Efficacy of Azithromycin or Clarithromycin for Prophylaxis of Viridans Group Streptococcus Experimental Endocarditis

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The efficacy of azithromycin or clarithromycin was compared to that of amoxicillin, clindamycin, or erythromycin for the prevention of viridans group streptococcus experimental endocarditis. Rabbits with catheter-induced aortic valve vegetations were given no antibiotics or two doses of amoxicillin at 25 mg/kg of body weight, azithromycin at 10 mg/kg, clarithromycin at 10 mg/kg, clindamycin at 40 mg/kg followed by clindamycin at 20 mg/kg, or erythromycin at 10 mg/kg. Antibiotics were administered 0.5 h before and 5.5 h after intravenous infusion of 5×10^5 CFU of *Streptococcus milleri*. Forty-eight hours after bacterial inoculation, the rabbits were killed and aortic valve vegetations were aseptically removed and cultured for bacteria. Infective endocarditis occurred in 88% of untreated animals, 1% of animals receiving amoxicillin, 9% of animals receiving erythromycin, 0% of animals receiving clindamycin, 2.5% of animals receiving clarithromycin, and 1% of animals receiving azithromycin. All five regimens were more effective ($P < 0.001$) than no prophylaxis. Erythromycin was less effective ($P < 0.05$) than amoxicillin or clindamycin. Azithromycin or clarithromycin was as effective as amoxicillin, clindamycin, or erythromycin for the prevention of viridans group streptococcus experimental endocarditis in this model.

The American Heart Association has recommended two doses of amoxicillin or, alternatively, for amoxicillin-intolerant patients, erythromycin or clindamycin for antimicrobial prophylaxis to prevent endocarditis after dental or upper respiratory procedures (5). The first dose is administered prior to the procedure and the second dose is administered 6 h later. Although data from case-control studies demonstrate the efficacy of antimicrobial prophylaxis for the prevention of endocarditis in high-risk patients (14, 15), data from prospective controlled studies comparing various antimicrobial options in humans are lacking (7). The current antimicrobial recommendations are based on the in vitro activities of the antimicrobial agents against viridans group streptococci, in vivo data from experimental models, and cost and convenience considerations. Although erythromycin has been recommended for endocarditis prophylaxis for many years, the large dosages recommended according to the current guidelines are poorly tolerated by many patients and may discourage compliance with the guidelines.

Azithromycin and clarithromycin are newer macrolide antimicrobial agents with activities against aerobic gram-positive cocci and some gram-negative bacilli. The mechanisms of antimicrobial activity of azithromycin and clarithromycin are similar to that of erythromycin or clindamycin, and both are active in vitro against viridans group streptococci (18, 24). Both azithromycin and clarithromycin are better tolerated than erythromycin, with significantly fewer adverse gastrointestinal reactions (1). Considering the dose-related gastrointestinal distress associated with erythromycin, azithromycin or clarithromycin may be an effective alternative macrolide to erythromycin for endocarditis prophylaxis in patients undergoing dental or upper respiratory procedures. The purpose of this study was to compare the prophylactic efficacy in vivo of amoxicillin, azithromycin, clarithromycin, clindamycin, or erythromycin for the prevention of viridans group streptococcus endocarditis with a rabbit experimental model.

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MATERIALS AND METHODS

Microorganisms. Thirty-five isolates of viridans group streptococci (1 *Gemella morbillorum*, 11 *Streptococcus mitis*, 15 *Streptococcus milleri*, 7 *Streptococcus sanguis*, and 1 *Streptococcus mutans* isolate) recovered from patients with bacterial endocarditis were studied in vitro. The MICs and minimum bactericidal concentrations were determined by a macrodilution technique in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (19, 20). A single isolate of *S. milleri*, with susceptibilities to amoxicillin, azithromycin, clarithromycin, clindamycin, and erythromycin matching the MICs at which 50% of isolates are inhibited (MIC$_{50}$s) for the 35 isolates, was chosen for study in vivo.

Concentrations of antibiotics in serum. Blood was drawn from five healthy rabbits at 0.5, 1.0, 1.5, 2.0, and 2.5 h after the administration of a single dose of amoxicillin, azithromycin, clarithromycin, clindamycin, or erythromycin and was assayed for antibiotic concentration. The prophylaxis dosages were chosen to approximate expected peak concentrations in human serum after the administration of recommended prophylactic dosages. The doses of antimicrobial agents used for prophylaxis were 25 mg of amoxicillin per kg of body weight orally, 10 mg of azithromycin per kg intravenously (i.v.), 10 mg of clarithromycin per kg i.v., 40 mg and then 20 mg of clindamycin per kg i.v., and 10 mg of erythromycin per kg i.v. Amoxicillin was administered per os to nonfasting rabbits by the technique of Marr et al. (17). Oral administration of azithromycin, clarithromycin, or erythromycin did not reproducibly result in adequate concentrations in rabbits due to variable bioavailabilities in lagomorphs. Parenteral lactobionate preparations of clarithromycin and erythromycin were therefore used to reproducibly achieve concentrations in serum of 2.0 to 3.0 μg/ml, and a parenteral dihydrate preparation of azithromycin was used to reliably achieve concentrations in serum of 0.5 to 1.0 μg/ml, approximating the expected concentrations in human serum after oral administration. Blood collected at timed intervals after clarithromycin administration to healthy rabbits was also assayed for the concentration of the 14-OH-clarithromycin metabolite. The clindamycin regimen of a second dose equal to one-half of the initial dose follows the recommendations of the American Heart Association (5). Therapeutic monitoring of antimicrobial concentrations was also performed for all rabbits in the prophylaxis experiment with blood

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TABLE 1. Susceptibilities of 35 clinical isolates of viridans group streptococci to the study antimicrobial agents

Antimicrobial agent	MIC (µg/ml)				MBC (µg/ml)[a]			
	Range	50%	90%	Study strain	Range	50%	90%	Study strain
Amoxicillin	<0.125–1	<0.125	0.5	<0.125	<0.125–32	<0.125	2.0	<0.125
Azithromycin	≤0.125–4	≤0.125	2.0	≤0.125	≤0.125–≥32	0.5	≥16	0.5
Clarithromycin	<0.125–1	<0.125	1.0	<0.125	0.125–16	0.125	32	0.125
Clindamycin	≤0.125–16	≤0.125	0.25	≤0.125	≤0.125–≥128	8	32	0.25
Erythromycin	<0.125–4	<0.125	2.0	<0.125	<0.125–32	<0.125	8	<0.125

[a] MBC, minimum bactericidal concentration.

obtained immediately prior to bacterial challenge. The concentrations of antibiotics in serum were determined by microbiological assay techniques as described by Chapin-Robertson and Edberg (2) for amoxicillin, azithromycin, clindamycin, and erythromycin and by reverse-phase high-pressure liquid chromatography as described by Chu et al. (3) for clarithromycin and 14-OH-clarithromycin.

Production of Nonbacterial valvular vegetations. Sterile vegetations were produced in New Zealand White rabbits (weight, 2.5 to 3.0 kg) by a modification of the technique described by Garrison and Freedman (11). After a preoperative examination and the application of bland ophthalmic ointment to both eyes, surgical anesthesia was induced with intramuscular administration of a combination of ketamine and xylazine. A midline incision was made in the neck, and the right carotid artery was exposed. The artery was ligated distally and clamped proximally, and a sterile polyethylene catheter was inserted into the artery and advanced proximally into the left ventricle. The distal end of the catheter was attached to a pressure-sensitive device, so that proper placement of the catheter across the aortic valve and into the left ventricle was indicated by a significant change in the arterial pressure gradient. The catheter was ligated in place and left in place for the duration of the experiment. Fibrin-platelet vegetations are visible on the aortic valve less than 6 to 12 h after catheter placement in this experimental model (8).

Determination of the ID$_{90}$. The inoculum of bacteria that infected 90% of untreated animals (ID$_{90}$) was determined by i.v. administration of 10-fold dilutions of 5×10^5 CFU of the study strain of streptococcus to groups of 10 rabbits 24 h after aortic catheter placement. The concentrations of bacteria in the bacterial challenge were quantitated by a serial dilution technique. Semiquantitative cultures of blood from six animals were performed at timed intervals after challenge with the estimated ID$_{90}$ of 5×10^5 CFU of the study strain to measure the timing and magnitude of bacteremia after bacterial challenge.

Antimicrobial prophylaxis for endocarditis. Experimental endocarditis prophylaxis was accomplished 24 h after catheter placement by administering prophylactic antibiotics, waiting 30 min, obtaining a blood specimen for therapeutic monitoring of the antibiotic concentration, and immediately administering an i.v. bacterial challenge. Six hours after administration of the initial antimicrobial dose (5.5 h after bacterial challenge), a second dose of antibiotic was administered. Forty-eight hours later, the animals were killed with 100 mg of pentobarbital per kg. The chest cavity was opened, the heart was excised and opened, and the aortic valves were aseptically removed. The vegetations were homogenized in a Stomacher 80, and the entire vegetation was cultured for viridans group streptococci in nutrient broth incubated at 35°C for 72 h. All cultures were subcultured to blood agar plates after incubation to document the presence of viridans group streptococci in positive cultures and to confirm that clear tubes were sterile. Six rabbits were challenged with an inoculum equal to the ID$_{90}$ and were sacrificed 48 h after bacterial challenge, and the aortic valve vegetations were quantitatively cultured to determine the bacterial density on the aortic valves when endocarditis did develop.

Statistical analysis. The results of the prophylaxis studies were expressed as the proportion of animals with sterile vegetations for each prophylaxis regimen and were analyzed by Fisher's exact test. Prior to initiating the animal studies, we estimated a 10% failure rate in the amoxicillin prophylaxis regimen. Assuming this failure rate, 80 animals in each prophylaxis arm provided a 90% probability of detecting a true difference in prophylaxis failure rate of 20% or greater (4).

RESULTS

Susceptibility testing. The results of susceptibility testing of the 35 clinical isolates of viridans group streptococci and of the strain studied in vivo are presented in Table 1. The strain used in vivo was susceptible to amoxicillin, azithromycin, clarithromycin, clindamycin, and erythromycin.

The mean concentrations of the five test antibiotics in the sera of five healthy rabbits are presented in Fig. 1. No 14-OH-clarithromycin was detected. Among the animals receiving prophylaxis, the median concentrations (range) of antimicrobial

agents in serum 0.5 h after dosing were 6.9 µg/ml (1.2 to 14.0 µg/ml) for amoxicillin, 0.7 µg/ml (0.2 to 1.2 µg/ml) for azithromycin, 2.7 µg/ml (1.5 to 3.9 µg/ml) for clarithromycin, 7.9 µg/ml (6.8 to 10.8 µg/ml) for clindamycin, and 2.4 µg/ml (1.1 to 5.1 µg/ml) for erythromycin. Data for one rabbit were excluded from analysis because the concentration of erythromycin was >25 µg/ml, possibly due to erythromycin contamination of the i.v. line used to draw the blood sample.

Eighteen of 20 animals (90%) challenged with 5×10^5 CFU of the study strain of viridans group streptococci developed infective endocarditis, whereas 6 of 10 animals challenged with 5×10^4 CFU developed infective endocarditis. An inoculum of 5×10^5 CFU (ID$_{90}$) was therefore chosen for the prophylaxis trials. With this inoculum, timed quantitative blood cultures for six rabbits after bacterial challenge demonstrated 100% positive cultures at 15 min, 50% positive cultures at 30 min and 1 h, 33% positive cultures at 1.5 h, and 0% positive cultures at 2 h. At 15 min, the number of organisms in blood ranged from 6 to 26 microorganisms/ml. Among six animals that received no antimicrobial prophylaxis and that developed infective endocarditis, the mean log$_{10}$ CFU per gram of aortic valve tissue 48 h after bacterial challenge was 10.4 ± 1.6.

Infective endocarditis occurred in 82 of 93 (88%) rabbits receiving no antimicrobial prophylaxis, 0 of 80 (0%) of animals receiving amoxicillin, 1 of 80 (1%) of animals receiving azithromycin, 2 of 80 (2.5%) animals receiving clarithromycin, 0 of 79 (0%) animals receiving clindamycin, and 7 of 80 (9%) animals receiving erythromycin. All five prophylaxis regimens effectively ($P < 0.01$) prevented infective endocarditis compared to no prophylaxis. Erythromycin was significantly less effective ($P < 0.05$) than amoxicillin or clindamycin; azithromycin or clarithromycin was as effective as amoxicillin, clindamycin, or
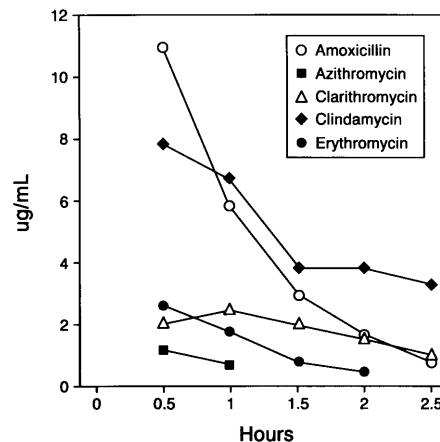

FIG. 1. Mean concentrations of study antimicrobial agents in rabbit serum at time intervals after a single dose was administered.
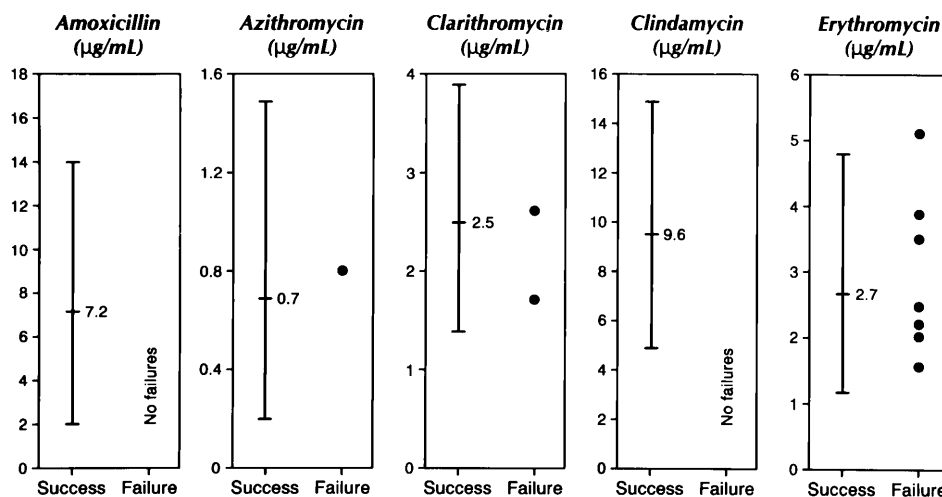
FIG. 2. Median and range of concentrations of antimicrobial agents in rabbit serum at the time of bacterial challenge in animals in which prophylaxis was successful (bars) and actual concentrations in animals failing prophylaxis (●).

erythromycin in this model. At the time of bacterial challenge, the concentrations of clarithromycin in the two animals failing prophylaxis was 1.7 and 2.6 µg/ml, respectively; among the seven erythromycin treatment failures, the concentration of erythromycin ranged from 1.6 to 5.1 µg/ml. The concentrations of antibiotics in animals failing prophylaxis (Fig. 2) were not significantly different from the concentrations of antibiotics in animals receiving effective prophylaxis (clarithromycin, 2.6 ± 0.61 µg/ml; erythromycin, 2.7 ± 0.88 µg/ml [mean ± standard deviation]). Failure of antimicrobial prophylaxis in the erythromycin or clarithromycin arms could not therefore be directly attributed to random variability in the concentration of antimicrobial agents at the time of bacterial challenge. More rapid antimicrobial clearance in animals failing prophylaxis, resulting in a shorter duration of inhibitory activity, cannot be excluded on the basis of these data.

DISCUSSION

The recommendation for the use of antimicrobial agents for the prevention of endocarditis is based on the observation that patients with hemodynamically significant turbulent abnormalities of blood flow are at increased risk of seeding of the endocardium when bacteremia due to organisms that have tropism for endocardial surfaces occurs. In the oropharynx and upper respiratory tract, the major microorganisms of concern are viridans group streptococci. The selection of specific antimicrobial agents for the prophylaxis of endocarditis is based on the susceptibility of viridans group streptococci in vitro and on studies with experimental animals, because no randomized, controlled data comparing alternative regimens in humans are available. Earlier, a series of elegant studies with experimental endocarditis models in the 1970s by Durack and colleagues (8, 9) demonstrated that vancomycin alone, ampicillin combined with streptomycin administered as a single dose, or penicillin administered in multiple doses effectively prevented tricuspid valve endocarditis in rabbits given a large (10^8) inoculum of *S. sanguis*. Erythromycin was effective with lower bacterial inocula (21). Subsequently, other investigators have confirmed that while bactericidal antimicrobial agents are required for large inocula, bacteriostatic antimicrobial agents are effective for inoculum sizes less than or equal to the ID_{90} (12). The mechanism of action of antimicrobial agents in preventing endocar-

ditis is not related to the prevention of bacteremia or solely to antibiotic-induced killing, but may involve interference with bacterial adhesion or events occurring after adhesion (10, 13, 16). The mechanisms of action may depend on the size of the inoculum (12). In humans, microorganisms causing endocarditis in patients failing prophylaxis may be susceptible to the antimicrobial agents used for prophylaxis (23).

In the current study, the efficacies of the newer macrolides azithromycin and clarithromycin was compared to that of amoxicillin, erythromycin, or clindamycin. Prophylaxis with all antimicrobial agents tested was more effective than no prophylaxis. Erythromycin was significantly less effective than amoxicillin or clindamycin under the experimental conditions used in the study. Several factors should be considered in interpreting these data. First, a single strain of viridans group streptococci was tested. While this strain was chosen on the basis of representative susceptibility in vitro to the study antimicrobial agents, the extent to which the in vivo response of this strain is characteristic of those of other strains is undetermined. However, Vermot et al. (22), using a smaller number of animals in an experimental rat model of endocarditis, also found with three different strains of viridans group streptococci that prophylactic clarithromycin was as effective as clindamycin. In that study, clarithromycin was dosed to mimic the concentrations in human serum following administration of a single dose of clarithromycin. Second, a bacterial inoculum that resulted in endocarditis in approximately 90% of the animals was given. This inoculum is larger than that ordinarily expected to result from most procedures in the oral cavity or upper respiratory tract in humans and resulted in a level of bacteremia an order of magnitude greater than that reported in humans. We detected 6 to 26 organisms per ml of arterial blood 15 min after bacterial challenge. Hall et al. (13) documented a level of bacteremia of 0.5 to 2 CFU/ml 15 min after dental extraction using a lysis filtration technique with 60 healthy patients. Additionally, the experimental model uses a foreign body at the infection site. These experimental conditions may result in a predisposition toward failure of the study antimicrobial agents in the study setting, which may represent a more difficult challenge than that ordinarily encountered in the routine clinical use of antimicrobial prophylaxis for infective endocarditis. The efficacies of azithromycin and clarithromycin compared to that of

of amoxicillin, clindamycin, or erythromycin under these experimental conditions suggest that these macrolides may be potential alternatives for prophylaxis for infective endocarditis due to viridans group streptococci. These findings are consistent with the inclusion of azithromycin and clarithromycin and the exclusion of erythromycin as alternatives to amoxicillin in the newly revised American Heart Association recommendations for prophylaxis for infective endocarditis (6).

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