Tolerance and Efficacy of Parenterally Administered Penicillin-Streptomycin and Orally Administered Amoxicillin or Penicillin V for Prophylaxis of Experimentally Induced Streptococcal Endocarditis

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A regimen of a single intramuscular dose of penicillin G-streptomycin was compared with regimens of three oral doses of amoxicillin and two oral doses of penicillin V to prevent Streptococcus sanguis endocarditis in rabbits with experimentally induced valvular heart lesions. Challenge doses of 10^4, 10^6, and 10^8 CFU of a strain of S. sanguis highly tolerant to penicillin and amoxicillin were used. The combination of penicillin and streptomycin was the only regimen tested that provided full protection even against the highest inoculum concentration. A single oral dose of penicillin V (36 mg/kg) or amoxicillin (50 mg/kg), two oral doses of penicillin V (36 and 18 mg/kg with a 7-h interval between doses), or six oral doses of amoxicillin (50 mg/kg followed by 8.5 mg/kg at 8-h intervals) protected recipients of the lowest inoculum concentration; protection diminished with increasing inocula. In contrast, administration of two high oral doses of amoxicillin (50 mg/kg) with a 10-h interval between doses provided full protection against challenge doses of 10^4 and 10^6 CFU, preventing endocarditis in 10 (66%) of 15 recipients of 10^6 CFU. All regimens evaluated were highly effective in preventing endocarditis when rabbits were challenged with 10^4 CFU. The combination of penicillin and streptomycin was the best regimen tested. Administration of two high oral doses of amoxicillin (50 mg/kg) with a 10-h interval between doses led to significantly fewer infections when compared with the other oral regimens when rabbits were challenged with 10^6 and 10^8 CFU.

Poor compliance with parenteral regimens for prophylaxis of infective endocarditis (IE) has led to current recommendations of orally administered penicillin V (17) or amoxicillin (19) regimens. Oral amoxicillin achieves higher and more sustained levels of antibiotic in serum than does penicillin V (16). However, we have found no differences between these two drugs for prophylaxis of IE in rabbits with experimentally induced valvular heart lesions challenged with a highly susceptible strain of Streptococcus sanguis biotype II (13, 14) when both antibiotics were administrated at dosages comparable to scheduled regimens recommended by the American Heart Association (17) and the Working Party of the British Society for Antimicrobial Chemotherapy (19). Further work evaluating protection against less susceptible strains is needed to elucidate the relative merits of each antibiotic and define the most cost-efficient regimen.

Approximately 20% of clinical isolates among the viridans group of streptococci are tolerant to the bactericidal action of penicillin (5, 8, 15). As these strains are inhibited by low concentrations of beta-lactam antibiotics but killed completely only at much higher concentrations, tolerance might lead prophylactic failures in patients at high risk of endocarditis. To study the role of tolerance and compare the effectiveness of currently recommended orally administered amoxicillin and penicillin V regimens in this setting, we repeated previous prophylactic experiments with a strain of S. sanguis that is highly tolerant to both antibiotics (MIC, 0.04 μg/ml; MBC, >128 μg/ml).

MATERIALS AND METHODS

Infecting organism. A strain of S. sanguis biotype II (S. sanguis HPE77) obtained from a patient with subacute bacterial endocarditis was used throughout these studies. For stock purposes, 1-ml samples of an 18-h culture of this strain were kept in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) enriched with 5% sheep erythrocytes and stored at −20°C. For each experiment, a sample was thawed, inoculated into 50 ml of the medium, and incubated for 18 h at 37°C.

In vitro susceptibility studies. The MICs and MBCs of amoxicillin, penicillin V, penicillin G, and streptomycin were measured by a standard twofold tube dilution method (13); the MIC was determined by visual inspection after 24 h of incubation at 37°C in a candle jar. The MBC was defined as the lowest concentration of antibiotic that killed 99.9% or more of the initial inoculum (10^6 CFU/ml).

Time kill curves with 10 μg of amoxicillin, penicillin V, penicillin G, or streptomycin per ml and the penicillin G-streptomycin combination (0.5 and 5 μg/ml, respectively) were performed in Mueller-Hinton broth by a modification of the method of Wolfe and Johnson (18) as previously described (13).

Assessment of prophylactic activity. Sterile vegetation were produced in New Zealand White rabbits weighing 3.0 to 3.8 kg by the procedure of Perlman and Freeman (12) as modified by Durack and Beeson (2). Briefly, a polyethylene catheter was introduced into the right carotid artery and advanced until it reached the aortic valve; the catheter was then secured in place for the duration of the experiment.

Seventy-two hours after catheterization, groups of 6 to 10
rabbits were inoculated via the marginal ear vein with approximately $10^6, 10^7$, or $10^8$ CFU of *S. sanguis*. In each of 45 sessions, one rabbit was randomly assigned to a control group, and the remainder were randomly assigned to a prophylactic regimen. Inocula were prepared from an 18-h culture by sedimenting the growth by centrifugation, washing the sediment three times with phosphate-buffered saline, suspending it in phosphate-buffered saline, and quantifying numbers by optical density measurements to attain $10^6$ CFU per unit of volume. Inocula of $10^6$ and $10^7$ CFU were obtained by serial dilution in phosphate-buffered saline. The actual numbers of CFU injected were verified by the culture of 0.1-ml portions from serial 10-fold dilutions in blood agar pour plates.

On the basis of the results obtained in previous studies with intramuscularly administered procaine penicillin G plus streptomycin and orally administered amoxicillin (13) and penicillin V (19) for prophylaxis of streptococcal endocarditis in fasting rabbits, 1 h before challenge subgroups of 15 animals received one of the following regimens.

(i) The first regimen was a single intramuscular dose of procaine penicillin G (80,000 U/kg of body weight) plus probenecid (25 mg/kg of body weight), together with streptomycin (20 mg/kg of body weight) (regimen PCN-SM). In previous studies in New Zealand White rabbits with similar weights, this regimen led to peak levels of penicillin G and streptomycin of $9.8 \pm 5.1$ and $54.2 \pm 10.1$ µg/ml of serum, respectively (13). These peak levels were similar to those achieved in humans after an intramuscular dose of crystalline penicillin G (1,000,000 U) plus procaine penicillin G (600,000 U) and streptomycin (1 g). Although levels of streptomycin decreased faster in rabbits, all levels of penicillin G in serum were roughly similar to those achieved in humans (13).

(ii) The second regimen was a single oral dose of amoxicillin (50 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) (regimen AMOX-1). As described in previous studies of rabbits with similar weights, the addition of probenecid to oral amoxicillin (50 mg/kg) led to peak levels of $21.0 \pm 5.2$ µg/ml of serum. These levels were roughly similar to those achieved in humans after an oral dose of 3 g of amoxicillin, decreasing at similar rates (13).

(iii) The third regimen was two oral doses of amoxicillin (50 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) with a 10-h interval between doses (regimen AMOX-2).

(iv) The fourth regimen was an oral dose of amoxicillin (50 mg/kg of body weight) plus probenecid (25 mg/kg of body weight), followed by five doses of amoxicillin (8.5 mg/kg of body weight) plus probenecid (10 mg/kg of body weight) with 8-h intervals between doses (regimen AMOX-6). In previous studies the addition of probenecid to oral amoxicillin (8.5 mg/kg) led to peak levels of $5.4 \pm 1.7$ µg/ml of serum. These levels were similar to those achieved in humans after an oral dose of 0.5 g of amoxicillin, decreasing at similar rates (13).

(v) The fifth regimen was a single oral dose of penicillin V (36 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) (regimen PCN-1). As described in previous studies of New Zealand White rabbits with similar weights, this regimen led to peak levels of penicillin V of $10.4 \pm 4.8$ µg/ml of serum. Both these peak levels and subsequent levels were similar to those achieved in healthy human adults after an oral dose of 2 g of penicillin V (14).

(vi) The sixth regimen was PCN-1 followed by an oral dose of penicillin V (18 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) with a 7-h interval between doses (regimen PCN-2). As described in previous studies of rabbits with similar weights, the addition of probenecid to oral penicillin V (18 mg/kg) led to peak levels of $6.7 \pm 3.4$ µg/ml of serum. These levels were roughly similar to those achieved in humans after an oral dose of 1 g of penicillin V, decreasing thereafter at similar rates (14).

Rabbits were sacrificed 72 h after the last dose of antibiotic (or after inoculation in control groups). The hearts were removed aseptically, and the vegetations were excised, weighed, and homogenized in 1.0 ml of tryptic soy broth (Difco Laboratories) in glass tissue grinders. Of this homog-enate, 0.1 ml was used for serial dilutions. Both the remaining 0.9- and 0.1-ml portions of various dilutions were incorpo-rated into blood agar plates containing 50 U of penicillinase per ml and incubated for 48 h at 37°C in a candle jar for enumeration of colonies. Vegetations were considered sterile if there was an absence of growth on the plate containing the undiluted homogenate.

**Statistical evaluation.** A univariate analysis was performed with the use of independent contingency tables that were generated by considering the distributions of noninfected rabbits among prophylactic groups challenged with the same inoculum size ($10^6$, $10^7$, or $10^8$ CFU). To test the hypothesis of overall equality of proportions between groups, a chi-square test was used. The Fisher exact test was used to compare proportions in two-way tables.

Overall comparisons of means were performed by using an analysis of variance; the method of Scheffé was used for multiple pairwise comparisons.

**RESULTS**

**Antibiotic susceptibility tests.** The MICs and MBCs, respectively, were 0.04 and $>128$ µg/ml for amoxicillin, penicillin V, and penicillin G; both were 32 µg/ml for streptomycin. Thus, the MICs of amoxicillin and penicillin were comparable to that obtained with the highly susceptible strain of *S. sanguis* (strain HPE93) used in previous prophylactic experiments (MIC, 0.4 µg/ml (13, 14), but *S. sanguis* HPE77 was highly tolerant to both antibiotics and thus a wide disparity in their respective MBCs was found (MBCs, $>128$ and 0.08 µg/ml for HPE77 and HPE93, respectively).

![FIG. 1. Bactericidal activity of amoxicillin (10 µg/ml) (AMOX), penicillin V (10 µg/ml) (PCN-V), penicillin G (10 µg/ml) (PCN-G), streptomycin (10 µg/ml) (SM), and the penicillin G-streptomycin combination (0.5 and 5 µg/ml, respectively) (PCN-G + SM) against *S. sanguis*. Each colony count represents the mean of three determinations.](http://aac.asm.org/)
TABLE 1. Results of prophylaxis with each regimen in rabbits challenged with a strain of S. sanguis that is tolerant to amoxicillin and penicillin

<table>
<thead>
<tr>
<th>Inoculum (CFU)</th>
<th>Control</th>
<th>PCN-SM</th>
<th>AMOX-1</th>
<th>AMOX-2</th>
<th>AMOX-6</th>
<th>PCN-1</th>
<th>PCN-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁴</td>
<td>8 (53)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (13)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>10⁵</td>
<td>15 (100)</td>
<td>0 (0)</td>
<td>5 (33)</td>
<td>0 (0)</td>
<td>5 (33)</td>
<td>10 (66)</td>
<td>4 (26)</td>
</tr>
<tr>
<td>10⁶</td>
<td>15 (100)</td>
<td>0 (0)</td>
<td>14 (93)</td>
<td>5 (33)</td>
<td>12 (80)</td>
<td>14 (93)</td>
<td>13 (86)</td>
</tr>
</tbody>
</table>

* p value for analysis of overall equality of proportions of infected to noninfected rabbits among orally treated prophylactic groups (AMOX-1, AMOX-2, AMOX-6, PCN-1, and PCN-2). NS, Not significant.
* Differences from all other groups (P < 0.05).
* Differs from control group and AMOX-1, AMOX-6, PCN-1, and PCN-2 groups (P < 0.05).
* Differs from PCN-SM and AMOX-2 groups (P < 0.05).

**Time kill curves.** The in vitro rates of killing of S. sanguis HPE77 by each antibiotic are shown in Fig. 1. The time kill curves with 10 μg of amoxicillin, penicillin V, and penicillin G per ml showed slight reductions in CFUs at 6 h, falling by less than 99.9% over 48 h. In contrast, penicillin (0.5 μg/ml) plus streptomycin (5 μg/ml) sterilized the culture in 12 h.

**Efficacy of prophylactic regimens.** The results for the various experimental groups are detailed in Table 1. Although all untreated animals inoculated with 10⁶ or 10⁷ CFU exhibited IE at necropsy, only 8 (53.3%; P < 0.01) of 15 recipients of 10⁷ CFU exhibited positive vegetation cultures.

None of the animals injected with 10⁴, 10⁵, or 10⁶ CFU of S. sanguis developed streptococcal endocarditis after prophylaxis with combined procaine penicillin G plus streptomycin.

Attempted prophylaxis with the PCN-1 regimen failed to prevent IE in 13.3% of 15 recipients of the lowest inoculum, with high infection rates when inocula of 10⁶ to 10⁸ CFU were used. Both the addition of a second penicillin V dose (regimen PCN-2) and the AMOX-1 regimen prevented IE in all rabbits challenged with 10⁴ CFU, but protection also diminished with increasing inoculum concentrations. Similar results were achieved when 5 oral doses of amoxicillin (8.5 mg/kg of body weight) were added to the single-oral-dose amoxicillin regimen at 8-h intervals (AMOX-6 regimen). In contrast, administration of two high oral doses of amoxicillin (50 mg/kg of body weight) with a 10-h interval between doses (AMOX-2 regimen) provided full protection when rabbits were challenged with 10⁶ and 10⁷ CFU, and only 5 (33.3%) of 15 recipients of 10⁷ CFU exhibited IE at necropsy. Thus, all oral regimens tested were highly effective in preventing IE when rabbits received 10⁶ CFU. However, there was a significant inequality of distributions of infected and noninfected animals among orally treated groups challenged with 10⁶ (P < 0.05) and 10⁷ (P < 0.01) CFU, since the AMOX-2 regimen led to significantly fewer infections than did the other oral regimens when rabbits were exposed to those inocula.

Table 2 shows mean (± standard deviation) log₁₀ CFU/g of culture per positive vegetation in each group. It should be noted that the mean bacterial densities in vegetations of amoxicillin-treated or penicillin-treated rabbits challenged with 10⁹ or 10⁸ CFU in which IE developed were significantly lower than those in control rabbits challenged with the same inoculum. Thus, although not fully effective, these antibiotics reduced the intensity of infection.

**DISCUSSION**

Poor compliance with the penicillin-streptomycin prophylactic regimen proposed for patients at high risk of IE led a working party of the British Society for Antimicrobial Chemotherapy to recommend that amoxicillin by administered orally in a 3-g dose before dental procedures (19). On the other hand, in 1984 the American Heart Association published its revised recommendations for prophylaxis against IE, suggesting penicillin V orally in a 2-g dose 1 h before dental procedures plus a 1-g dose 6 h afterward (17). In previous work we evaluated protection achieved with each recommended regimen in the rabbit model of experimental endocarditis; similar results were obtained when animals were challenged with a highly susceptible strain of S. sanguis (13, 14).

Tolerance to the lethal action of penicillin among strains of the viridans group of streptococci has been increasingly recognized. Holloway et al. (8) reported that 19% of viridans group streptococci cultured from the gingivae of children and from their blood after dental extraction were tolerant to penicillin. Pulliam et al. (15) found that for 16 of 80 blood culture isolates of viridans group streptococci the MBCs of penicillin were at least 10-fold greater than the MICs. A report by Horne and Tomasz (9) indicated that all of nine S. sanguis strains tested were tolerant to penicillin, and that by Glauser et al. (5) indicated that one-third of streptococcal strains causing endocarditis were tolerant to amoxicillin. Since tolerance in oral streptococci seems to be a quite

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**TABLE 2. Mean log₁₀ CFU/g of culture per positive vegetation**

<table>
<thead>
<tr>
<th>Inoculum (CFU)</th>
<th>Control</th>
<th>AMOX-1</th>
<th>AMOX-2</th>
<th>AMOX-6</th>
<th>PCN-1</th>
<th>PCN-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁴</td>
<td>7.59 ± 0.61</td>
<td>6.03 ± 1.09</td>
<td>5.65 ± 0.81b</td>
<td>5.32 ± 1.20c</td>
<td>5.47 ± 0.90b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>10⁵</td>
<td>7.39 ± 0.61</td>
<td>5.91 ± 0.68</td>
<td>5.05 ± 0.56c</td>
<td>5.77 ± 0.75c</td>
<td>5.50 ± 1.03c</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>10⁶</td>
<td>7.40 ± 0.99</td>
<td>5.91 ± 0.68</td>
<td>5.05 ± 0.56c</td>
<td>5.77 ± 0.75c</td>
<td>5.50 ± 0.79c</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Overall P value for analysis of variance; NS, not significant.
* Differs from control group (P < 0.05).
* Differs from control group (P < 0.01).
common phenomenon, some of these strains being completely killed only at antibiotic concentrations higher than peak levels achieved in serum after prophylactic regimens currently used, prophylactic failures can conceivably occur when high-risk patients are exposed to bacteremia due to these strains. These considerations led to comparative studies in the rabbit model of endocarditis on the efficacy of recommended prophylactic regimens against strains of viridans group streptococci that are tolerant to penicillin and amoxicillin.

All regimens tested were highly effective in preventing endocarditis when rabbits were challenged with 10^6 CFU of a S. sanguis strain that was tolerant to beta-lactams. Thus, tolerance did not have detectable in vivo implications for prophylaxis in rabbits with low-grade bacteremia, the pattern in most dental procedures. It is unlikely that successful prophylaxis against S. sanguis HPE77 IE with oral regimens was achieved through bacterial killing, since incubation with penicillin V or amoxicillin (10 μg/ml) for 48 h only reduced colony counts to less than 99.9% of the original inoculum. These results are consistent with recent data suggesting that single intravenous doses of bactericidal (1, 4, 5) or bacteriostatic agents (6) protect a considerable number of animals against streptococcal endocarditis in the absence of bacterial killing, provided that the infective inoculum is lower than the minimum able to induce IE in 90% of control animals. However, comparison of these results with results obtained when inocula of 10^6 or 10^8 CFU were used with those in previous studies with a highly susceptible strain of S. sanguis (13, 14) indicates that tolerance significantly increased the prophylactic failure rates when oral regimens were used.

Previous in vivo studies showed that penicillin combined with streptomycin was very effective in the prevention of experimental endocarditis by a strain of S. sanguis that was highly susceptible to penicillin (3, 11). More recently, Hess et al. (7) extended these earlier observations to tolerant streptococci, showing that a combination of penicillin plus streptomycin provides better bactericidal activity in vitro and protects better against IE caused by these strains than does penicillin alone in vivo. Our results confirm these observations; the PCN-SM regimen (the only one that achieved bacterial killing in vitro) was significantly more effective than all other regimens against the highest inoculum, since none of 15 animals studied developed IE.

Our results indicate that protection achieved with oral regimens decreased with increasing inocula. An inequality of overall distribution of infected versus noninfected rabbits among orally treated prophylactic groups challenged with 10^6 and 10^8 CFU was found; the AMOX-2 regimen prevented IE significantly better than any other oral regimen. Enhancing protection by lengthening the duration of high levels of amoxicillin in serum might be related to a progressive decrease in viable bacterial counts in vegetations with hours of inhibition (as happens in time kill curves in vitro), facilitating the action of host defenses against organisms attached to them. In contrast, when we attempted to increase the protection achieved with the single-dose amoxicillin regimen by the administration of five subsequent low doses, the efficacy was not significantly improved. This could be related to limited antibiotic penetration into vegetations (10); very low inner concentrations were achieved after oral administration of amoxicillin (8.5 mg/kg).

Our findings indicate that intramuscular injection of penicillin G-streptomycin, a synergistic combination with high bactericidal activity against viridans group streptococci, seems the most effective regimen to use. Both the single high-concentration oral dose of amoxicillin proposed by the British Society for Antimicrobial Chemotherapy (19) and the two oral doses of penicillin V suggested by the American Heart Association (17) protected acceptably well against low-grade bacteremia, the pattern in most dental procedures. However, when rabbits were challenged with 10^6 and 10^8 CFU, lengthening the duration of serum levels of amoxicillin by means of a second high-concentration oral dose enhanced protection when compared with the other oral regimens. Thus, administration of a second high-concentration oral dose of amoxicillin might be a second-choice alternative to parenteral penicillin G-streptomycin regimens for high-risk patients who might carry oral streptococci that are tolerant to penicillin.

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LITERATURE CITED


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