LY146032, Alone and in Combination with Gentamicin, for the Treatment of Enteroococcal Pyelonephritis in the Rat Model

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The in vivo and in vitro activity of LY146032 against Streptococcus faecalis GK was examined. The following MICs and MBCs in micrograms per milliliter were obtained: ampicillin, 0.8 and 1.5; vancomycin, 0.8 and 50; gentamicin, 12 and 25; and LY146032, 0.8 and 6. A time-kill-curve study involving approximately 10^6 organisms per ml showed a drop in the number of organisms of almost 2 log_{10} in the tube containing LY146032 (2 μg/ml) plus gentamicin (4 μg/ml) compared with bacterial numbers for the control at 4 h of incubation. However, regrowth was observed at 24 and 48 h, and no in vitro synergism was observed with the combination. A sample (1 ml) of overnight growth of this enteroococcal strain at a concentration of 10^7 was then injected intravenously into 184 male Wistar rats weighing about 100 g each. After 12 days, 10 rats were sacrificed and the remaining ones were randomized into four treatment groups: (i) untreated control, (ii) LY146032 (3 mg) given subcutaneously, (iii) gentamicin (0.8 mg) given intramuscularly, and (iv) LY146032 plus gentamicin at the same dosages as when the drugs were used singly. The rats received antibiotics for 4 weeks twice daily, and approximately 10 rats in each group were sacrificed for quantitative kidney cultures at 1, 2, 4, and 6 weeks after the start of therapy. At the end of the 4- and 6-week periods, significantly better results were obtained with the combination of LY146032 plus gentamicin than with no treatment or treatment with single antibiotic.

LY146032 is a novel cyclic lipopeptide antibiotic derived from A219786, a complex of acidic antibiotics containing the same peptide core (3). It has been shown to be highly bactericidal against all species of streptococci and staphylococci tested, including enterococci (4; N. Allen, W. Alborn, Jr., J. Hobbs, Jr., and H. Percifield, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1081, 1984). This bactericidal activity against enterococci is more impressive than that of vancomycin, to which most strains of enterococci are generally tolerant. The possibility exists, therefore, that LY146032 may be used as a single-agent therapy for serious enterococcal infections. Currently, systemic enterococcal infections are generally treated with penicillin or ampicillin in combination with an aminoglycoside such as gentamicin. The alternative for patients who are allergic to penicillin or who cannot receive the drug for other reasons is a combination of vancomycin plus an aminoglycoside. The potential nephro- and ototoxicity of these two drugs in combination, however, is of concern to most clinicians.

The rat model of enterococcal pyelonephritis has been an established model of systemic enterococcal infection that is easily reproducible and has been used for therapeutic experiments in the past (5, 6, 9). We employed this model to test the efficacy of LY146032 for the therapy of an experimental pyelonephritis due to a strain of Streptococcus faecalis. LY146032 was evaluated as a single agent as well as in combination with gentamicin for this infection.

(A preliminary report of this work has been presented [Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, A51, p. 9].)

MATERIALS AND METHODS

Bacteria. The bacterium that was used was S. faecalis GK. We selected this strain for the study because of its impressive reproducibility in producing enterococcal pyelonephritis when given intravenously to male Wistar rats (5, 6, 9). This model of enterococcal infection is also difficult to treat and generally requires 4 to 6 weeks of therapy with a combination of ampicillin and gentamicin (9).

In vitro studies. The laboratory standard powders of the following antibiotics were used: sodium ampicillin (Bristol Laboratories, Syracuse, N.Y.), gentamicin (Schering Corp., Kenilworth, N.J.), and LY146032 (Eli Lilly & Co., Indianapolis, Ind.).

MICs and MBCs were determined by the methods outlined by Barry (1) and by the macrotube dilution method, with calibrated pipettes to transfer 0.01-ml aliquots to blood agar plates for MBC determinations (defined as 99.9% or more of kill). The original inoculum was prepared by allowing the test organism to grow for 4 to 6 h in Mueller-Hinton broth. Subsequently, 0.01 ml of this suspension was added to 5 ml of Mueller-Hinton broth. Then a 0.1-ml aliquot was added to 1.8 ml of Mueller-Hinton broth containing the antibiotic dilutions for MIC determinations. The inoculum used was approximately 10^3 organisms per ml. Calcium and magnesium supplements were added to achieve final concentrations of 50 and 25 mg/liter of broth media, respectively, as recommended by the manufacturer of LY146032. Care was exercised not to touch the sides of the test tubes when aliquots were obtained for MBC determinations. Aliquots of 0.01 ml were spread on the surface of one-half of a blood agar plate with the aid of a sterile glass rod for colony counting for MBC determinations. The lowest antibiotic
concentration showing no growth was determined as the MBC.

Time-kill studies for LY146032 were performed with inocula of $10^8$ to $10^9$ bacteria in the logarithmic phase of growth, by a modification of the method of Krogstad and Moellerling (8). The antibiotic concentrations used were clinically achievable levels but did not exceed one-half of the MBC for the individual antibiotics and were one-half of that value when combinations were used as suggested by Hallander et al. (7). Calcium and magnesium supplementation was performed in a manner similar to that of the MIC procedures. Calibrated pipettes were used to transfer 0.1-ml aliquots to Mueller-Hinton plates for colony counts at the time of mixture (0 h) and subsequently at 4, 24, and 48 h after mixture of the antibiotic solution and bacteria. Techniques in plating similar to those used for MBC determinations were followed. Very gentle mixing at the lowest setting of a vortex mixer was done before obtaining aliquots. Care was exercised not to touch the sides of the tubes when aliquots were removed for subculture. Sterile, unwashed, and unsiliconized test tubes were used, each containing 10 ml of Mueller-Hinton broth, the test inoculum, and (i) no antibiotic (control), (ii) gentamicin (4.0 μg/ml), (iii) LY146032 (2 μg/ml), or (iv) LY146032 (1 μg/ml plus gentamicin [2.0 μg/ml]). Tenfold dilutions of the aliquots were made in normal saline solution. The tubes and plates were incubated at 35°C, and colony counts were done after overnight incubation. Synergy was defined as a decrease of $\geq 2 \log_{10}$/ml in bacterial growth with an antibiotic combination as compared with results for the more bactericidal antibiotic used alone at 24 h of incubation. The MIC, MBC, and time-kill studies were performed in duplicate.

In vivo studies. Pyelonephritis was produced in 184 randomly bred male Wistar rats weighing 100 to 150 g by intravenous injection (via the tail vein) of an overnight growth of the S. faecalis test strain. Aliquots of the inoculum were subcultured on brain heart infusion agar for colony counts after overnight incubation. Two weeks after infection, before institution of therapy, 10 rats were sacrificed under general anesthesia for quantitation of renal infection. The kidneys were removed, weighed, and homogenized in Ten Brack tissue grinders (Corning Glass Works, Corning, N.Y.), and serial 10-fold dilutions made in sterile normal saline solution. Aliquots of 0.1 ml were taken from the homogenate, and alternate dilutions were made thereafter up to $10^{-7}$ dilution for colony counts on brain heart infusion agar. The colony counts were made from 1-ml aliquots incorporated into 20 ml of molten brain heart infusion agar kept at 55°C before pouring after 48 h of incubation. The lower limit for detection of bacteria in the kidneys was 30 CFU/ml of homogenate.

Treatment was started on the remaining rats. These rats were randomly and equally divided into four groups defined by the antibiotics they received twice daily: 1, control (no antibiotics); 2, gentamicin (0.6 mg); 3, LY146032 (3.0 mg [10 mg/kg], as suggested by the manufacturer, with allowance made for weight gains during the study)); and 4, LY146032 (3.0 mg) plus gentamicin (0.6 mg). Injections were given subcutaneously for LY146032 (as suggested by the manufacturer) and intramuscularly for gentamicin on the hind limb (alternately) at 8:30 a.m. and 4:00 p.m. for 4 consecutive weeks. This regimen is similar to that used in previous studies (5, 6, 9) and has been quite convenient for the investigators. Subgroups of about 10 rats from each group were sacrificed at 1, 2, and 4 weeks after initiation of therapy and 2 weeks after the end of therapy. Kidney infection was expressed in numbers of bacteria ($\log_{10}$) for each kidney, and the mean value for the two kidneys was calculated for each rat. The mean values for each treatment group were then calculated. Kidneys with undetectable infection were given a value equivalent to the lower limits of detection. Statistical comparisons were done by the Student t test.

In a separate study, levels of antibiotics in serum and urine samples were determined for noninfected rats after antibiotic administration. After 2 days of therapy, four rats from groups 1 to 3 were sacrificed under penthrane inhalation anesthesia at intervals of 1 and 16 h after antibiotic administration. Urine was aspirated from the bladder at the time of sacrifice, when heart blood samples were also collected. Serum and urine specimens were assayed for antibiotic levels by the disk diffusion method of Edberg and Sabath (2), with B. subtilis as the test organism.

RESULTS AND DISCUSSION

In vitro studies. The MICs and MBCs (in micrograms per milliliter) of various antibiotics for the S. faecalis GK used in this study were as follows: ampicillin, 0.8 and 1.5; gentamicin, 12 and 25; vancomycin, 0.8 and 50; and LY146032, 0.8 and 6.

In the in vitro time-kill-study results are shown in Fig. 1. No synergy was observed between LY146032 and gentamicin at 4, 24, or 48 h of incubation.

In vivo studies. The results of quantitative bacterial counts for the kidneys cultured from the sacrificed rats at 1, 2, and 4 weeks of antibiotic therapy and 2 weeks after cessation of therapy are shown in Fig. 2. At the end of the 4-week course of antibiotic therapy, the bacterial counts obtained from the group treated with combination therapy were significantly lower than those from the control, the LY146032-, and the gentamicin-treated groups. Of 10 rats in the combination therapy group, 5 had no detectable kidney infection at 4 weeks, and 3 had infection in only one kidney. In contrast, all but one rat each in the gentamicin-treated and control groups and all rats in the LY146032-treated group had detectable kidney infections. Two rats in the gentamicin group, one rat in the LY146032 group, and none of the
controls had undetectable infection in one kidney. Two weeks after cessation of therapy, 5 of 11 rats in the combination therapy group had undetectable bilateral kidney infections and an additional 3 more had undetectable infections in one kidney each. In contrast, only three of the LY146032 group, the control group, and none of the gentamicin group had undetectable bilateral kidney infection. In addition, two of the gentamicin group and none of the LY146032 or control group had one kidney with undetectable infection. The undetectable bilateral kidney infections in a few control rats could represent either spontaneous cure (which had been unusual in previous studies) or, more likely, random failure to inject the inoculum intravenously during the initial infection.

The levels of antibiotics in serum and urine samples from a separate group of rats are shown in Table 1. High antibiotic levels in urine specimens were observed up to 16 h after antibiotic injection. The peak levels in sera observed at 1 h after LY146032 administration were lower than expected from the data supplied by the manufacturer but still exceeded the known MIC for the test organism.

LY146032, either alone or in combination with gentamicin in clinically achievable concentrations, was not bactericidal to S. faecalis GK when the time-kill in vitro test for antibiotic synergy was used. Despite the unimpressive results of the in vitro studies, however, the combination of the two drugs demonstrated synergy when used for a 4-week course of therapy against the rat model of enterococcal pyelonephritis from S. faecalis GK. LY146032 given alone at the dosage used, however, was ineffective for this rat model of enterococcal pyelonephritis. It is possible that increasing the dosage of LY146032 given to the rats may improve the results observed with the drug when given alone.

Since this study involved only one strain of S. faecalis, one should exercise caution in the interpretation of the results obtained. Further animal studies should be performed to evaluate the efficacy of LY146032, alone and in combination with aminoglycosides, for experimental enterococcal infections.

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