LY146032 Compared with Penicillin G in Experimental Aortic Valve Endocarditis Caused by Group G Streptococci

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Rabbits with group G streptococcal aortic valve endocarditis received no therapy (controls); repeated doses of procaine penicillin G, 300 mg/kg (body weight) per day, administered intramuscularly; or LY146032, a new peptide antibiotic, 20 mg/kg per day, administered intravenously. Penicillin G and LY146032 reduced mean intravenous group G streptococcal densities significantly below those observed in controls at both day 3 and day 6 of therapy. Penicillin G effected a more rapid clearance of intravenous streptococci than LY146032 by day 3, but not by day 6, of therapy.

Lancefield group G streptococci are increasingly recognized as important causes of serious infections in both immunocompromised and immunocompetent adults (2, 9, 11, 16, 17). The most problematic of these infections have been endocarditis, septic arthritis, and osteomyelitis (8, 9, 11, 17). Penicillin G, with or without an aminoglycoside for synergistic efficacy, has been the cornerstone of treatment for such infections (9, 16, 17). However, the usefulness of treatment with penicillin G in patients with such infections may be limited by adverse drug reactions or by β-lactam tolerance on the part of the infecting strain (14). There is limited experience in vivo with nonpenicillin antibiotic regimens in treating serious group G streptococcal infections. LY146032, a newly developed peptide agent, has potent in vitro bactericidal activity against beta-hemolytic streptococci (7). In this study, we compared the efficacies of LY146032 and penicillin G in the treatment of experimental aortic valve endocarditis caused by a group G streptococcal strain.

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The group G streptococcal strain used in this study was isolated from the blood of a patient with endocarditis. It was identified as a group G streptococcus according to the criteria of Lancefield (10). The MICs and MBCs of both penicillin G and LY146032, as determined by the broth macrodilution technique (12), at ~10² CFU/ml of inoculum in cation-supplemented Mueller-Hinton broth were <0.125 and 0.125 μg/ml, respectively.

The bactericidal rates of penicillin G and LY146032 for the group G streptococcal strain were examined by the timed-kill technique. The group G streptococcal strain in logarithmic phase (final inoculum, ~10³ CFU/ml) was tested in penicillin G- or LY146032-containing (1 or 10 μg/ml, respectively) cation-supplemented Mueller-Hinton broth. Subcultures were obtained at 0, 4, and 24 h after inoculation. Timed-kill curves were then constructed to compare surviving log₁₀ CFU per milliliter versus incubation time (hours). A total of 48 New Zealand White rabbits (2 to 2.5 kg) were anesthetized with ketamine hydrochloride (Bristol Laboratories, Syracuse, N.Y.) and xylazine (Miles Laboratories, Inc., Shawnee, Kans.) and then underwent transcarotid-to-left-ventricular catheterization as previously described (6). The catheters remained in place for the duration of the experiment. Group G streptococci (~10⁶ CFU) were administered intravenously to each animal 24 h after catheterization. Positive blood cultures for the organism at 24 h were considered presumptive confirmation of endocarditis after intravenous challenge (1). Animals were randomized to receive (i) no therapy (controls), (ii) LY146032 (10 mg/kg, twice a day, intravenously), or (iii) procaine penicillin G (150 mg/kg, twice a day, intramuscularly). Treatment was administered for either 3 or 6 days, at which time the animals were killed by rapid intravenous pentobarbital hypersedation (~150 mg). Blood cultures were performed at the time of death, and the heart was removed aseptically. Aortic valve vegetations were removed, homogenized, and quantitatively cultured as previously described (4). The animals were killed at ~18 h after the last antibiotic dose. This timing, as well as serial dilution of the vegetation homogenate during quantitative culturing, minimizes the antibiotic carry-over effect. Animals which expired >6 h before their assigned sacrifice time were not analyzed further (15). Vegetations which were culture negative were considered to contain up to 2 log₁₀ CFU/g (mean vegetation weight, ~0.01 g) (3).

Blood samples for penicillin G and LY146032 serum levels were obtained at ~1 and 18 h postdose. Bacillus subtilis ATCC 6633 was used to determine penicillin G levels by agar well diffusion bioassay, and Micrococcus luteus ATCC 9341 was used for determining LY146032 levels. The lower limits of sensitivity of the bioassays for penicillin G and LY146032 were 0.125 and 0.31 μg/ml, respectively.

The same serum samples used for the assessment of antibiotic concentrations were used for determination of bactericidal activities at ~1 h postdose as determined by a previously described microdilution technique (13). Normal pooled rabbit serum was the diluent for the assay. The highest serum dilution causing >99.9% killing of the infecting streptococcal strain was used as the bactericidal titer (13).

The chi-square test with the Yates correction factor was used for comparing proportional data, and analysis of variance was used for comparing mean log₁₀ CFU per gram of vegetation in the three therapy groups. P values of <0.05 were considered significant.

Penicillin G and LY146032 at both 1 and 10 μg/ml exerted a complete bactericidal effect by 24 h of incubation;
LY146032 at 10 μg/ml caused the most rapid killing of the group G streptococcal strain, resulting in negative cultures by 4 h postdose (Fig. 1).

Between 20 and 25% of the animals died before their assigned sacrifice times (all within the first 72 h postinfection) in the three treatment groups, with no significant differences observed between controls and penicillin G and LY146032 recipients. Both antibiotic regimens were completely effective, resulting in negative cultures of blood taken from all animals at sacrifice (12 of 12 and 13 of 13 sterile cultures from penicillin G and LY146032 recipients, respectively, versus 4 sterile cultures from 12 controls; \( P < 0.0025 \) for both penicillin G and LY146032 versus controls).

Both penicillin G and LY146032 significantly reduced mean vegetation streptococcal densities at day 3 of therapy compared with untreated controls (\( P < 0.001 \) and \( P < 0.02 \), respectively). Penicillin G was somewhat more effective than LY146032 in this regard (\( P < 0.10 \) and \( P > 0.05 \), respectively). By day 6 of treatment, penicillin G and LY146032 were equally effective in significantly reducing intravegetation group G streptococcal densities (\( P < 0.0005 \) versus controls; Table 1).

There was a trend towards a more rapid bactericidal effect with penicillin G than with LY146032 in rendering vegetation culture negative (Table 1). By day 3 of treatment, 70% of sampled vegetations were culture negative for penicillin G recipients as compared with 56% of those from LY146032 recipients (\( P > 0.05 \)). However, by day 6 of treatment, all but one vegetation was rendered culture negative by penicillin G or LY146032 treatment.

Mean levels of penicillin G in serum (± standard error of the mean) at 1-h postdose were 2.9 ± 0.4 μg/ml, whereas no penicillin G was detectable at 18 h postdose (<0.125 μg/ml). Mean levels of LY146032 in serum (± standard error of the mean) at ~1 and 18 h postdose were 3.1 ± 1.34 and 2.38 ± 0.4 μg/ml, respectively. Despite significantly higher levels of antibiotic in serum achieved in LY146032 recipients compared with penicillin G recipients, the bactericidal activity of serum samples obtained at 1 h post-LY146032 dose was significantly lower than activity observed in penicillin G recipients (reciprocal geometric mean titer ± standard error of the mean, 4.8 ± 0.79 versus 60 ± 3.2, respectively; \( P < 0.001 \)).

This study confirmed that both LY146032 and penicillin G are highly effective in eradicating group G streptococci from aortic-valve vegetations and blood in animals with experimental endocarditis. Although there was a somewhat better effect with LY146032 than with penicillin G in kill curves in vitro and concentrations of LY146032 in serum were higher than those of penicillin G in vivo, serum bactericidal activity 1 h postdose was actually significantly greater for penicillin G than for LY146032, and the efficacies of the drugs in sterilizing aortic vegetations in vivo were similar. These findings could be explained by the much higher degree of serum protein binding of LY146032 by rabbit serum (85 to 87%; G. Brier, Eli Lilly Research, personal communication) compared with binding of penicillin G (35 to 58% [5]).

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**LITERATURE CITED**