NOTES

Treatment of Clostridium difficile Colitis in Hamsters with a Lipopeptide Antibiotic, LY146032

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LY146032, an acidic lipopeptide antibiotic which inhibits the biosynthesis of cell wall peptidoglycan, was found to be effective in delaying death in a hamster model of pseudomembranous colitis. A dose of 0.05 mg/day was effective. The equivalent protection with vancomycin required a dose 100-fold higher, i.e., 5 mg/day.

LY146032, a new acidic lipopeptide antibiotic which inhibits the biosynthesis of cell wall peptidoglycan, has antibacterial activity against gram-positive organisms including Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, group D streptococci, and Clostridium difficile (M. L. Cimino, D. R. Schaeberg, and R. Fekety, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 897, 1986; P. Gilligan, K. Jack, and N. Wannamaker, 26th ICAAC, abstr. no. 900, 1986). By the agar dilution method, the MICs of LY146032 and vancomycin were about the same when tested against 100 strains of C. difficile (C. E. Nord, 26th ICAAC, abstr. no. 905, 1986). In this study, we evaluated the efficacy of LY146032 in a hamster model of pseudomembranous colitis caused by C. difficile and compared the efficacy with that of vancomycin.

Male Syrian hamsters were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. The weight of the animals varied from 50 to 70 g. They were fed on Agway chow and caged in groups of five. A few (2 to 3 days) after the animals arrived, they were administered C. difficile organisms (10⁶ bacteria in a 0.1-ml volume) by orogastric intubation. Five days after this challenge, all animals were given 1 mg of clindamycin phosphate intraperitoneally. Ten animals were used in each treatment group, with 12 animals used as the controls. All trials were set up and run concurrently.

At 24 h after clindamycin injection, doses of vancomycin or LY146032 at 5, 0.5, or 0.05 mg/day were given in a 0.1-ml volume by orogastric intubation. The treatment period was 5 days.

The animals were observed daily, and their stools were collected daily for toxin assay. The total period of observation lasted for 14 days after the clindamycin challenge.

Toxin detection was done by tissue culture assay, with MRC-5 cells in microplate. The presence of cytopathic changes which were neutralized by Clostridium sordellii antitoxin was considered a positive test for C. difficile toxin (5).

In vitro susceptibility was measured in an anaerobic chamber by the microtiter method, with 50 μl of a 10⁻⁷ dilution of 6-h growth in prereduced brain heart infusion broth. The MBC was determined by subculturing 50 μl from each well onto blood agar plates under anaerobic conditions and observing growth after a 48-h incubation at 37°C.

Both vancomycin and LY146032 were supplied by Eli Lilly & Co., Indianapolis, Ind.

After clindamycin challenge, all control animals died within 3 days. Vancomycin treatment prolonged the survival time in a dose-related manner (Fig. 1). With a dosage of 0.05 mg/day, survival was prolonged by 2 days; at a 0.5-mg/day level, the survival time was 6 days; and at 5 mg/day, survival was prolonged to 10 days. LY146032 feeding caused prolongation of survival time to 10.5 days at all doses tested, even at the lowest dose (0.05 mg/day).

The in vitro MICs for the C. difficile strain were 16 μg/ml (LY146032) and 0.5 μg/ml (vancomycin). MBCs were 32 and 2 μg/ml, respectively.

Toxin in the stools was detectable 1 to 2 days before the death of the animal. Stools collected from the few surviving animals remained toxin negative throughout the study period.

Investigation of the cause of death in hamsters challenged with clindamycin indicated that toxin-producing strains of C. difficile were responsible for a lethal enterocolitis (2). Subsequently, this animal model has been used for testing the efficacy of therapeutic agents against enterocolitis (1, 3, 4). Vancomycin, among other agents, was found to be highly effective in preventing death in hamsters with enterocolitis.

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FIG. 1. Effect of LY146032 and vancomycin on mortality from C. difficile colitis in hamsters on the day after challenge: ●, Control; ○, LY146032 (LY); ■, Vancomycin (Van); 1, 5 mg daily; 2, 0.5 mg daily; 3, 0.05 mg daily.
These observations were confirmed in patients with C. difficile colitis.

In our study, vancomycin treatment prolonged the survival time in a dose-dependent manner. With 10-fold increases in the vancomycin dose, there were 2-fold increases in survival times. Treatment with LY146032, irrespective of the dose tested, caused a prolongation of survival time equivalent to that of the highest vancomycin dose. Thus, a dose of 0.05 mg of LY146032 per day was as effective as 5 mg of vancomycin per day in delaying death from C. difficile colitis in the hamster. This result is remarkable since in vitro susceptibility of the strain of C. difficile to LY146032 was 32-fold less than to vancomycin.

Death from clindamycin-induced colitis can be prevented by vancomycin as long as the agent is administered, but death invariably occurs within 5 to 10 days after treatment is stopped, even when the antibiotic is continued for up to 12 weeks (4). In this respect, LY146032 therapy was not different from that with vancomycin and other effective agents.

The reason LY146032 is more efficient than vancomycin in treating colitis in hamsters is not known. It is possible that LY146032 persisted in the gut for a longer time because of its stability or its adherence to the mucosal cells. It is also possible that the effects of LY146032 on the intestinal flora are somewhat different from the effects of vancomycin. Anaerobic gram-positive cocci were susceptible to both antibiotics, but unlike vancomycin, LY146032 appears to be bactericidal (R. N. Jones, A. L. Barry, and C. Thornsberry, 26th ICAAC, abstr. no. 887, 1986). Another possibility is that LY146032 is more active than vancomycin against Clostridium spores. These speculations cannot be resolved now.

The MIC of LY146032 for this strain of C. difficile is higher than that reported by Nord (26th ICAAC). Nord measured MICs by the agar dilution method, whereas we used microtiter plates. Our data are in the same range as those with other strains of C. difficile studied in our laboratory and are in agreement with those reported by Cimino et al. (26th ICAAC). It is possible that the difference in MICs determined in this study and in that of Nord is related to the difference in calcium concentrations in the agar and the liquid medium, since the presence of calcium in the medium reduced MICs by as much as 32-fold (6).

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