Influence of gentamicin dosing interval on the efficacy of penicillin-containing regimens in experimental *Enterococcus faecalis* endocarditis

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The influence of the gentamicin dosing regimen was studied in experimental *Enterococcus faecalis* endocarditis. After inoculation, animals received penicillin, or penicillin plus once-daily gentamicin, or penicillin plus thrice-daily gentamicin, or no treatment. After the treatment period, bacterial densities within the vegetations (mean ± SEM) were 6.06 ± 0.30, 5.42 ± 0.29, 4.98 ± 0.10 and 9.97 ± 0.16 log cfu/g for the four groups. All regimens produced significant reductions in bacterial density when compared with controls; penicillin plus thrice-daily gentamicin resulted in a significant difference from penicillin alone. Although once-daily regimens have proved effective in trials involving other organisms, such regimens do not appear to be so optimal for the treatment of enterococcal endocarditis.

**Introduction**

*Enterococci* are isolated from 10–20% of cases of bacterial endocarditis and therapy generally consists of a combination of a penicillin or vancomycin plus an aminoglycoside.\textsuperscript{1,2} For the penicillins, data suggest that serum concentrations should be maintained above the MIC throughout the dosing interval to prevent the loss of efficacy, since no post-antibiotic effect has been observed for these agents against *enterococci*.\textsuperscript{3} Although the optimal dosing regimen for the penicillins has been described, the most appropriate administration schedule for the aminoglycosides remains controversial.

In this study, we simulated the pharmacokinetic profile of once-daily and thrice-daily gentamicin regimens to determine their efficacy in combination with penicillin for the treatment of experimental *Enterococcus faecalis* endocarditis.

**Materials and methods**

**Bacteria and antibiotics**

A clinical isolate of *E. faecalis* was used throughout the study. Antimicrobials for the determination of the MIC and MBC were standard analytical powders (Sigma Chemical Co., St Louis, MO, USA). Antibiotics utilized for treatment studies were commercially available products (penicillin G potassium, Marsam Pharmaceuticals, Cherry Hill, NJ, USA; penicillin G procaine suspension, Wyeth Labs, Philadelphia, PA, USA; gentamicin, SoloPak Labs, Village, IL, USA).

**Susceptibility studies**

MICs and MBCs for the *E. faecalis* isolate were determined in cation-supplemented Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) by the microdilution method.

**Pharmacokinetic study of gentamicin**

Before the initiation of the endocarditis study, a group of healthy rabbits was used for the pharmacokinetic study of gentamicin. A single im dose was administered to groups of animals and blood samples were collected over an 8 h post-dose period. Gentamicin concentrations were deter-
mined using a TDxFLx analyser (Abbott Laboratories, N. Chicago, IL, USA). The pharmacokinetic parameters for gentamicin were generated using a non-linear least squares regression method (PCNONLIN, Statistical Consultants Inc., Lexington, KY, USA) and these data were used to simulate the concentration–time profile observed in humans receiving 7 mg/kg once-daily. A series of 6.5, 1.0, 0.9, 0.8 and 0.7 mg/kg doses (2 h interval between doses) was administered to simulate the once-daily regimen. Peak concentrations (mean ± s.d.) with this regimen (19.1 ± 0.46 mg/L) were similar to those (20.3 ± 2.5 mg/L) observed in humans. The total once-daily dose was fractionated (2.16, 0.72 and 0.4 mg/kg with 2 h interval between doses repeated at 8 h) to simulate the tds regimen. The Figure displays the concentration–time profile of gentamicin in rabbits compared with that observed with doses of 7 and 1.7 mg/kg in humans.

Experimental endocarditis

The study was approved by the Institutional Animal Use and Care Committee at our institution. New Zealand White rabbits were used and endocarditis was induced by modifications of previously described techniques. Ninety-six hours after catheterization, animals were inoculated intravenously with $10^8$ cfu and 24 h later were randomized to various treatment groups for 3 days of im therapy: penicillin alone (aqueous penicillin G (400,000 U) plus procaine penicillin G (600,000 U) twice daily); penicillin plus once-daily gentamicin; penicillin plus thrice-daily gentamicin; or no treatment. This penicillin regimen was chosen because it produced concentrations that were maintained above the MIC for our isolate.

Antimicrobial therapy blood samples were obtained to confirm the concentration of gentamicin. At the conclusion of treatment animals were killed and the vegetations excised, homogenized, serially diluted and subcultured on to agar plates containing penicillinase to eliminate penicillin carryover. Vegetations were considered sterile when the culture showed no growth after incubation (48 h); negative specimens were those with $<100$ cfu (limit of sensitivity).

Statistical analysis

Differences in bacterial counts among groups were examined by analysis of variance, followed by the multiple comparison method of Scheffe. A $P$ value of $<0.05$ was considered significant. All results are expressed as mean ± S.D. or mean ± S.E.M.

Results

Susceptibility studies

The MIC/MBC were 4/8 and 32/64 mg/L of penicillin and gentamicin, respectively.

Experimental endocarditis

The bacterial density of the aortic vegetations after 3 days of treatment are displayed in the Table. There were significantly fewer bacteria in the vegetations of the treated animals than in those in the untreated control group ($P < 0.05$). Penicillin plus tds gentamicin was significantly more effective than penicillin alone ($P < 0.05$), but was not
Experimental enterococcal endocarditis

Table. Bacterial density (mean ± s.e.m.) of aortic vegetations after 3 days of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Log (cfu/g of vegetation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (control)</td>
<td>6</td>
<td>9.97 ± 0.16</td>
</tr>
<tr>
<td>Penicillin</td>
<td>11</td>
<td>6.06 ± 0.30^a</td>
</tr>
<tr>
<td>Penicillin plus once-daily gentamicin</td>
<td>12</td>
<td>5.42 ± 0.29^a</td>
</tr>
<tr>
<td>Penicillin plus tds gentamicin</td>
<td>10</td>
<td>4.98 ± 0.10^a,b</td>
</tr>
</tbody>
</table>

^a^ Statistically significant difference from control (P < 0.05).
^b^ Statistically significant difference from penicillin alone (P < 0.05).

statistically different from the penicillin plus once-daily gentamicin regimen.

Gentamicin serum concentrations from animals during the last dosing interval were similar to those observed in the healthy animals during the design of the once-daily and tds regimens.

Discussion

Since the aim of studies involving experimental models is to evaluate efficacy and generate data that might be extrapolated to humans, it is necessary to use clinically relevant concentrations. Differences in the half-life between animals and humans preclude a direct extrapolation of animal results to human situations when using the same dosing regimen. To overcome this limitation, we mimicked the pharmacokinetic profile of a 7 mg/kg once-daily gentamicin regimen in humans by administering the drug using multiple im doses in rabbits. After 3 days of treatment, our results show that, when combined with penicillin, the conventional regimen was more effective than the once-daily regimen, despite the similar drug exposure (e.g., area under the concentration–time curve) for both regimens.

Many studies have been conducted to evaluate the role of aminoglycosides in the treatment of E. faecalis endocarditis; however, these studies have not taken into account the alteration in the pharmacokinetic profile of the test agent between species as the dosing interval commonly employed in the animal models was similar to that used in humans. Henry et al. determined the efficacy of penicillin combined with a low dose of streptomycin (3.5 mg/kg) compared with a higher dose of streptomycin (10 mg/kg) tid in the treatment of streptomyein-susceptible enterococcal endocarditis in rabbits. The two dosages combined with penicillin were not significantly different; however, both were more effective than penicillin alone. Carrizosa & Levison reported that the killing of enterococci in cardiac vegetations with streptomycin-resistant endocarditis treated for 5 days with penicillin combined with gentamicin, 1 or 5 mg/kg, produced a similar reduction in bacterial vegetation densities. However, after 10 days, the bacterial density was lower in the animals treated with the higher dose.

Fantin & Carbon evaluated the efficacy of penicillin, alone or in combination with various dosing regimens of netilmicin for the treatment of E. faecalis endocarditis in rabbits. For a given total daily dose of aminoglycoside, the dosing regimen with more frequent injections was more effective than a single dose regimen in reducing bacterial titres in the vegetations. The netilmicin high dose tds regimen was more effective than a lower dose when each was combined with penicillin. These data suggest that the most effective aminoglycoside regimen was one which provides a prolonged exposure of the organism to both agents. This is in agreement with the report that penicillin enhances the uptake of aminoglycosides. In addition, Hessen et al. reported that for enterococci the combination of penicillin and gentamicin exhibited a more prolonged post-antibiotic effect than did penicillin alone.

In this study, we used the penicillin regimen reported by Fantin & Carbon; our results using a different aminoglycoside agree with their observations. As after tds dosing with netilmicin, gentamicin was detectable for a greater duration of the dosing interval and resulted in the greatest reduction of the bacterial load. These data suggest that for optimal bactericidal and synergistic activity of penicillin- and aminoglycoside-containing regimens prolonged exposure of the organism to both agents is required. Although the use of once-daily gentamicin has been safe and effective in human trials involving other organisms, such a schedule does not appear to be optimal for the treatment of enterococcal endocarditis.

References


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