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

M. S. ROUSE, J. M. STECKELBERG,* C. M. BRANDT,‡ R. PATEL, J. M. MIRO,‡ AND W. R. WILSON

Infectious Diseases Research Laboratory, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

Received 14 January 1997/Returned for modification 7 April 1997/Accepted 9 May 1997

The efficacy of azithromycin or clarithromycin was compared to that of amoxicillin, clindamycin, or erythromycin for the prevention of viridans group streptococcal 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 × 10⁸ 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 streptococcal 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 (6). 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 antemicrobial 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 streptococcal endocarditis with a rabbit experimental model.

(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 (MIC₅₀) 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., 4.0 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 dihydroxy 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

* Corresponding author. Phone: (507) 284-2511. Fax: (507) 255-7767.
† Present address: Institut für Medizinische Mikrobiologie, der Medizinischen Fakultät der FWH Aachen, Aachen, Germany.
‡ Present address: Infectious Diseases Unit, Hospital Clinic I Provincial, 08036-Barcelona, Spain.

Copyright © 1997, American Society for Microbiology
TABLE 1. Susceptibilities of 35 clinical isolates of viridans group streptococci to the study antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)*</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
<th>Study strain</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
<th>Study strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>&lt;0.125–1</td>
<td>&lt;0.125</td>
<td>0.5</td>
<td>&lt;0.125</td>
<td>0.125–32</td>
<td>&lt;0.125</td>
<td>2.0</td>
<td>&lt;0.125</td>
<td>0.125–2</td>
<td>0.125–128</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>&lt;0.125–4</td>
<td>&lt;0.125</td>
<td>2.0</td>
<td>&lt;0.125</td>
<td>0.125–32</td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125–32</td>
<td>0.125–32</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>&lt;0.125–1</td>
<td>&lt;0.125</td>
<td>1.0</td>
<td>&lt;0.125</td>
<td>0.125–32</td>
<td>0.125–32</td>
<td>32</td>
<td>0.125</td>
<td>0.125–32</td>
<td>0.125–32</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&lt;0.125–16</td>
<td>&lt;0.125</td>
<td>0.25</td>
<td>&lt;0.125</td>
<td>0.125–32</td>
<td>8</td>
<td>32</td>
<td>0.25</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>&lt;0.125–4</td>
<td>&lt;0.125</td>
<td>2.0</td>
<td>&lt;0.125</td>
<td>0.125–32</td>
<td>&lt;0.125</td>
<td>8</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
</tr>
</tbody>
</table>

* MBC, minimum bactericidal concentration.

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 × 10⁵ CFU of the study strain of viridans group streptococci developed infective endocarditis, whereas 6 of 10 animals challenged with 5 × 10⁴ CFU developed infective endocarditis. An inoculum of 5 × 10⁵ CFU (ID₉₀) 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 log₁₀ 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 regimes 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
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 (10⁸) 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 ID₉₀ (12). The mechanism of action of antimicrobial agents in preventing endocarditis 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 azithromycin 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 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).

ACKNOWLEDGMENTS

J. M. Miro was supported in part by grants from the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC-Upjohn) and from the Hospital Clinic I Provincial de Barcelona. C. M. Brandt was supported in part by a grant from Ortho McNeil. The study was supported in part by grants from Pfizer and Abbott.

REFERENCES