Postantibiotic Effect of Penicillin plus Gentamicin versus Enterococcus faecalis In Vitro and In Vivo

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A persistent suppression of bacterial growth following a brief exposure to an antibiotic (postantibiotic effect [PAE]) has been described for a variety of antibiotics and microorganisms. If a PAE is present in vivo, antibiotic levels in tissue at the site of infection may decrease below the MIC without bacterial regrowth in the latter portion of the dosing interval. In the present studies, a PAE was sought in vitro and in vivo for penicillin G plus gentamicin versus Enterococcus faecalis. The studies demonstrated that increasing concentrations of gentamicin caused an increased rate of bactericidal action and an increasingly prolonged PAE in vitro. The combination of penicillin and gentamicin, in addition to more rapid killing, exhibited a more prolonged PAE than did penicillin alone. However, unlike these in vitro findings, no PAE could be demonstrated in vivo in rats with experimental left-sided enterococcal endocarditis treated with penicillin plus gentamicin. This suggests that antibiotic vegetation levels should be maintained above the MIC throughout the dosing interval to prevent loss of efficacy as a result of bacterial regrowth.

Persistent suppression of bacterial growth after a brief exposure to an antibiotic, i.e., a postantibiotic effect (PAE), has been described for a variety of antibiotics and organisms (12). A PAE, if present in vivo, should permit antibiotic levels in tissue to fall such that antibacterial activity could be absent for a portion of the dosing interval without loss of drug efficacy as a result of bacterial regrowth. A number of factors, including inoculum size, antibiotic concentration, medium, and conditions of incubation, influence the development of a PAE. One aspect of the PAE that has not been well studied is the effect of antibiotic combinations. This information would be relevant, for example, in the therapy of enterococcal endocarditis, which requires the combination of penicillin and gentamicin to sterilize the vegetation.

We report here a study of the PAE with penicillin and gentamicin versus five strains of enterococci in vitro and versus one of these strains in a rat model of experimental left-sided endocarditis.

MATERIALS AND METHODS

In vitro experiments. Five strains of Enterococcus faecalis isolated from the blood of patients with endocarditis were used.

The MIC and MBC were determined by the broth dilution method with Mueller-Hinton broth (MHB) supplemented with calcium and magnesium (5). The antibiotics were diluted in twofold steps in tubes; each tube contained 0.5 ml of MHB. The bacterial inoculum was added to each tube in 0.5 ml of MHB diluted from an 18-h culture in MHB to yield a concentration of 10⁵ CFU of E. faecalis per ml. The MIC was defined as the lowest concentration of antibiotic that prevented turbidity after 18 h of incubation at 37°C. The MBC was defined as the lowest concentration of antibiotic that decreased the inoculum by 99.9% within 18 h, as determined by plating 0.1-ml portions of the MIC dilutions.

The rate of killing and the presence of a PAE of penicillin (at 20 μg/ml) and gentamicin (at 3, 6, 12, and 24 μg/ml) each alone and in various combinations, were determined in vitro in MHB. Bacterial suspensions of 10⁷ CFU/ml were prepared by inoculating 1 ml of stock culture into fresh broth and incubating it for 6 to 8 h at 37°C. The bacteria were then exposed to antibiotic(s) at various concentrations for 2 h. The antibiotic(s) was then removed by repeated centrifugation and suspension of the organisms in fresh medium, as described by McDonald et al. (6). The absence of antibiotic was documented by a disk diffusion assay. The antibiotic-free bacterial suspension was reincubated at 37°C. The number of viable organisms per milliliter was determined at time zero, after 2 h of incubation (immediately after suspension in antibiotic-free medium), and hourly for the next 8 to 10 h. The PAE was quantitated by calculating the difference in time required for the number of drug-exposed and untreated control organisms to increase 10-fold above the number present immediately after removal of the antibiotic(s) (6).

In vivo experiments. To induce endocarditis, the right carotid artery of a rat was cannulated, and the catheter was advanced into the left ventricle (9). At 24 h later, 10⁶ CFU of E. faecalis 1 in 1 ml of saline were injected into the tail vein. All rats infected with this inoculum developed endocarditis on the aortic valve. At 18 h later, the rats were treated intramuscularly with one dose of antibiotic(s). The rats were killed at various times thereafter, and the vegetations were removed and homogenized in 0.5 ml of MHB. The homogenate was cultured quantitatively, and the antibiotic concentration was determined by a disk diffusion assay (8).

Statistical analysis. One-way analysis of variance followed by a test for trend was used to determine the significance of differences among PAEs with varying gentamicin concentrations. One-way analysis of variance, followed by t tests, with the Bonferroni correction to allow for having performed multiple comparisons, was used to determine the significance of differences among PAEs with penicillin or gentamicin, alone and in combination.

RESULTS

In vitro experiments. The MIC and MBC for each organism are listed in Table 1. The rate of bactericidal action and the PAE for various concentrations of the antibiotics are...
shown in Table 2. The mean initial inoculum of all strains was 6.8 CFU/ml (range, 6.4 to 7.1 CFU/ml). Increasing the concentrations of gentamicin (3 to 24 μg/ml) caused a greater rate of bactericidal action and increasingly prolonged PAE from a mean of 0.5 h to 3.7 h (P < 0.001). The combination of penicillin and gentamicin caused more rapid bactericidal action than either penicillin or gentamicin alone and resulted in a significant prolongation of the PAE in comparison with the PAE with each drug alone (P < 0.01).

In vivo experiments. Figure 1 shows the pharmacodynamic effects and Table 3 gives the antibiotic concentrations in vegetations following administration of single intramuscular doses of aqueous penicillin G (50 mg/kg) plus gentamicin (4 mg/kg) to rats with experimental enterococcal endocarditis. Peak levels of penicillin G in vegetation were 43.2 μg/ml. The levels remained above the MIC of penicillin for strain 1 (about 3 μg/ml) for about 3 h. The peak level of gentamicin in vegetation was 2.1 μg/ml at 1 h.

Vegetation enterococcal counts decreased approximately 1 log_{10} CFU/g (P < 0.01, t test) during the first hour, but then rose rapidly (approximately 0.5 log_{10} CFU/h); they exceeded pretreatment enterococcal counts at 3 h after treatment. Hence, no PAE was demonstrated in the rat endocarditis model, although the antibiotic levels in vegetation were equal to or greater than the concentrations at which a PAE had been observed in vitro (Table 2).

**DISCUSSION**

The experimental model of aortic-valve endocarditis is appropriate for demonstrating pharmacodynamic effects, since the aortic-valve vegetation is a secluded site in which host defense mechanisms apparently are of little importance in eliminating bacteria (2). Therefore, clearance of organisms from the vegetation is related primarily to the rate and extent of the bactericidal effect of antimicrobial therapy and the duration of the PAE. For example, a small bactericidal effect after each dose would require an accumulation of the bactericidal effects of multiple doses to significantly reduce the bacterial population in the vegetation. Additionally, the absence of a PAE would require relatively short dosing intervals to maintain antibiotic concentrations in tissue above the MIC and thereby prevent bacterial regrowth in the latter portion of each dosing interval. In contrast, a rapid and large bactericidal effect following a single dose of antibiotic would reduce the bacterial population in the vegetation to relatively small numbers. This small residual bacterial population would require a relatively long interval to recover to its original numbers. The presence of a long PAE in vivo would extend the inhibitory effect on the small residual population and allow the use of longer dosing intervals, during which the drug levels in tissue could fall below the MIC without loss of efficacy (1, 3, 4, 12).

Therapy of enterococcal endocarditis requires the combination of penicillin plus an aminoglycoside to enhance the rate and extent of bactericidal action to sterilize the vegetation (14). Penicillin G is usually administered as an intermittent intravenous infusion of 3 × 10^6 units over 30 min every 4 h, ideally resulting in peak and trough levels in serum of approximately 200 to 400 μg/ml and about 1 μg/ml, respectively (7). Gentamicin is commonly administered intermittently by intravenous infusion in a dose of 1 mg/kg every 8 h; this dose results in peak levels in serum of 3 μg/ml, declining within 4 h to 1 μg/ml (14). If the antibiotics diffuse readily into vegetations, intermittent simultaneous administration of penicillin and gentamicin would result in levels of both antibiotics in vegetation that (i) are bactericidal only during the initial portion of the dosing interval and (ii) may be subinhibitory during the latter portion of the dosing interval. With the loss of inhibitory levels, regrowth of the organisms might be expected. A clinically significant PAE, however, would maintain bacteriostasis despite the fall in levels to subinhibitory concentrations. Our experiments demonstrate in vitro PAEs for penicillin and for gentamicin versus the enterococcus. Both the rate of bactericidal action and the duration of the PAE of gentamicin were concentration dependent. The combination of penicillin plus gentamicin showed more rapid killing than did penicillin or gentamicin alone; the combination also showed a prolongation of the PAE.

However, the animal experiments failed to confirm the presence of a PAE in vivo following administration of the combination of penicillin plus gentamicin. This finding is similar to those of other studies in which no PAE was found.

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**TABLE 1.** Antimicrobial susceptibility of enterococci

<table>
<thead>
<tr>
<th>Strain</th>
<th>Penicillin G</th>
<th>Gentamicin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (μg/ml)</td>
<td>MBC (μg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>3.1</td>
<td>&gt;50</td>
</tr>
<tr>
<td>2</td>
<td>3.1</td>
<td>&gt;50</td>
</tr>
<tr>
<td>3</td>
<td>3.1</td>
<td>&gt;50</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>&gt;50</td>
</tr>
<tr>
<td>5</td>
<td>3.1</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* Geometric mean of four determinations for strain 1; otherwise, results of one determination.

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**TABLE 2.** Fall in bacterial count and PAE following a 2-h exposure to antibiotics in vitro

<table>
<thead>
<tr>
<th>Antibiotic (amt [μg/ml])</th>
<th>Strain 1*</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
<th>Strain 5</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall in count*</td>
<td>PAE</td>
<td>Fall in count</td>
<td>PAE</td>
<td>Fall in count</td>
<td>PAE</td>
</tr>
<tr>
<td>Gentamicin (3)</td>
<td>0</td>
<td>0.7</td>
<td>0.9</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Gentamicin (6)</td>
<td>0</td>
<td>1.1</td>
<td>2.4</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Gentamicin (12)</td>
<td>0</td>
<td>2.0</td>
<td>2.9</td>
<td>0.3</td>
<td>1.8</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Gentamicin (24)</td>
<td>3.4</td>
<td>5.2</td>
<td>5.0</td>
<td>3.1</td>
<td>2.8</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Penicillin G (20)</td>
<td>0.4</td>
<td>1.8</td>
<td>0.4</td>
<td>2.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Gentamicin (3) + penicillin G (20)</td>
<td>3.6</td>
<td>3.5</td>
<td>2.1</td>
<td>4.6</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Gentamicin (6) + penicillin G (20)</td>
<td>3.6</td>
<td>4.1</td>
<td>2.3</td>
<td>4.9</td>
<td>1.8</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* In additional experiments, PAE was 1.8 h for penicillin (20 μg/ml) plus gentamicin (1 μg/ml) and 3.8 h for penicillin (20 μg/ml) plus gentamicin (2 μg/ml).
+ Fall in bacterial count is expressed as log_{10} CFU per milliliter.
+ PAE is expressed in hours.
in vivo when penicillin was used to treat other streptococcal infections, e.g., in the rabbit meningitis model (10) and in neutropenic mice with thigh infections (13). Additionally, our previous experiment failed to find a PAE in either left- or right-sided Pseudomonas aeruginosa endocarditis in rats treated with imipenem alone or imipenem in combination with gentamicin, despite the presence of an in vitro PAE (3a). To our knowledge, there are no previous studies evaluating the PAE in enterococci, either in vitro or in vivo.

It is unlikely that the experimental model alone is a factor, since ciprofloxacin exhibited a PAE against P. aeruginosa in this model (4). Nor is it likely that the inoculum size accounts for the discrepancy, since initial inocula were similar in vitro and in vivo (6.8 CFU/ml and 7.0 CFU/g, respectively). One explanation may be that the metabolic state of the organisms in vivo is different from that in vitro, so that rapid resynthesis of penicillin-binding proteins occurs in vegetations, allowing immediate regrowth of tissue organisms following antibiotic removal, as implied by Tuomanen (11). These metabolic differences in organisms would allow some antimicrobial agents, such as ciprofloxacin (but not others, such as beta-lactams), to exhibit a PAE in vivo. Alternatively, it is possible that the antibiotic concentration measured in the vegetation, although equivalent to the concentration shown to produce a PAE in vitro, is not present in the immediate environment of the bacterial cell, perhaps because (i) the bacteria are sequestered within a biofilm or (ii) there is an uneven distribution of antibiotic in the vegetation (A. C. Cremieux, J. M. Vallois, B. Maziere, M. Ottaviani, A. Bouvet, and C. Carbon, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother, abstr. no. 922, 1987). Additionally, since increasingly long PAEs were seen with increasing concentrations of gentamicin in vitro, an increased dose of gentamicin in vivo might have produced a detectable PAE. In any case, the absence of a PAE in this model suggests that antibiotic concentrations in vegetation should be maintained above the MIC, and perhaps above the MBC, throughout the dosing interval to prevent a loss of efficacy in the treatment of enterococcal endocarditis.

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LITERATURE CITED


