therapy are available. Imipenem should not be used for treatment of meningitis because of its tendency to cause convulsions. Meropenem does not appear to possess this property and is a therapeutic option (9). If results of toxicological and pharmacokinetic studies point to development of LY 333328, clinical studies of the treatment of meningitic and nonmeningitic pneumococcal infections are indicated on the basis of these in vitro results.

TABLE 2. Broth microdilution MICs for strains tested in time-kill experiments

Strain no. (characteristic)[a]	MIC (μg/ml) of:					
	LY 333328	Vancomycin	Teicoplanin	Ceftriaxone	Rifampin	Imipenem
1 (S)	0.008	0.25	0.25	0.125	0.06	0.008
2 (S)	0.004	0.5	0.25	0.06	0.03	0.004
3 (S)	0.002	0.5	0.03	0.016	0.008	0.008
4 (I)	0.002	0.5	0.06	0.125	0.06	0.008
5 (I)	0.001	0.25	0.06	0.25	16.0	0.03
6 (I)	0.016	0.25	0.06	1.0	32.0	0.06
7 (R)	0.008	0.25	0.06	0.5	0.008	0.25
8 (R)	0.004	0.25	0.06	2.0	0.008	0.25
9 (R)	0.004	0.25	0.25	2.0	64.0	0.5

[a] S, penicillin susceptible; I, penicillin intermediate; R, penicillin resistant.

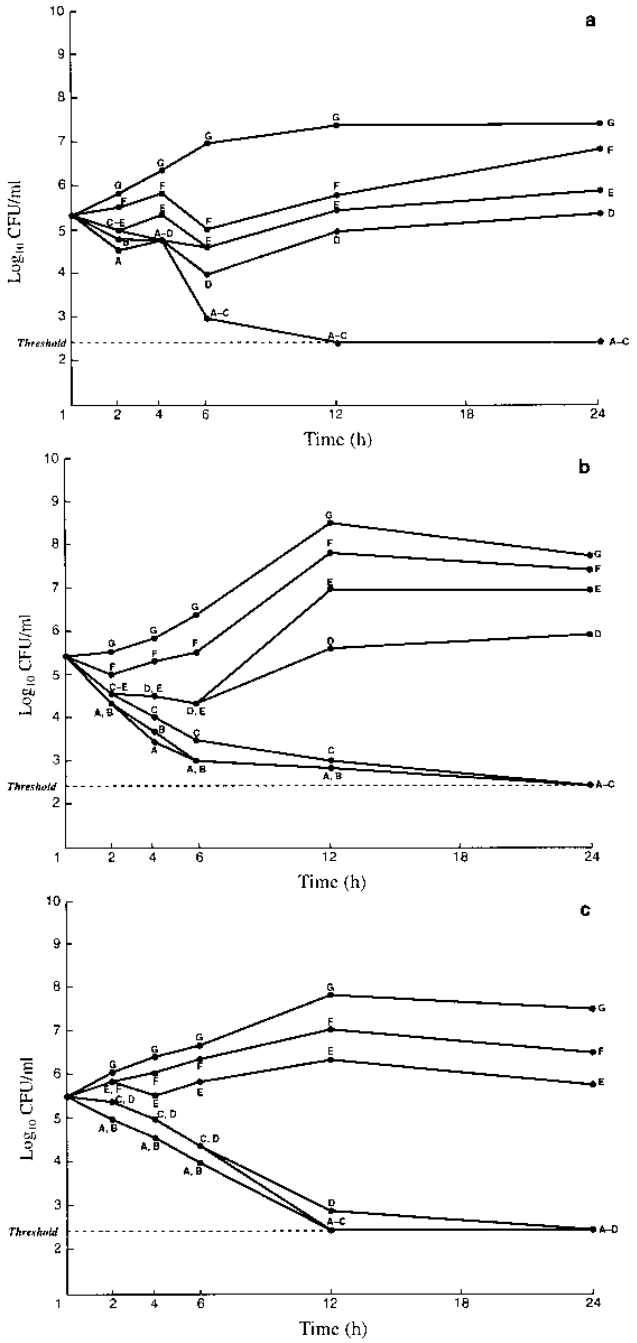

FIG. 1. Time-kill kinetics of LY 333328 (a), vancomycin (b), and teicoplanin (c) against a penicillin-resistant pneumococcus (strain 9) at 0, 2, 4, 6, 12, and 24 h. Drug concentrations (in micrograms per milliliter) are as follows. (a) A, 0.015; B, 0.008; C, 0.004 (broth microdilution MIC); D, 0.002; E, 0.001; F, 0.0005; G, none (growth control). (b) A, 1.0; B, 0.5; C, 0.25 (broth microdilution MIC); D, 0.125; E, 0.06; F, 0.03; G, none (growth control). (c) A, 1.0; B, 0.5; C, 0.25 (broth microdilution MIC); D, 0.125; E, 0.06; F, 0.03; G, growth control.

This study was supported by a grant by Eli Lilly & Co.
We thank Thalia Nicas for advice.

REFERENCES

1. **Appelbaum, P. C.** 1992. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clin. Infect. Dis. **15:**77–83.
2. **Block, S., J. Hedrick, P. Wright, R. Finger, R. Leggiadro, M. Appleton, S. Kahn, and R. Hutcheson.** 1994. Drug-resistant *Streptococcus pneumoniae*—Kentucky and Tennessee, 1993. Morbid. Mortal. Weekly Rep. **43:**23–25, 31.
3. **Boylan, C. J., T. I. Nicas, D. A. Preston, D. L. Zeckner, B. J. Boyll, P. A. Raab, D. L. Mullen, N. J. Snyder, L. L. Zornes, R. E. Stratford, M. J. Zweifel, S. C. Wilkie, M. J. Rodriguez, R. C. Thompson, and R. D. G. Cooper.** 1995. Efficacy of semisynthetic glycopeptides active against vancomycin-resistant enterococci in a mouse infection model, abstr. F255, p. 157. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
4. **Breiman, R. F., J. C. Butler, F. C. Tenover, J. A. Elliott, and R. R. Facklam.** 1994. Emergence of drug-resistant pneumococcal infections in the United States. JAMA **271:**1831–1835.
5. **Friedland, I. R., and G. H. McCracken, Jr.** 1994. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. N. Engl. J. Med. **331:**377–382.
6. **Goldstein, F. W., P. Geslin, J. F. Acar, and the French Study Group.** 1994. Comparative activity of teicoplanin and vancomycin against 400 penicillin susceptible and resistant *Streptococcus pneumoniae*. Eur. J. Clin. Microbiol. Infect. Dis. **13:**33–34.
7. **Jacobs, M. R.** 1992. Treatment and diagnosis of infections caused by drug-resistant *Streptococcus pneumoniae*. Clin. Infect. Dis. **15:**119–127.
8. **Jacobs, R. F., S. L. Kaplan, G. E. Schutze, A. S. Dajani, R. J. Leggiadro, C.-S. Rim, and S. K. Puri.** 1996. Relationship of MICs to efficacy of cefotaxime in treatment of *Streptococcus pneumoniae* infections. Antimicrob. Agents Chemother. **40:**895–898.
9. **Klugman, K. P., R. Dagan, and the Meropenem Meningitis Study Group.** 1995. Randomized comparison of meropenem with cefotaxime for treatment of bacterial meningitis. Antimicrob. Agents Chemother. **39:**1140–1146.
10. **Lin, Y., R. E. Stratford, L. L. Zornes, W. L. Confer, V. Vasudevan, T. W. Jones, T. I. Nicas, D. A. Preston, C. J. Boylan, D. L. Zeckner, B. J. Boyll, P. A. Raab, N. J. Snyder, M. J. Zweifel, S. C. Wilkie, M. J. Rodriguez, R. C. Thompson, and R. D. G. Cooper.** 1995. Non-clinical pharmacokinetics of LY333328, a semisynthetic glycopeptide antibiotic active against vancomycin-resistant enterococci, abstr. F254, p. 157. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
11. **National Committee for Clinical Laboratory Standards.** 1995. Performance standards for antimicrobial susceptibility testing. M100-S5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. **Nicas, T. I., J. E. Flokowitsch, D. A. Preston, D. L. Mullen, J. Grissom-Arnold, N. J. Snyder, M. J. Zweifel, S. C. Wilkie, M. J. Rodriguez, R. C. Thompson, and R. D. G. Cooper.** 1995. Semisynthetic glycopeptides active against vancomycin-resistant enterococci: activity against staphylococci and streptococci in vitro and in vivo, abstr. F248, p. 156. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
13. **Nicas, T. I., D. L. Mullen, J. Grissom-Arnold, N. J. Snyder, M. J. Zweifel, S. C. Wilkie, M. J. Rodriguez, R. C. Thompson, and R. D. G. Cooper.** 1995. Semisynthetic glycopeptides active against vancomycin-resistant enterococci: in vitro activity against enterococci, abstr. F249, p. 156. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
14. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1994. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin, and vancomycin by using time-kill methodology. Antimicrob. Agents Chemother. **38:**2065–2072.
15. **París, M. M., O. Ramilo, and G. H. McCracken, Jr.** 1995. Management of meningitis caused by penicillin-resistant *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **39:**2171–2175.
16. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1992. Susceptibilities of penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae* to RP 59500, vancomycin, erythromycin, PD 131628, sparfloxacin, temafloxacin, Win 57273, ofloxacin, and ciprofloxacin. Antimicrob. Agents Chemother. **36:**856–859.
17. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1996. Activities of RPR 106972 (a new oral streptogramin), cefditoren (a new oral cephalosporin), two new oxazolidinones (U-100592 and U-100766), and other oral and parenteral agents against 203 penicillin-susceptible and -resistant pneumococci. Antimicrob. Agents Chemother. **40:**481–484.
18. **Viladrich, P. F., F. Gudiol, J. Liñares, R. Pallarés, I. Sabaté, G. Rufí, and J. Ariza.** 1991. Evaluation of vancomycin for therapy of adult pneumococcal meningitis. Antimicrob. Agents Chemother. **35:**2467–2472.