Activity of LY146032 In Vitro and in Experimental Enterococcal Pyelonephritis

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The efficacy of LY146032 (LY), a new lipopeptide antibiotic, was compared with that of vancomycin, ciprofloxacin, ceftaxime, imipenem, and gentamicin and combinations of LY-ceftaxime, LY-imipenem, and LY-gentamicin against 15 strains of Streptococcus (Enterococcus) faecalis by microtiter dilution and checkerboard techniques. LY was effective within a very narrow range of drug concentrations (from 0.125 to 2.0 $\mu$g/ml) and was more active than other agents tested against S. faecalis. Enhanced inhibition of S. faecalis was seen more frequently with combinations of either penicillin or ampicillin and an aminoglycoside than with combinations of LY and gentamicin, imipenem, or ceftaxime. The in vivo efficacy of LY was compared with that of vancomycin and ampicillin alone and combinations of vancomycin-gentamicin, ampicillin-gentamicin, and LY-gentamicin in a rat model of chronic enterococcal pyelonephritis. At a dose of 10 mg/kg given twice daily, LY reduced the number of organisms per kidney significantly compared with that in infected untreated controls within 48 h after the initiation of therapy. At 20 mg/kg given once a day, LY was less effective but reduced colony counts significantly after 4 days of therapy, and its activity was comparable to that of vancomycin or vancomycin-gentamicin given twice daily. LY may be a promising agent for the treatment of enterococcal infections.

LY146032 (LY), a recently developed biosynthetic antibacterial agent belonging to a new class of antibiotics known as lipopeptides, exhibits inhibitory and bactericidal activity against gram-positive bacteria. Limited therapeutic options are available against some of these organisms. At present, enterococci, including Streptococcus (Enterococcus) faecalis, must be treated with vancomycin in penicillin-allergic patients. Combination therapy is often used in serious enterococcal infections. We studied the in vitro activity of LY and a number of antibiotics, individually and in various combinations, against S. faecalis.

In addition, the in vivo potential of LY was studied in a model of chronic enterococcal pyelonephritis. This model has been well studied and has been used previously to determine effective modes of therapy and parameters for successful treatment (5, 6). Its progressive renal infection mimics human enterococcal pyelonephritis, and it is useful for evaluating the longer treatment courses that are usually required for serious human enterococcal infections.

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MATERIALS AND METHODS

**Bacterial strains.** A total of 15 strains of S. faecalis were collected from the microbiology laboratory of Yale-New Haven Hospital.

**Antibiotics.** Antibiotics were generously provided as standard powders by the following manufacturers: LY, vancomycin, and penicillin by Eli Lilly & Co., Indianapolis, Ind.; ceftaxime by Roche Diagnostics, Div. Hoffmann-La Roche, Inc., Nutley, N.J.; imipenem by Merck & Co., Inc., Rahway N.J.; ampicillin by Bristol Laboratories, Syracuse, N.Y.; and ciprofloxacin by Miles Pharmaceuticals, West Haven, Conn. Gentamicin was obtained as a solution at 40 mg/ml (Elkins-Sinn, Inc., Cherry Hill, N.J.).

**Antibiotic susceptibility testing.** MICs were determined by a microtiter broth dilution technique (9) in microtiter plates with 96 U-shaped wells (Dynatech Laboratories, Inc., Alexandria, Va.) containing the antibiotics diluted in Mueller-Hinton broth supplemented with 50 mg of Ca$^{2+}$ and 25 mg of Mg$^{2+}$ per liter of medium. For the MIC determinations 100 $\mu$l of twofold serial dilutions of each antibiotic was added to each well. One well in each row contained only broth to serve as an inoculation and growth control. Bacteria were grown overnight and then diluted in fresh broth to a density of approximately 10$^6$ CFU/ml, as determined by spectrophotometry. A semiautomatic inoculator (Dynatech) was used to deliver 5 x 10$^6$ CFU to each well containing 100 $\mu$l of drug or broth (final concentration of bacteria, 5 x 10$^5$ CFU/ml). The MIC was taken as the lowest concentration of drug with no visible growth after 18 h of incubation at 37°C. MBCs were determined by inoculating Mueller-Hinton agar plates with a 1.5-$\mu$l fraction from each well of the MIC plate with an automatic inoculator (Dynatech). Agar plates were incubated at 37°C for 24 h. The MBC was read as the lowest antibiotic concentration which gave no visible growth.

Organisms were screened for high-level gentamicin resistance by streaking a colony on enriched nutrient agar plates containing gentamicin at a concentration of 2,000 $\mu$g/ml (10). Resistant organisms showed confluent growth, whereas those inhibited by 2,000 $\mu$g of gentamicin per ml or less showed no growth.

The microtiter broth dilution technique described above was also used to test antibiotic combinations. One drug of the combination being tested was placed horizontally in the tray and the other drug of the combination was placed vertically, resulting in a checkerboard array of drug combi-
The concentrations chosen for each drug ensured a range of from one-eighth to two times the MIC wherever possible. The same inoculum was delivered to each well, with one well per row serving as a drug-free control. After incubation at 37°C for 24 h, the lowest concentration of drugs showing no visible growth was taken as the best combination.

**Synergy criteria.** Synergy data were evaluated by using previously published methods (3, 12, 13, 15), by which the fractional inhibitory concentrations (FICs) and FIC indices (FIXs) were calculated. The FIC for a single antibiotic was determined as the ratio of that drug in combination divided by the MIC when that drug was used alone. The FIX was calculated as the numerical sum of the two FICs for a given combination. The following criteria were used: FIX less than or equal to 0.5, synergy; FIX greater than 4.0, antagonism.

**In vitro studies.** (i) **Animals.** White female pathogen-free Sprague-Dawley (Camm) rats (weight, 110 to 135 g) were used.

(ii) **Levels in serum.** Concentrations of LY in serum were determined by a microbiological assay by the agar well diffusion method (2), with *Micrococcus luteus* ATCC 9341 used as the test organism. Antibiotic medium 1 (Penassay seed agar [Difco Laboratories, Detroit, Mich.]) was the test medium. Standards were prepared in pooled normal rat serum. Assay plates were incubated at 37°C overnight. Animals received 10 mg of LY per kg subcutaneously, and blood was drawn by cardiac puncture at 20, 40, 60, 120, 240, and 360 min after single-dose administration of the drug.

Concentrations of vancomycin in serum were determined by a fluorescence polarization immunoassay technique (TDX analyzer; Abbott Laboratories, North Chicago, Ill.) by using commercial vancomycin standards (Abbott). Animals received 20 mg of vancomycin per kg, and blood was drawn as described above for LY.

(iii) **Organism.** *S. faecalis* ATCC 23241, kindly supplied by Phyllis Guze and also referred to as strain GK, has been described in detail by Guze et al. (5, 6) and has been used previously to produce chronic, nonobstructed enterococcal infection in rat kidneys.

**Drug efficacy studies.** Inocula containing 10⁸ CFU/ml were prepared from an 18-h brain heart infusion broth culture. The exact number in each inoculum was subsequently determined by the standard serial 10-fold dilution agar pour plate technique. Animals were challenged intravenously with a 1.0-ml inoculum. This inoculum is known to infect the renal medulla of normal rats (5). Twenty-four hours later the animals were divided into eight groups and were given either saline only (controls) or antibiotic therapy initiated with LY at 10 mg/kg (LY10), LY at 20 mg/kg (LY20), LY10 plus gentamicin at 1.5 mg/kg, vancomycin at 20 mg/kg, vancomycin at 20 mg/kg plus gentamicin at 1.5 mg/kg, ampicillin at 30 mg per rat per injection, and ampicillin at 30 mg per rat plus gentamicin at 1.5 mg/kg. Animals received LY20 once daily; all other drugs were administered twice daily. Vancomycin and LY were given subcutaneously; ampicillin and gentamicin were given intramuscularly. Drugs were administered for up to 13 days.

Animals were sacrificed at 6 and 24 h (controls only) and at 3, 5, 8, and 14 days after infection. Kidneys were removed and homogenized in a tissue grinder with tryptic (Difco) soy broth, and serial 10-fold dilutions were prepared and cultured by the agar pour plate technique. Colony counts were determined after incubation for 24 and 48 h at 37°C. By this technique as few as 10 CFU/g of kidney tissue can be detected (1). Statistical analysis was performed by using the Fisher exact or the Student *t* test. A *P* value of ≤0.05 was considered significant.

### RESULTS

**In vitro studies.** The cumulative distribution of the MIC data for the most effective antibiotics against strains of *S. faecalis* is shown in Fig. 1. While the vancomycin and LY MICs for 90% of strains tested were similar against *S. faecalis*, the vancomycin MIC for 50% of strains tested was approximately 4 times higher than that of LY. LY was inhibitory within a very narrow range of drug concentrations (0.125 to 2.0 μg/ml) against all organisms tested. LY and vancomycin were the only antibiotics to inhibit 100% of the *S. faecalis* strains at 2 μg/ml. High-level gentamicin resistance (>2,000 μg/ml) was observed with 1 of our 15 strains of *S. faecalis*. MBCs, determined by agar plating, showed that LY was bactericidal at concentrations less than or equal to 4 times the MIC for all organisms studied.

The FIXs of various combinations of antibiotics against 15 strains of *S. faecalis* are shown in Table 1. The combination of LY-gentamicin was synergistic against eight *S. faecalis* isolates and demonstrated enhanced activity against a total of 14 of the *S. faecalis* isolates. One isolate had a high level of gentamicin resistance, and the FIX could not be determined. Similar results with these 15 strains were observed with all combinations containing gentamicin (Table 1). In contrast, enhanced inhibitory activity was seen against all 15 strains when tested against both nongentamicin combinations (LY-imipenem and LY-ceftriaxone). The concentrations of ceftriaxone in combination with LY, however,

![Graph showing cumulative (CUM) distribution of MICs](http://aac.asm.org/)

**TABLE 1.** FIXs of various combinations of antibiotics against 15 strains of *S. faecalis*

<table>
<thead>
<tr>
<th>Combination</th>
<th>No. of strains with the following FIXs:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.50</td>
</tr>
<tr>
<td>LY-gentamicin</td>
<td>8</td>
</tr>
<tr>
<td>Vancomycin-gentamicin</td>
<td>9</td>
</tr>
<tr>
<td>Penicillin-gentamicin</td>
<td>13</td>
</tr>
<tr>
<td>Ampicillin-gentamicin</td>
<td>13</td>
</tr>
<tr>
<td>LY-imipenem</td>
<td>4</td>
</tr>
<tr>
<td>LY-ceftriaxone</td>
<td>10</td>
</tr>
</tbody>
</table>

* Gentamicin-resistant strain.
which were required to achieve enhanced activity, were so high that this combination (LY-ceftiraxone) would have no clinical relevance.

**In vitro studies with strain GK enterococcus.** In vitro MICs of the various antibiotics against *S. faecalis* GK, which was used in our in vivo experiments, were as follows: LY, 0.5 µg/ml; ampicillin, 2.0 µg/ml; gentamicin, 5.0 µg/ml; vancomycin, 1.0 µg/ml. Strain GK did not exhibit high-level gentamicin resistance.

**Levels in serum.** The mean drug levels for LY and vancomycin are shown in Table 2; each value reported represents the mean drug concentration observed in two rats. Levels in serum in excess of the MIC for strain GK enterococcus were maintained for more than 6 h with LY and for up to 4 h with vancomycin.

**In vivo studies with strain GK enterococcus.** Intravenous challenge with 10⁹ CFU of *S. faecalis* GK resulted in persistent renal infection in the saline-treated animals, with the mean log₁₀ CFU per gram of kidney (± standard error of the mean) rising progressively from 5.67 ± 0.20 at 24 h after inoculation to 7.03 ± 0.24 at 14 days (Fig. 2 and Table 3). After 48 h of therapy (day 3 from challenge) only rats treated with LY10, ampicillin, or LY10-gentamicin had bacterial counts significantly lower than those of controls *(P < 0.001 to 0.005). Forty-eight hours of treatment with LY10 or ampicillin was significantly better than that with vancomycin *(P < 0.005 and P < 0.025, respectively). The differences between treatment with either LY10 or ampicillin and treatment with LY20 were not statistically significant. All experimental groups had significantly lower counts than controls *(P < 0.001 to 0.01) after 4 or more days of therapy. Four days of therapy with ampicillin or ampicillin-gentamicin resulted in bacterial counts that were significantly lower than that with all other treatment groups *(P < 0.001). Treatment with LY10 for 4 days resulted in significantly lower counts than that with vancomycin *(P < 0.005) or LY20 (P < 0.01). After 7 to 13 days of therapy there was no statistically significant difference between LY10 and ampicillin, although the CFU per gram of rat kidney remained lower with ampicillin. Both LY10 and ampicillin produced counts that were significantly lower than those with vancomycin or LY20 at 7 and at 13 days of therapy *(P < 0.001 to 0.05). The addition of gentamicin to LY10, ampicillin, or vancomycin did not reduce further the number of organisms in the kidney at all points examined.

In previous studies it has been documented that pyelonephritis correlates with >5 log₁₀ CFU/g of kidney (1, 5). Kidneys with at least that number of bacteria were considered infected. All kidneys cultured from control rats had mean bacterial counts greater than 5 log₁₀ CFU/g of kidney (Table 3). After 2 days of therapy only LY10 and LY10-gentamicin significantly reduced the number of infected kidneys compared with controls *(P = 0.016 and P = 0.002, respectively). After 13 days of therapy fewer infected kidneys were seen in all treated rats (4 of 46) when compared with those in controls *(P < 0.0001). In fact, throughout the course of these experiments, only a few infected kidneys were seen with LY10 (1 of 23), ampicillin (2 of 24), LY10-gentamicin (3 of 24), and ampicillin-gentamicin (4 of 24), with no significant differences among these groups. Although the group treated with LY20 had more infected kidneys (8 of 25) than the groups treated with LY10, ampicillin, LY10-gentamicin, or ampicillin-gentamicin, this difference was significant only when compared with those treated with LY10 *(P = 0.024). More kidneys remained infected with vancomycin (14 of 24) or vancomycin-gentamicin (12 of 24) than with LY20 treatment, but the differences between these groups were not statistically significant. Amoxicillin, ampicillin-gentamicin, LY10, and LY10-gentamicin, however, were all significantly more effective in reducing infection than either vancomycin or vancomycin-gentamicin *(P < 0.001 to 0.03). Although all treatments reduced mean bacterial counts significantly, only ampicillin and ampicillin-gentamicin effectively sterilized the kidneys (Table 3). After 2 days of therapy no sterile kidneys were found in any treatment group or controls. After 4 days of therapy all ampicillin- or ampicillin-gentamicin-treated kidneys were sterile (Table 3). There were no other treatment groups for which sterile kidneys were found until day 13 of therapy, at which time 5 of 12 kidneys from LY10 and LY10-gentamicin and 3 of 12 kidneys from vancomycin and vancomycin-gentamicin treatment groups were sterile compared with 12 of 12 kidneys from ampicillin and ampicillin-gentamicin treatment groups.

![FIG. 2. Comparison of therapeutic regimens in rats with enterococcal pyelonephritis. Animals were inoculated with strain GK enterococcus. Twenty-four hours later antibiotic therapy was started. Animals were sacrificed at the indicated times, and their kidneys were cultured to determine the CFU per gram of kidney. Each point is the mean value for six kidneys. The various treatment groups were controls (○), LY10 administered twice daily (○), LY20 administered once daily (×), ampicillin (30 mg) (A) administered twice daily (△), vancomycin (20 mg/kg) (V) administered twice daily (□), ampicillin (30 mg/kg)-gentamicin (1.5 mg/kg) (A+G) administered twice daily (△), vancomycin (20 mg/kg)-gentamicin (1.5 mg/kg) (V+G) administered twice daily (■), and LY10-gentamicin (1.5 mg/kg) (LY+G) administered twice daily (●).](http://aac.asm.org/)

### TABLE 2. Antibiotic levels in rat serum

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Drug level (µg/ml) in serum*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LY</td>
</tr>
<tr>
<td>20</td>
<td>9.0</td>
</tr>
<tr>
<td>40</td>
<td>15.0</td>
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<td>60</td>
<td>22.0</td>
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<td>120</td>
<td>34.0</td>
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<tr>
<td>240</td>
<td>22.5</td>
</tr>
<tr>
<td>360</td>
<td>12.9</td>
</tr>
</tbody>
</table>

*Each value represents the mean drug concentration observed in two rats. Animals were given a single subcutaneous injection of LY-10. Blood was drawn by cardiac puncture at the indicated times. Animals were given a single subcutaneous injection of vancomycin at 20 mg/kg.

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(P < 0.005 and P < 0.0004, respectively). No control kidneys were found to be sterile.

DISCUSSION

LY demonstrated significant in vitro activity against S. faecalis. Only LY and vancomycin were effective against all the strains of S. faecalis tested. Synergy was seen in vitro with combinations of LY and other antibiotics against most of the strains of S. faecalis tested. Also, LY was observed to be bactericidal at concentrations near the MIC for S. faecalis. Because serious enterococcal infections are frequently treated with combination therapy by using a cell wall-active agent and an aminoglycoside (8). LY may potentially offer alternative single-agent therapy, particularly if the enterococcus has a high level of gentamicin resistance.

The activity of LY against enterococci was confirmed in vivo by using a rat model of pyelonephritis. This model produced a chronic and progressive infection similar to the course of pyelonephritis with this organism observed in humans. Also, this model is particularly useful for evaluating longer courses of therapy for enterococcal infections, like those that are required for the treatment of human pyelonephritis or endocarditis (8). Eliopoulos and colleagues (4) have shown that LY is effective in reducing cardiac vegetation in a rat model of enterococcal endocarditis, but therapy could only be given for 5 days in that lethal model. Nevertheless, both LY and vancomycin were shown to be effective in reducing colony counts compared with controls (4).

In our model, LY was the only antibiotic that was able to reduce bacterial counts in the kidneys significantly compared with controls after only 2 days of therapy. After 13 days of therapy, all treatment groups, including LY20 administered once daily, had at least a 3 log10 reduction in bacterial counts per g of kidney tissue. LY10 and LY-gentamicin were also effective in eliminating pyelonephritis (i.e., <5 log10 CFU/g of kidney) in over 90% of the kidneys examined after only 2 days of therapy. Ampicillin, however, was the most effective antibiotic used in the animal model because it reduced colony counts per gram of kidney significantly and produced sterile kidneys. Similar observations have been reported previously by other workers (7, 11, 14).

In our model LY10 given twice daily was more effective than vancomycin given at 20 mg/kg twice daily both in producing lower absolute colony counts and fewer infected kidneys after 13 days of therapy. Higher peak levels of LY10 than vancomycin (20 mg/kg) were obtained in serum, and higher levels of LY were seen at 6 h after drug administration. These higher levels in serum combined with a lower MIC of LY than of vancomycin for strain GK enterococcus may explain the enhanced efficacy of LY compared with that of vancomycin.

When given in a once daily dose, LY20 was effective in reducing bacterial colony counts and in eliminating pyelonephritis compared with controls; but it was less effective than other forms of therapy, particularly when LY was given in two divided doses totaling 20 mg/kg per day. This observation suggests that the 24-h interval for therapy at this dose is too long for maximum therapeutic benefit. The once daily dose of LY, however, was comparable to that of vancomycin given twice daily.

These results confirm that LY is active in vitro and has bactericidal activity against enterococci. Its in vivo efficacy was demonstrated in a rat model of chronic enterococcal pyelonephritis. LY may be a promising agent for the treatment of enterococcal infections.

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LITERATURE CITED


