Optimal Aminoglycoside Dosing Regimen for Penicillin-Tobramycin Synergism in Experimental Streptococcus adjacens Endocarditis

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The combination of penicillin and aminoglycoside is the recommended therapy for endocarditis caused by nutritionally variant streptococci (NVS). However, the optimal aminoglycoside dosing regimen remains controversial. We compared the efficacies of four regimens of tobramycin alone or combined with procaine penicillin in the therapy of rabbits with endocarditis caused by Streptococcus adjacens, a new species of NVS. Animals were injected intramuscularly for 4 days with procaine penicillin (150,000 U/kg of body weight twice daily) or tobramycin at a low dose (3 mg/kg every 24 h) or a high dose (12 mg/kg every 24 h) either once or three times daily (t.i.d.) alone or in combination with procaine penicillin. Additional groups of animals were treated with the combination regimens for a shorter period of time (2 days) in order to demonstrate a possible difference in the rapidity of efficacy between the regimens. The MICs and MBCs were 0.015 and 1 μg/ml and 8 and 16 μg/ml for penicillin and tobramycin, respectively. The mean peak tobramycin levels in plasma were 2.4 ± 1.3 (1 mg/kg t.i.d.), 5.4 ± 3.7 (4 mg/kg t.i.d.), and 25 ± 9.3 (12 mg/kg once daily). The mean penicillin levels in serum were always above the MIC. In vitro kill curves plotted at the time that peak concentrations were reached in plasma showed a concentration-dependent killing effect of tobramycin alone but not in combination with penicillin. In vivo, low-dose tobramycin was significantly less effective than the high dose. Results for the combinations of the different dosing regimens of tobramycin with procaine penicillin were not significantly different. Our results suggest that (i) against susceptible strains of streptococci, aminoglycoside alone exhibits a concentration-dependent killing effect both in vitro and in vivo; (ii) against NVS strains, combinations of penicillin and high- or low-dose tobramycin are equally effective; and (iii) an aminoglycoside given once daily or at a low dose t.i.d. with penicillin could be a cost-effective alternative with reduced toxic risk for patients with NVS endocarditis when the bacteria are susceptible to the killing activities of both compounds.

Nutritionally variant streptococci (NVS), which include two distinct species, Streptococcus defectivus and Streptococcus adjacens (3), have been incriminated in 5% of cases of human bacterial endocarditis (21). Complication and relapse rates are higher among patients with NVS endocarditis than among patients with endocarditis caused by other viridans group streptococci (4). Despite low penicillin MICs for NVS, penicillin alone is not very effective in patients with NVS endocarditis (4). Experimental studies have shown that the combination of an aminoglycoside with penicillin increases the efficacy of the latter compound (2, 7). As for enterococci, administration of a combination of penicillin and aminoglycoside for at least 4 weeks has been recommended by some authors investigators (8a). However, the mechanism of the apparent synergistic effect in vivo could be different from that described by Moellering and Weinberg (16) for enterococci. In vitro data on viridans group streptococci have suggested that this β-lactam–aminoglycoside potentiation is not due to the enhancement of aminoglycoside uptake by penicillin (15). Because the penicillin-aminoglycoside combination is recommended for a prolonged period of time, patients are exposed to the potential nephrotoxic and ototoxic side effects of the aminoglycoside. Thus, it is important to define the optimal aminoglycoside dosing regimen that will provide maximal efficacy and minimal toxicity.

The use of low doses of aminoglycoside has been suggested to diminish the toxicities of aminoglycosides (25). However, lowering of the aminoglycoside dose could seem paradoxical in view of the clear dose-dependent killing effect of aminoglycosides on gram-negative bacilli that has been demonstrated in vitro (6), experimentally (10, 24), and in some clinical trials (17). Previous reports on the importance of the aminoglycoside dosing regimen associated with penicillin for the therapy of streptococcal endocarditis have yielded conflicting results. In experimental NVS endocarditis (12), lowering of the dose of gentamicin did not diminish the efficacy of its combination with procaine penicillin. In the same study (12) the highest dose of streptomycin was significantly more effective than the low dose. A dose-dependent efficacy of an aminoglycoside in combination with penicillin has also been reported in an experimental Enterococcus faecalis endocarditis (9) and Pseudomonas aeruginosa endocarditis in patients (20).

Another way to attenuate aminoglycoside toxicity would be to inject the antibiotic once daily (11). In experimental enterococcal endocarditis, an aminoglycoside given once daily in combination with penicillin was less effective than the same daily dose given three times daily (t.i.d.) (9). However, the effect of increasing the interval between doses on the penicillin-aminoglycoside synergism against NVS endocarditis has never been evaluated in vivo.

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In the study described here, we evaluated the efficacy of tobramycin alone or combined with procaine penicillin at different dosing regimens: a low dose given once daily or t.i.d. and a high dose given once daily or t.i.d. Tobramycin was selected over another aminoglycoside because its pattern of diffusion into vegetations is known (8) and its in vitro activity is similar to that of gentamicin, which has been studied extensively (2, 12).

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MATERIALS AND METHODS

Test strain. A well-defined strain of *S. adjacens* (ATCC 49175), GaD<sup>T</sup>, isolated from the cardiac valve of a patient who underwent surgery after 2 weeks of treatment with penicillin G and streptomycin was used. This strain was selected because its in vitro susceptibility to antibiotics was representative of NVS. It had previously been used to induce endocarditis in rabbits to evaluate antibiotic therapies prescribed to patients (2, 7) and to determine the diffusion patterns of antibiotics into large vegetations (8). The strain was grown at 37°C in a chemically defined medium enriched with 2% Todd-Hewitt dialysate (CDMT) (5).

In vitro antibacterial susceptibility tests. MICs and MBCs were determined in triplicate by using the tube macrodilution method in CDMT containing a lower concentration of cysteine (5 mg/liter) to minimize penicillin inactivation (18). The inocula were diluted from log-phase cultures to obtain a final concentration of 10<sup>6</sup> CFU/ml. The antibiotics tested were penicillin G (Specia, Paris, France) and tobramycin (Eli Lilly, Saint Cloud, France). MBCs were determined after 24 h of incubation of 0.1 ml from each clear broth tube plated onto 5% sheep blood Columbia agar (Bio-Merieux, Marcy l'Etoile, France) enriched with pyridoxal hydrochloride (100 μg/ml). The MIC was defined as the lowest concentration of antibiotic that prevented turbidity in the test tube after 24 h of incubation, and the MBC was defined as the lowest concentration of antibiotic that reduced the organism count by 99.9% of the original count. Tolerance was defined as an MBC/MIC ratio of greater than 32 (22).

Bactericidal rates were determined with log-phase inocula of 10<sup>7</sup> CFU/ml in CDMT with the lower concentration of cysteine containing, alone or in combination, penicillin G (20 μg/ml) and tobramycin (2, 8, and 18 μg/ml), i.e., at concentrations similar to the peak levels obtained in the sera of treated animals. Samples were removed from the tubes after 0, 3, 6, and 24 h and were serially diluted 10-fold in sterile saline, with 0.1 ml of each dilution being plated onto enriched agar plates and incubated for 48 h. Penicillinase was added to agar for penicillin-containing samples. Each experiment was run twice in duplicate and included controls of growth without antibiotic. In vitro synergy was defined as a 100-fold increase of killing by the combination over that obtained with the single most effective agent and a 1,000-fold decrease in the bacterial count compared with that of the inoculum. The minimal detectable number of CFU per milliliter was 10.

Experimental endocarditis. A modified version of the method of Perlman and Freedman (19) was used to induce endocarditis in female New Zealand White rabbits, each weighing between 2 and 3 kg (2). A polyethylene catheter was inserted through the right carotid artery into the left ventricular cavity and was left in place throughout the experiment. Twenty-four hours after placement of the catheter (day 1) animals were inoculated via the marginal ear vein with 10<sup>6</sup> CFU of strain GaD<sup>T</sup> in 1 ml. When the catheter was correctly placed, endocarditis was obtained in 100% of the inocules, as demonstrated by positive blood cultures on day 7 and by the presence of infected vegetations on the aortic valves at the time of sacrifice. Blood cultures were performed as described previously (2).

Therapeutic studies. (i) Treatment and evaluation of therapy. Six days after infection (day 7), the following treatment regimens injected intramuscularly (i.m.) were started: procaine penicillin alone (150,000 U/kg of body weight twice daily [b.i.d.]) or tobramycin at a low dose (1 mg/kg t.i.d. or 3 mg/kg once daily) or a high dose (4 mg/kg t.i.d. or 12 mg/kg once daily) alone or combined with procaine penicillin. Each regimen was administered for 4 days. Additional groups of rabbits were treated with the combination regimens for 2 days in order to discern a possible difference in the rapidity of the antibacterial effect between these treatments. Animals were killed on day 9 or on day 11, 8, 12, or 24 h, after the last doses of 1 and 4 mg of tobramycin per kg, penicillin, or 3 and 12 mg of tobramycin per kg, respectively. One control rabbit died on day 8; the others were sacrificed at the time corresponding to the end of therapy (day 11).

At the time of sacrifice, all vegetations were excised, weighed, homogenized in 0.5 ml of saline, and quantitatively cultured on pyridoxal-enriched blood agar plates at 37°C for 48 h. The lowest detectable bacterial counts ranged from 2 to 3.5 log<sub>10</sub> CFU/g of vegetation, depending on the size of the vegetation. The vegetation was considered sterile when the culture showed no growth after 48 h of incubation at 37°C, and the CFU count was recorded as the lowest detectable bacterial count. Drug carryover was avoided by serial dilutions and spreading the subculture (50 μl) on agar plates or by the addition of penicillinase to agar for penicillin-containing samples.

(ii) Serum antibiotic levels. To determine peak and trough concentrations in plasma, blood was drawn from the ear vein 1 h after the injection of antibiotic on day 10 and at the time of sacrifice, respectively; blood was stored at −70°C. Penicillin concentrations were measured by agar disk diffusion by using *Bacillus subtilis* ATCC 6633 as the test strain; these concentrations have been reported previously (2). A radiodiffusion assay was used to measure tobramycin concentrations. The lower limits of detection per milliliter of plasma were 0.1 μg of penicillin and 0.015 μg of tobramycin; assay reproducibilities were ±7 and ±6%, respectively.

Statistics. Results are expressed as means ± standard deviations. Bacterial counts in vegetations from the various experimental groups were compared by an analysis of variance; this was followed by the Scheffe test for multiple comparisons. A P value of <0.05 was considered significant.

RESULTS

In vitro studies. The MICs and MBCs of penicillin and tobramycin were 0.015 and 1 μg/ml and 8 and 16 μg/ml, respectively, indicating tolerance to penicillin and low-level resistance to tobramycin. Penicillin G was bactericidal, reducing bacterial counts from 7 log<sub>10</sub> CFU/ml to 4.9 log<sub>10</sub> CFU/ml after 24 h of incubation, as shown by in vitro killing curves (Fig. 1A). When tobramycin was tested alone, an increase in the drug concentration increased the rate of killing and the final bactericidal effect. The combination of penicillin with the lowest concentration of tobramycin tested (2 μg/ml) (Fig. 1B) was synergistic, as defined in Materials and Methods. In combination with penicillin, an increase in
the tobramycin concentration increased the rate of killing within the first few hours but not the final bactericidal effect measured at 24 h.

**Therapeutic studies.** The antibiotic concentrations measured in the sera of treated animals are given in Table 1. Mean penicillin levels were always above the MIC for the strain studied. Mean peak tobramycin levels ranged from 2.4 to 25.2 μg/ml, depending on the dose administered (1 to 12 mg/kg).

In all treated groups except those given low-dose tobramycin once daily, the mean bacterial counts in vegetations were significantly reduced compared with those in control animals (P < 0.05 to 0.001) after 4 days of therapy (Table 2). For the same daily dose, the efficacies of the once-daily and t.i.d. dosage regimens were similar. A high-versus low-dose–effect comparison was then made by considering the daily dose independently of the dosing regimen. Given as monotherapy, the low-dose tobramycin regimen was significantly less effective than the high-dose regimen (P < 0.01). Penicillin plus low-dose or high-dose tobramycin was significantly more effective than penicillin alone (P < 0.05) or tobramycin alone (P < 0.01) at the same dose. The efficacies of all combination groups were comparable.

Evaluation of the efficacies of the combined therapies after 2 days of treatment showed no significant differences (Table 3).

**DISCUSSION**

NVS endocarditis, like enterococcal endocarditis, is a good model for the study of in vivo synergistic effects of combinations of penicillin and an aminoglycoside. Indeed, even if penicillin alone has a significant effect compared with the results for the controls in an experimental model (2, 7), monotherapy is not sufficient to cure NVS endocarditis in patients (4); a combination of penicillin with an aminoglycoside for the first 2 weeks of a 4-week course of therapy is currently recommended (1). However, the optimal aminoglycoside dosing regimen remains to be determined.

In the present study we showed that tobramycin alone exhibits a concentration-dependent bactericidal effect in vitro on killing curves obtained at concentrations close to

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**TABLE 1. Antibiotic concentrations measured in serum after 4-day regimens given i.m.**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Regimen</th>
<th>Concen (μg/ml)</th>
<th>Peaka</th>
<th>Troughb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine penicillin</td>
<td>150,000 U/kg b.i.d.</td>
<td>22.2 ± 11.3</td>
<td>0.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1 mg/kg t.i.d.</td>
<td>2.4 ± 1.3</td>
<td>0.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>4 mg/kg t.i.d.</td>
<td>5.4 ± 3.7</td>
<td>0.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>12 mg/kg once daily</td>
<td>25.2 ± 9.3</td>
<td>0.5 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

a Samples were obtained 1 h after the last injection of antibiotic.
b Samples were obtained 8, 12, and 24 h after the last dose of tobramycin (1 and 4 mg/kg t.i.d.), procaine penicillin (b.i.d.), and tobramycin (12 mg/kg once daily), respectively.

**TABLE 2. Result of a 4-day antibiotic treatment of experimental endocarditis caused by S. adjacens**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Regimen</th>
<th>log(_{10}) CFU/g (mean ± SD) [no. of rabbits]</th>
<th>Avg log(_{10}) CFU/g (mean ± SD) [no. of rabbits]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>b.i.d.</td>
<td>7.5 ± 0.9 (6)</td>
<td>4.5 ± 0.7a (6)</td>
</tr>
<tr>
<td>Procaine penicillin, 300,000 U/kg/day</td>
<td>t.i.d.</td>
<td>5.4 ± 0.7a (6)</td>
<td>5.6 ± 1.0 (12)</td>
</tr>
<tr>
<td>Tobramycin, low dose (3 mg/kg/day)</td>
<td>Once daily</td>
<td>5.8 ± 1.2 (6)</td>
<td></td>
</tr>
<tr>
<td>Tobramycin, high dose (12 mg/kg/day)</td>
<td>t.i.d.</td>
<td>4.1 ± 1.3b (6)</td>
<td>4.2 ± 1.5 (7)</td>
</tr>
<tr>
<td>Procaine penicillin + tobramycin, low dose</td>
<td>t.i.d.</td>
<td>2.4 ± 0.4 (7)</td>
<td>2.8 ± 0.5 (7)</td>
</tr>
<tr>
<td>Procaine penicillin + tobramycin, high dose</td>
<td>Once daily</td>
<td>3.2 ± 0.1b (6)</td>
<td></td>
</tr>
</tbody>
</table>

- a Data are averages for the t.i.d. and once-daily treatment regimens combined.
- b P < 0.01 compared with control.
- c P < 0.05 compared with control.
- d P < 0.01 compared with tobramycin low-dose monotherapy.
- e P < 0.05 compared with penicillin alone, and P < 0.01 compared with tobramycin monotherapy at the same dosage.

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**TABLE 3. Result of a 2-day antibiotic treatment of experimental endocarditis caused by S. adjacens**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Regimen</th>
<th>log(_{10}) CFU/g (mean ± SD) [no. of rabbits]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine penicillin + tobramycin, low dose</td>
<td>t.i.d.</td>
<td>4.0 ± 1.3 (5)</td>
</tr>
<tr>
<td>Procaine penicillin + tobramycin, high dose</td>
<td>Once daily</td>
<td>3.8 ± 0.9 (5)</td>
</tr>
</tbody>
</table>

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**FIG. 1.** Bactericidal kinetics comparing the effect of antibiotics alone (A) or in combination (B) on the *S. adjacens* GaD. Abbreviations: Pen, penicillin G (20 μg/ml); Tob 2, tobramycin (2 μg/ml); Tob 8, tobramycin (8 μg/ml); Tob 20, tobramycin (20 μg/ml).
peak levels in rabbit plasma for the different regimens. Also, in vivo, the mean bacterial counts per gram of vegetations measured in animals receiving tobramycin alone were lower with the high-dose (12 mg/kg/day) than with the low-dose (3 mg/kg/day) regimen. The concentration-dependent killing effect of aminoglycosides has been clearly demonstrated in vitro (6) and in experimental models (10, 24), mostly when tested alone against gram-negative bacilli. Vogelman et al. (24) have indeed shown that the logarithm of the area under the curve, i.e., the dose administered, was the most important parameter of aminoglycoside efficacy in a thigh infection experimental model. In our study, treatment with low-dose tobramycin t.i.d. reduced significantly the bacterial counts compared with those in the controls. This could be surprising, considering the fact that with this low dose, the peak serum tobramycin concentration in animals was lower than the MIC for the test strain. Gentamicin has been shown to be active in vivo against enterococci, despite the low-level resistance observed in vitro, with MICs and MBCs of 16 and 32 μg/ml, respectively, for the Streptococcus faecalis strain tested (23). In that study, the peak concentration of gentamicin in serum (5.8 ± 2.1 μg/ml) was also far lower than the MIC. Because long-term therapy exposes patients to toxic amounts of drug, however, the use of aminoglycosides in monotherapy in patients with endocarditis is unrealistic.

In combination with penicillin, low and high doses of tobramycin were significantly more effective than penicillin alone. In contrast to tobramycin alone, the two doses of tobramycin were equally effective when given in combination with procaine penicillin. This observation is in accordance with the in vitro time-kill curves obtained at concentrations similar to the peak levels in plasma, if the end point after 24 h of incubation is considered. However, a possible difference between the in vivo effects of the regimens of penicillin in combination with high and low doses of tobramycin could have been masked by the fact that after eight i.m. injections, almost all vegetations in these animals are sterile. We therefore compared the two dosage regimens earlier in the course of the same treatment (2 days, four i.m. injections). At that time, most of the vegetations still contained organisms, but the CFU counts in the two groups did not differ significantly.

Contradictory findings concerning the importance of the aminoglycoside dose in combination with penicillin for the therapy of NVS and other bacterial endocarditis have been published. In an experimental NVS endocarditis, Henry et al. (12) have shown that the dose of streptomycin, but not that of gentamicin, influences the efficacy of the combination with procaine penicillin. One explanation could be that, in vitro, the peak streptomycin level in serum obtained with the low-dose regimen (5 μg/ml) did not act synergistically with penicillin. In contrast, even the low-dose peak level of gentamicin in serum (0.7 μg/ml) was synergistic in vitro with penicillin. Thus, the influence of the aminoglycoside dose combined with penicillin could depend on the respective concentrations required to obtain in vitro synergism and the peak aminoglycoside level in serum. The good correlation between the in vivo results and the in vitro killing rate obtained at peak concentrations in serum is favored by the homogeneous diffusion of the aminoglycoside like tobramycin into vegetations, with local concentrations being similar to concentrations in plasma (8). In enterococcal infections, the influence of the dose of an aminoglycoside in combination with penicillin varies with the strain tested and the doses used (9, 26). The relationship between in vitro killing curves obtained at peak concentrations in plasma and in vivo results is not clear, probably because the trough aminoglycoside concentration is also an important parameter of in vivo efficacy (9, 13). In our study, tobramycin monotherapy (3 or 12 mg/kg) was equally effective when it was administered as a single daily dose or t.i.d. This could be due to a postantibiotic effect of tobramycin on NVS streptococci. However, a postantibiotic effect of an aminoglycoside alone on streptococci has never been studied because, as emphasized previously, these organisms are poorly susceptible in vitro to aminoglycosides.

In combination with procaine penicillin, tobramycin given once daily was as effective as the t.i.d. regimen for the same total dose, whether determined after four or eight i.m. injections. This result contrasts with those obtained with S. faecalis (9). This difference is probably related to the different penicillin-aminoglycoside mechanisms of synergy in NVS and enterococci (15). Because penicillin enhances the uptake of aminoglycoside in enterococci (15, 16), both antibiotics must simultaneously be in prolonged contact with the bacteria. In contrast, aminoglycoside uptake by viridans group streptococci (15) remains unchanged in the presence or absence of penicillin. The precise mechanism of this apparent synergism remains to be defined (15). Both penicillin and tobramycin are active alone. Therefore, the persistent, simultaneous presence of the two antibiotics in contact with the bacteria during the interval between two doses is not essential for obtaining an optimal in vivo bactericidal effect. These observations are comparable to the results obtained with an infection caused by gram-negative bacilli (14), which showed that as long as the concentration of the β-lactam remained above the MIC, a once-daily dose of aminoglycoside was as effective as conventional (t.i.d.) therapy. The retained efficacy of penicillin in combination with an aminoglycoside given once daily against NVS endocarditis is also in accordance with some experimental results obtained in other studies of endocarditis caused by viridans group streptococci (7a). It must be emphasized, however, that when penicillin is combined with the low dose of tobramycin, bacterial counts in vegetations after the 4-day treatment were slightly more important with the once-daily than with the t.i.d. regimen. Even if this difference was not significant under our experimental conditions, extrapolation to humans should be done with caution.

Once-daily administration or low-dose tobramycin regimens in combination with procaine penicillin could be cost-effective alternatives, with reduced toxic risk for patients with NVS endocarditis.

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REFERENCES


