Azithromycin (AZM) differs from other macrolide antibiotics in that it has unusual pharmacokinetics characterized by rapid tissue penetration with simultaneous low levels in serum (5). The favorable tissue pharmacokinetics makes AZM a good candidate for prophylaxis against infective endocarditis (IE). This study was designed to evaluate the prophylactic efficacy of AZM against the most common etiologic agents of IE, namely, viridans group streptococci (Streptococcus oralis) and Staphylococcus aureus.

The two microorganisms used in this study were isolated from blood cultures of patients with endocarditis and were identified by standard methods. MICs and minimal bactericidal concentrations (MBCs) of AZM, vancomycin (VAN), oxacillin (plus 2% NaCl), and ampicillin (AMP) were determined by a microdilution technique in 0.1-ml volumes, as described previously (12). Nonbacterial thrombotic endocarditis of the aortic valve was induced in female white rabbits weighing 2.3 to 3.5 kg by using the model described by Perlman and Freedman, with the polyethylenecatheter left in place throughout the experiment (13). Twenty-four hours after catheterization, rabbits were randomly assigned to a control group, a group receiving a single dose of 50 mg of AZM per kg of body weight per os, a group receiving a single dose of 30 mg of VAN (supplied by Eli Lilly, Indianapolis, Ind.) per kg of body weight intravenously, and a group receiving a single dose of 40 mg of AMP (supplied by Bristol-Myers, Squibb, Athens, Greece) per kg of body weight intravenously. AMP or VAN was given for prophylaxis against S. oralis or S. aureus, respectively. AZM was supplied by Pfizer (Groton, Conn.) as a suspension at a concentration of 40 mg/ml and was given to the animals through an esophageal catheter (Nelaton catheter no. 10; Uno Plast, Denmark) after the intramuscular administration of 25 mg of ketamine HCl per kg. The dose of AZM used was chosen because in pilot studies it was found to achieve peak levels in serum in rabbits similar to those obtained in humans after a single per os dose of 500 mg (5). The doses of VAN and AMP were chosen because they have been used in previous studies of successful endocarditis prophylaxis (11). Animals treated with AZM and animals treated with AMP or VAN were challenged 1 and 0.5 h later, respectively, with an inoculum of \( \sim 10^7 \) CFU of S. oralis or of \( \sim 10^6 \) CFU of S. aureus. The inocula were suspended in 1-ml volumes of saline and injected via the marginal ear vein. The rabbits were sacrificed 5 days (120 h) after bacterial challenge. The processing of vegetations and criteria for IE were described previously (12).

AZM levels were determined in serum samples obtained at 0.5, 1, 2, and 4 h postdosing. An agar well diffusion bioassay technique was applied (6). Micrococcus luteus ATCC 9341 was used as the test organism, and normal rabbit serum was used as the diluent. The lower limit of detection of this assay was 0.3 \( \mu \)g/ml. AMP and VAN levels were determined as previously described (12). AZM levels were determined in aortic valve vegetation samples obtained 1, 2, 6, 12, and 24 h (n = 3, at each time point) postdosing. An agar well bioassay technique described previously (2) was applied. The small pieces of sterile vegetations used were placed into wells of agar plates after homogenization. To compare the differences between sterile (successful prophylaxis) and nonsterile vegetations, the Fisher exact test for probabilities was used. A \( P \) value of \(< 0.05 \) was considered significant.

The MICs and MBCs of AZM, VAN, AMP, and oxacillin for both species are shown in Table 1. S. oralis was tolerant to AZM but not to AMP, while S. aureus was methicillin resistant (MRSA), with borderline susceptibility to AZM (MIC = 1 \( \mu \)g/ml) and intermediate sensitivity to erythromycin (MIC = 2 \( \mu \)g/ml). The pharmacokinetics of AZM, AMP, and VAN are presented in Table 2. The results of prophylaxis against the two species tested are shown in Table 1. Of the control animals challenged with S. oralis or S. aureus, 90 or 94%, respectively, developed infected vegetations. AZM prevented endocarditis due to S. oralis or S. aureus in 94 and 59% of the animals, respectively (significantly different \( P < 0.001 \)) from the value obtained for the control group for both comparisons. The mean log\(_{10}\) CFU of S. aureus per gram of vegetation for the unprotected animals was less than the value for the control animals (4.7 versus 9.2). VAN prevented endocarditis in all but one animal challenged with MRSA (significantly different from the value obtained for the control group \( P < 0.001 \)) and from the value obtained for the group treated with AZM \( P < 0.018 \)). Finally, AMP prevented endocarditis due to S. oralis in
72% of the challenged animals (significantly different from the value obtained for the control group \(P < 0.001\) but not significantly different from the value obtained for the group treated with AZM \(P = 0.177\)).

The present study differs from past evaluations of AZM in experimental endocarditis prophylaxis (7, 14), as it is the first to our knowledge that evaluates the prophylactic efficacy of a single dose of AZM against a tolerant strain of viridans group streptococci, as well as against a methicillin-resistant strain of \(S. aureus\). The prophylactic efficacy of AZM against \(S. oralis\) is satisfactory and superior to that of AMP (94 versus 72% sterility rate, respectively). Although the statistical difference between the groups treated with the two drugs is not significant \((P = 0.177)\), it is important to mention that the above results were observed against an AZM-tolerant streptococcal strain. This is probably due to sustained levels of AZM in valve tissue that are higher than the MIC for more than 24 h. Berney and Francioli (1) suggested that in the absence of bacterial killing, prolifing inhibition of bacterial growth on the surface of vegetation, rather than inhibition of bacterial adherence, may be the mechanism of successful antibiotic prophylaxis against endocarditis due to tolerant streptococci, by allowing the bacteria to be cleared from the damaged valves.

Staphylococci are not very common pathogens of endocarditis following iatrogenic bacteremia in outpatients (3). However, certain groups of patients, namely, patients with diabetes mellitus, chronic renal failure, or chronic skin conditions and intravenous drug addicts, are often colonized with \(S. aureus\), a fact responsible for the increased incidence of staphylococcal infections in these particular groups of patients (10). Moreover, the abovementioned groups are colonized mostly with MRSA strains which are rarely susceptible to macrolides (8). There are no studies in the literature on the prophylactic efficacy of newer macrolides against staphylococcal endocarditis. In the present study, the prophylactic efficacy of AZM against \(S. aureus\) is not as impressive as that against \(S. oralis\). The sustained (for at least 24 h) supra-MIC tissue concentrations of AZM are probably responsible for AZM’s prophylactic action, possibly through mechanisms related to prolonged growth inhibition of intracellular bacteria (inside the cells that constitute the vegetation). However, a large number of the bacteria of the vegetations reside primarily in the extracellular space of that tissue, where concentrations of AZM are equal to those in serum but last longer (7). These concentrations do not exceed the MIC for \(S. aureus\), and they are probably not sufficient to inhibit completely its growth, a fact possibly responsible for the low (59%) prophylactic efficacy of AZM. However, it must be stated that prevention is far more difficult to achieve in animals than in humans because of the large inoculum used to ensure that endocarditis almost always develops in untreated control animals and the presence of a foreign body (intracardiac catheter) (4). The inoculum of iatrogenic bacteremia in humans is very low (<10 CFU/ml), and only a small minority of cases of endocarditis are attributable to health care procedures (4, 15). In addition, only a small number of patients at risk of developing endocarditis harbor an intravascular foreign body, and it has been documented that removal of the catheter increases the effectiveness of VAN in experimental endocarditis prophylaxis by 1,000-fold (9).

In conclusion, AZM is undoubtedly protective against streptococcal endocarditis, while it is possible to exert prophylactic efficacy against staphylococcal endocarditis in humans, where conditions are not as “difficult” as those in experimental animal models. However, prophylactic use of AZM in groups of patients at high risk of developing staphylococcal endocarditis cannot be recommended. On the other hand, prevention could be achieved even in cases of streptococcal tolerance, possibly through high and sustained tissue drug levels. The observations

### Table 1. Results of prophylaxis in animals challenged with \(S. oralis\) or \(S. aureus\) (MRSA)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC/MBC (µg/ml)</th>
<th>No. of sterile vegetation cultures/total no. (%)</th>
<th>Log(_{10}) CFU/g of heart tissue (mean ± SD)</th>
<th>MIC/MBC (µg/ml)</th>
<th>No. of sterile vegetation cultures/total no. (%)</th>
<th>Log(_{10}) CFU/g of heart tissue (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>–</td>
<td>1/10 (10)</td>
<td>8.0 ± 1.0</td>
<td>–</td>
<td>1/17 (6)</td>
<td>9.2 ± 1.4</td>
</tr>
<tr>
<td>AMP</td>
<td>&lt;0.12/0.5</td>
<td>13/18 (72)</td>
<td>6.1 ± 1.9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VAN</td>
<td>0.5/1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>AZM</td>
<td>&lt;0.06/0.2</td>
<td>16/17 (94)(^{d,e})</td>
<td>5.43</td>
<td>1/1</td>
<td>17/18 (94)(^{d,e})</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/64</td>
<td>10/17 (59)(^{d,e})</td>
<td>4.7 ± 1.5</td>
</tr>
</tbody>
</table>

\(^{a}\) Oxacinil MIC and MBC = 64 and 128 µg/ml, respectively; erythromycin MIC = 2 µg/ml.

\(^{b}\) Significantly different from the value obtained for the control group \((P < 0.001)\).

\(^{c}\) NS, not studied.

\(^{d}\) Not significantly different from the value obtained for the group treated with AMP \((P = 0.177)\).

\(^{e}\) Value for the one animal in each group in which prophylaxis failed.

### Table 2. Levels of AMP, VAN, and AZM in serum or heart valve tissue

<table>
<thead>
<tr>
<th>Antibiotic (dose, administration route(^{a}))</th>
<th>Level (µg/ml mean ± SD) in serum at the following time (h) postdosing:</th>
<th>Level (µg/g) in heart valve tissue at the following time (h) postdosing:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>AZM(^{b}) (50 mg/kg, p.o.)</td>
<td>&lt;0.3</td>
<td>0.91 ± 0.25</td>
</tr>
<tr>
<td>AMP(^{b}) (40 mg/kg, i.v.)</td>
<td>13.5 ± 6.8</td>
<td>7.3 ± 3.2</td>
</tr>
<tr>
<td>VAN(^{b}) (30 mg/kg, i.v.)</td>
<td>71.5 ± 13.7</td>
<td>51.1 ± 7.1</td>
</tr>
</tbody>
</table>

\(^{a}\) Abbreviations: p.o., per os; i.v., intravenous.

\(^{b}\) Lower limit of detection of AZM in serum (agar well bioassay), 0.3 µg/ml. AZM was not detected in heart valve tissue 1 h after the dose was given.

\(^{c}\) Lower limit of detection (agar well bioassay), 0.1 µg/ml.

\(^{d}\) Lower limit of detection (fluorescence polarization immunoassay), 0.6 µg/ml.

\(^{e}\) ND, not done.
of the present study in combination with the results of the previously mentioned experimental studies (7, 14) and the very satisfying safety profile of AZM suggest that the antibiotic can be used with expected success in endocarditis prophylaxis in the community. The above are in agreement with the recent American Heart Association guidelines that recommend AZM at a single dose of 500 mg 1 h prior to dental, oral, respiratory tract or esophageal procedures in patients allergic to penicillin and at risk of developing bacterial endocarditis (3).

REFERENCES