ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1997, p. 1407–1408 Vol. 41, No. 6
0066-4804/97/$04.00+0

Time-Kill Curves for a Semisynthetic Glycopeptide, LY333328, against Vancomycin-Susceptible and Vancomycin-Resistant *Enterococcus faecium* Strains

The purpose of this study was to characterize the time-kill curves for LY333328 (LY), an investigational semisynthetic glycopeptide, against three clinical *Enterococcus faecium* isolates: #23-1 (vancomycin susceptible), #559 (probe-positive *vanB*), and #560 (probe-positive *vanA*) (3). Susceptibilities were determined in triplicate by broth macrodilution as described by the National Committee for Clinical Laboratory Standards (1). The respective MICs for strains #23-1, #559, and #560 were as follows: LY, 0.63, 0.25, and 1 μg/ml; vancomycin, 0.5, 16, and 1,024 μg/ml; teicoplanin, 0.25, 1, and 64 μg/ml; gentamicin, 16, >2,048, and 2,048 μg/ml; ampicillin, 2, 128, and 128 μg/ml; and ciprofloxacin, 8, >128, and >128 μg/ml.

Time-kill experiments were conducted in triplicate in cation-supplemented Mueller-Hinton broth (CSMHB). Experiments were performed over 24 h at LY concentrations of 1, 10, and 100 times the MIC for all strians and vancomycin concentrations of 1, 10, and 100 times the MIC for isolate #23-1 and 1 and 10 times the MIC for isolate #559. Initial inocula of approximately 10^7 CFU/ml were used and verified by performing colony counts. Samples (0.1 ml) were collected at 0, 1, 2, 4, 8 (or 10), and 72 h; serially diluted in normal saline at 4°C; aliquoted in duplicate onto blood agar plates; and incubated at 35°C for 72 h. Potential antibiotic carryover was assessed by running concurrent samples which were washed twice to remove antibiotic. Washing was performed by centrifuging at 4,000 × *g* for 10 min, decanting the supernatant, and resuspending the pellet in CSMHB. After 24, 48, and 72 h of incubation, colonies (10 to 100 per plate) were counted, providing a lower limit of detection of 10^2 CFU/ml.

Colony count variation at each time point, within and between duplicate experiments and between unwashed and washed samples, was less than 10%. Colony counts were not changed with incubation beyond 24 h. LY activity against all strains as demonstrated by maximal bacterial killing at any time point and by bacterial counts at 24 h is summarized in Table 1. Time-kill curves for LY against strain #560 are depicted in Fig. 1. LY at 1 times the MIC produced modest bacterial killing (i.e., <1.5 log kill at any time point), and bacterial counts at 24 h approached or exceeded the initial inocula for all strains. LY at 10 times the MIC was bactericidal (i.e., ≥3 log kill) against all strains at 4 h and maintained bacterial counts ≥2.5 $\log_{10}$ CFU/ml below the initial inocula at 24 h. LY at 100 times the MIC produced a 4.5 to 5.5 log kill within 1 to 2 h, yielding undetectable bacterial counts (i.e., <100 CFU/ml) at 24 h for all strains. Vancomycin at all concentrations against all strains studied demonstrated bacteriostatic activity and maintained bacterial counts at approximately the initial inocula.

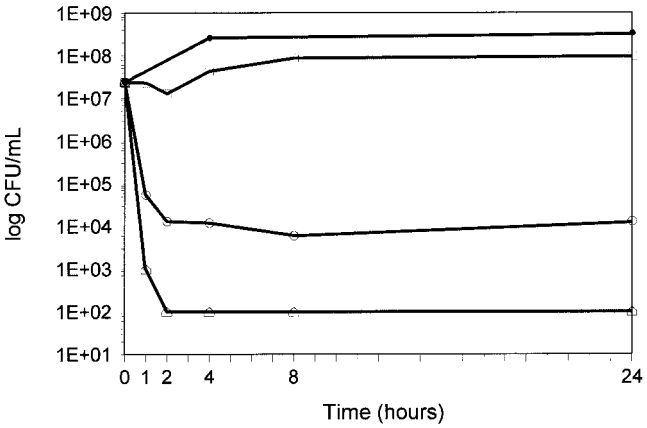

FIG. 1. Time-kill curves for LY at concentrations of 1, 10, and 100 times the MIC against *E. faecium* #560 (*vanA*). Log CFU/milliliter values are as follows: 1E+09, 1×10^9; 1E+08, 1×10^8, etc. ●, growth control; □, LY at 1 times the MIC; ○, LY at 10 times the MIC; ⌒, LY at 100 times the MIC.

In summary, we observed bactericidal, dose-dependent LY activity which was maintained against multiple-drug-resistant *vanA E. faecium*. These data are consistent with those of Schwalbe et al., who also reported dose-dependent killing against a *vanA* vancomycin-resistant enterococcal strain (2). Those investigators concluded that LY concentrations of ≥16 times the MIC were required to produce a 3 $\log_{10}$ decrease in viable colonies at 24 h.

TABLE 1. Time-kill curve parameters characterizing LY activity at 1, 10, and 100 times the MIC against *E. faecium*

Strain	Result ($\log_{10}$ CFU/ml)[a] for LY at:					
	1× MIC		10× MIC		100× MIC	
	Maximal kill at any time point	Bacterial kill at 24 h	Maximal kill at any time point	Bacterial kill at 24 h	Maximal kill at any time point	Bacterial kill at 24 h
#23-1[b]	−1.50	−0.03	−3.92	−2.64	>−5.18	>−5.18
#559[c]	−1.48	−0.34	−3.24	−2.59	>−4.85	>−4.85
#560[d]	−0.27	0.60	−3.30	−3.27	>−5.38	>−5.38

[a] Bacterial count after incubation minus the initial inoculum (a negative value indicates net kill and a positive value indicates net growth).
[b] Vancomycin-susceptible *E. faecium*; LY MIC = 0.63 μg/ml.
[c] Probe-positive *vanB E. faecium*; LY MIC = 0.25 μg/ml.
[d] Probe-positive *vanA E. faecium*; LY MIC = 1 μg/ml.

The study of more strains, use of additional LY concentrations within the range defined by our study, and measurement of free-antibiotic activity in serum rather than broth are warranted. Furthermore, investigations of LY in combination with penicillins and aminoglycosides are required.

This study was supported by Lilly Research Laboratories and a University of Manitoba research grant. S.A.Z. is supported by an Eli Lilly Canada postdoctoral fellowship. J.A.K. is supported by a PMAC-HFR/MRC postdoctoral fellowship.

REFERENCES

1. **National Committee for Clinical Laboratory Standards.** 1995. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. National Committee for Clinical Laboratory Standards, Villanova, Pa.
2. **Schwalbe, R. S., A. C. McIntosh, S. Qaiyumi, J. A. Johnson, R. J. Johnson, K. M. Furness, W. J. Holloway, and L. Steele-Moore.** 1996. In vitro activity of LY333328, an investigational glycopeptide antibiotic, against enterococci and staphylococci. Antimicrob. Agents Chemother. **40:**2416–2419.
3 **Zelenitsky, S., B. Booker, J. Karlowsky, D. Hoban, A. Kabani, M. Zeckel, and G. Zhanel.** 1996. Bactericidal activity of an investigational glycopeptide, LY333328, against vancomycin-sensitive and vancomycin-resistant *Enterococcus faecium*, abstr. F200, p. 135. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

Sheryl A. Zelenitsky
James A. Karlowsky
George G. Zhanel
Faculty of Pharmacy and Department of Medical Microbiology, Faculty of Medicine
University of Manitoba
Winnipeg, Manitoba, Canada

Daryl J. Hoban
Department of Clinical Microbiolgy
Health Sciences Centre
Winnipeg, Manitoba, Canada

Thalia Nicas
Lilly Research Laboratories
Indianapolis, Indiana