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Activity of LY146032 against Enterococci with and without High-Level Aminoglycoside Resistance, Including Two Penicillinase-Producing Strains

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We tested the activity of LY146032 (LY) against 57 strains of enterococci collected from Chile, Thailand, and the United States. Some of the strains were resistant to high levels of gentamicin or streptomycin (or both), and two produced beta-lactamase (Bla^+). MICs of LY ranged from 0.5 to 8 μg/ml, and MBCs ranged from 1 to 64 μg/ml. In time-kill assays, a 2 to 3 $\log_{10}$ killing effect was observed with LY against two Bla^+ strains of *Streptococcus* (*Enterococcus*) *faecalis* and against three strains that were highly resistant to streptomycin and gentamicin. Synergism was demonstrated with LY and streptomycin against a Bla^+ strain lacking high-level streptomycin resistance. These in vitro results suggest that LY should be studied further for possible use in treatment of enterococcal infections.

LY146032 (LY), a new lipopeptide compound that inhibits bacterial cell wall synthesis (1), has recently been shown to have good activity against enterococci, with MICs ranging from 0.25 to 8 μg/ml and MBCs near the MICs for the enterococci tested (2, 3, 13). This is potentially significant since an increasing number of enterococci have been found to be resistant to high levels of all aminoglycosides and are thus resistant to penicillin-aminoglycoside synergism (5, 9, 14). Two penicillinase-producing strains of *Streptococcus* (*Enterococcus*) *faecalis* have recently been identified (7, 8), further complicating the therapy of enterococcal infections. In this study, we determined the inhibitory and bactericidal activity of LY against two penicillinase-producing enterococci and other enterococci with and without high-level aminoglycoside resistance that were isolated from three distinct geographic locations.

MATERIALS AND METHODS

Bacterial strains. Enterococci highly resistant (MIC, >2,000 μg/ml) to streptomycin only (Sm^r) (21 strains), gentamicin only (Gm^r) (1 strain), both gentamicin and streptomycin (30 strains), or neither (5 strains) were collected from Santiago, Chile; Bangkok, Thailand; and Houston, Tex. All of the Gm^r strains were identified as *S. faecalis* with the API 20S system (Analytab Products, Plainview, N.Y.). Strain PA is a clinical isolate of *S. faecalis* which is highly resistant to all aminoglycosides and produces penicillinase (Bla^+) (4, 7). Strain 67 × 22 is a Gm^r Bla^+ transconjugant derived from mating strain HH22 (8) with enterococcal strain 67, a rifampin- and fusidic acid-resistant derivative of a clinical enterococcal isolate; this Bla^+ transconjugant is not highly Sm^r and therefore was used instead of strain HH22 in synergy experiments. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Streptococcus pyogenes* ATCC 19615 were also included.

Susceptibility testing and synergy studies. MICs were determined by broth micro- and macrodilution methods according to the protocol of the National Committee for Clinical Laboratory Standards (11), with the modification that Mueller-Hinton broth supplemented with 50 μg of calcium per ml (as recommended by Eli Lilly Research Laboratories) was used. The antibiotics used were penicillin G (E. R. Squibb & Sons, Princeton, N.J.), vancomycin and LY (Eli Lilly Research Laboratories, Indianapolis, Ind.), imipenem (Merck Sharp & Dohme Research Laboratory, Rahway, N.J.), streptomycin (Sigma Chemical Co., St. Louis, Mo.), and gentamicin (Elkins-Sinn Pharmaceuticals). Agar dilution assays were performed with a Steers replicator and Mueller-Hinton agar without additional calcium (11). MBCs (11) were determined by spreading 50 μl onto one-fourth of a brain heart infusion agar plate. Possible antibiotic carry-over was investigated by spreading approximately 10^5 CFU/ml onto one-fourth of a Mueller-Hinton plate previously spread with 50 μl of LY at various concentrations (12).

Time-kill and synergy experiments were performed according to the procedure described by Moellering et al. (6) with Mueller-Hinton broth supplemented with 50 μg of calcium per ml. Penicillin (10 U/ml), streptomycin (20 μg/ml), and vancomycin, imipenem, and LY (10 μg/ml each) were used. Samples were removed and serially diluted, and 100 μl of the appropriate dilutions was spread onto the entire surface of a brain heart infusion agar plate which was then incubated for 48 h at 37°C. Assuming maximal plating efficiency, the minimum number of detectable CFU per milliliter would be 10. Lack of antibiotic carry-over was demonstrated as outlined above.

RESULTS AND DISCUSSION

LY MICs and MBCs for enterococcal strains. The geometric mean MIC of LY for the 57 strains tested by agar dilution was 2.5 μg/ml with a range of 0.5 to 8 μg/ml. The geometric mean MIC for the 18 strains tested by microdilution was 3.2 μg/ml with the same range as given above. Twelve strains were tested by macrodilution, and the geometric mean MIC was 1.2 μg/ml (range, 0.25 to 8 μg/ml). These results concur with those previously obtained for enterococci (2, 3, 13). For 12 strains tested by all three methods, the MICs for 50 and 90% of these strains were, respectively, 2 and 4 μg/ml by

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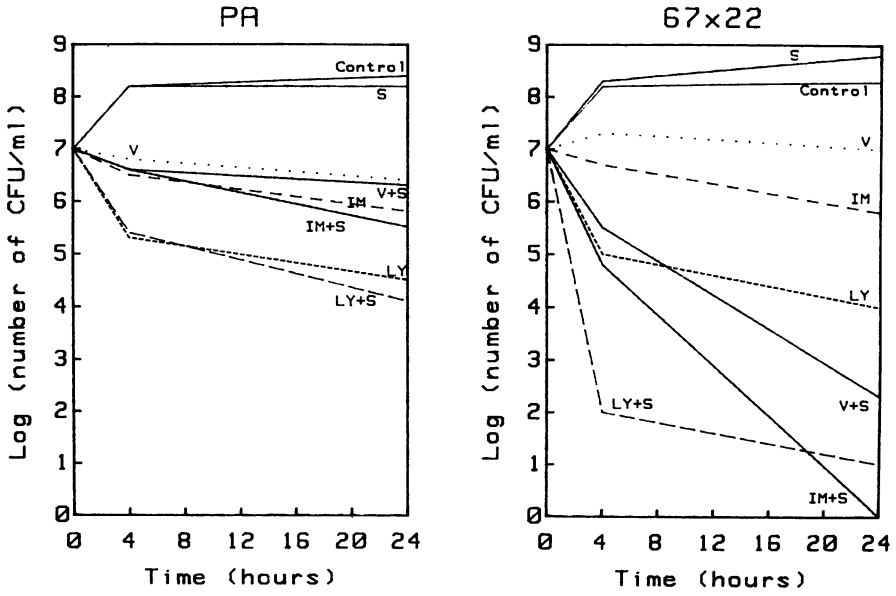

FIG. 1. Time-kill experiments with Bla$^+$ enterococcal strains PA (Sm^r Gm^r Bla$^+$) and 67 × 22 (Sm^s Gm^r Bla$^+$) with LY. The antibiotics used were streptomycin (S), 20 μg/ml; and vancomycin (V), imipenem (IM), and LY, 10 μg/ml. The lowest number of detectable CFU per milliliter was 10.

agar dilution, 1 and 2 μg/ml by broth macrodilution, and 4 and 8 μg/ml by broth microdilution. MICs for the two Bla$^+$ strains were 0.5 and 1 μg/ml by agar dilution and 2 μg/ml by broth microdilution.

The geometric mean MBC for the 12 strains tested by broth macrodilution was 6.7 μg/ml, and that for the 18 strains tested by broth microdilution was 7.4 μg/ml. These results are also in agreement with the MBCs previously reported for this compound against enterococcal strains (1). For 12 strains tested by both methods, the MBCs for 50 and 90% of the strains by broth microdilution were 8 and 16 μg/ml, respectively (range, 4 to 25 μg/ml); those by broth macrodilution were 8 and 32 μg/ml, respectively (range, 1 to 64 μg/ml). There was no difference in the MICs or MBCs for the highly Sm^r or Gm^r strains as compared with the more susceptible strains or in the strains collected from the different geographic locations. Our larger sample size did not produce an antibiotic carry-over effect.

As a control for our methods of antibiotic susceptibility testing, broth microdilution MICs and MBCs of vancomycin and penicillin were determined for 16 strains. MICs were ≤8 μg/ml, and MBCs were >256 μg/ml. In the agar dilution assay, the MICs for *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. pyogenes* ATCC 19615 were >1,280, 1, and <0.125 μg/ml, respectively, which correlate well with results previously reported (11).

Effect of inoculum on LY MIC for aminoglycoside-resistant enterococci. Since some infections involve a large number of bacteria and since various agents have decreased efficacy at high inocula (10, 13), we tested the effect of low and high inocula on the MICs of LY. For each of the seven strains tested, including PA (Bla$^+$), there was an eightfold or smaller increase in LY MIC with an inoculum of 10^7 CFU/ml as compared with an inoculum of 10^3 CFU/ml. Five of the seven strains showed a fourfold or smaller increase in LY MIC with the higher inoculum. Fass and Helsel have also reported little or no inoculum effect when LY was tested against several enterococcal strains at various bacterial concentrations (3).

Time-kill curves and synergy studies of Bla$^+$ *S. faecalis* 67 × 22 and PA. In time-kill curves, a 2 to 3 $\log_{10}$ reduction in CFU per milliliter was consistently demonstrated in repeated experiments with LY against strain 67 × 22, and a 3 to 4.5 $\log_{10}$ reduction was demonstrated with strain PA (both strains are Bla$^+$). A 1 to 2 $\log_{10}$ killing effect was demonstrated with imipenem against both strains, and a <0.5 $\log_{10}$ killing effect was seen with vancomycin. These results are demonstrated by the representative experiment shown in Fig. 1. Three other aminoglycoside-resistant strains were also tested in time-kill experiments with similar results; that is, a 2 to 4 $\log_{10}$ killing effect was seen with LY alone.

A synergistic effect of LY plus streptomycin was demonstrated with strain 67 × 22 (Bla$^+$ Gm^r), which is not highly Sm^r, but not with strain PA, which is highly Sm^r. No synergism was shown for strain 67 × 22 when LY was combined with gentamicin. Synergism was demonstrated against 67 × 22 but not PA when either vancomycin or imipenem was combined with streptomycin. Log-phase cultures of strains 67 × 22 and PA gave the same results in time-kill and synergy studies as did overnight cultures. Although it may be reassuring that synergism can occur with Bla$^+$ strains if high-level aminoglycoside resistance is not present, both Bla$^+$ clinical isolates described to date (PA and HH22) have been highly resistant to all aminoglycosides (7, 8) and do not show synergism with any aminoglycoside.

In conclusion, LY seems to hold promise for the treatment of infections caused by enterococci, including those which are aminoglycoside resistant or penicillinase producing. Our in vitro results suggest that LY used alone may be useful as a substitute for penicillin or ampicillin in patients who are allergic to penicillin or against Bla$^+$ strains for therapy of enterococcal urinary tract and soft-tissue infection. It also appears that LY may be effective in combination with an aminoglycoside for enterococcal endocarditis. Whether the bactericidal activity is sufficient for LY alone to cure enterococcal endocarditis should be investigated further; this would be particularly important for infections caused by

strains highly resistant to all aminoglycosides, for which there is no standard regimen that is bactericidal.

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