Cephalosporin-Aminoglycoside Synergism in Experimental Enterococcal Endocarditis

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Despite in vitro demonstrations of synergism against enterococci, combinations of cephalosporin and aminoglycoside antibodies have been ineffective in the therapy of enterococcal endocarditis. Penicillin-gentamicin, cephalothin-gentamicin, and cefazolin-gentamicin were used to treat enterococcal endocarditis in rabbits. A direct relation was observed between the rate of cure and the degree by which the peak serum concentration of penicillin and the cephalosporin exceeded the minimal inhibitory concentration of the enterococcus. Thus, cephalosporin doses which produce serum concentrations which exceed the minimal inhibitory concentration of the enterococcus by several orders of magnitude may, in combination with aminoglycosides, be effective in treating human enterococcal endocarditis.

Serious infections caused by the enterococci are relatively resistant to antimicrobial therapy, and the use of single antibiotics such as penicillin or ampicillin in enterococcal endocarditis results in a significant incidence of treatment failure (6). Since the studies of Hunter in 1947, the combination of penicillin with an aminoglycoside antibiotic has been used successfully in the treatment of enterococcal endocarditis (3, 14). Synergistic killing of the enterococci with such combinations has been repeatedly documented (8). Cephalosporin antibiotics closely resemble penicillin, both in chemical structure and in mechanism of action (13), and in vitro synergism between cephalothin and streptomycin against enterococci has been demonstrated (1). However, trials of cephalothin, alone and in combination with streptomycin, have been unsuccessful in treatment of enterococcal endocarditis (10). Thus, in the patient who is allergic to penicillin, the combination of vancomycin and an aminoglycoside must be used (10). Since nephro- and ototoxicity may occur with vancomycin, a therapeutic regimen with less potential toxicity would be preferable in the treatment of enterococcal endocarditis in the penicillin-allergic patient. The clinical failure of cephalosporins to act synergistically with aminoglycoside antibiotics against enterococci has previously been investigated in vitro (11). The availability of a rabbit model of enterococcal endocarditis has provided the opportunity to evaluate in vivo the effect of cephalosporins when used in combination with an aminoglycoside in the treatment of enterococcal endocarditis.

MATERIALS AND METHODS

Aortic valve enterococcal endocarditis was produced by a modification of the method of Garrison and Freedman (2). White male Skippack rabbits (2.5 kg) were anesthetized with intravenous nembutal and the right carotid artery was exposed. A 19-gauge plastic cannula was inserted into the artery, threaded into the left ventricle, and left in place overnight. Animals were then given an intravenous injection of 10^8 enterococci in Trypticase soy broth (BBL) and the left ventricular catheter was removed immediately.

Daily blood cultures were obtained either until the time of spontaneous death or until blood cultures had been sterile for a 21-day period. Animals were autopsied either at the time of spontaneous death or after blood cultures had been sterile for 21 consecutive days. Kidneys and spleens were examined histologically and valve vegetations were weighed and quantitatively cultured.

The enterococcus used in all experiments was obtained from a clinical isolate, previously shown to be resistant to 2,000 μg of streptomycin and kanamycin per ml, but sensitive to 2,000 μg of gentamicin per ml. The minimal inhibitory concentrations (MIC) of various antibiotics for this organism were: penicillin G, 4 μg/ml; cefazolin, 24 μg/ml; and cephalothin, 26 μg/ml. All MICs were determined by the standard broth-dilution technique in Trypticase soy broth (BBL) (7). All bacteria recovered from blood cultures and cardiac vegetations were identified by the formation of typical colonies on blood agar, the ability to grow in bile-esculin medium (EMB broth),

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and the pattern of aminoglycoside susceptibility.

Tests of synergism were performed in dextrose-phosphate broth medium (Albimi-Pfizer). A 1-ml aliquot of an overnight culture of the enterococcus was added to 19 ml of dextrose-phosphate broth, producing a final concentration of approximately 10^7 organisms per ml. Appropriate antibiotics were added in concentrations equal to the MIC for the organism and the cultures were incubated at 37 C. Samples of 0.5 ml were removed at 0, 4, and 24 hours for determination of viable organisms by standard serial dilution technique (7) and subsequent subculture on brucella agar plates with 5% horse blood. Synergy was defined as a 100-fold increase in killing after 24 hours of incubation by the combination as compared with the most effective antibiotic used alone.

Animals were divided into seven treatment categories (Table 1). The first group, consisting of eight animals, served as controls and received no therapy. Animals in all other groups received gentamicin, 3.5 mg/kg, intramuscularly either three or four times daily, as noted, depending upon the frequency with which the accompanying antibiotic was administered. Animals in group 2 were given penicillin G, 10^4 U/kg, intramuscularly three times daily, while those in group 3 were given penicillin G, 2.5 x 10^4 U/kg, intramuscularly also thrice daily. Animals in group 4 received cephalothin, 40 mg/kg, intramuscularly three times daily, and those in group 5 were given cephalothin, at a dose of 80 mg/kg, intramuscularly four times daily. Groups 6 and 7 received cefazolin in addition to gentamicin, those in group 6 receiving 16 mg/kg intramuscularly thrice daily and those in group 7 receiving 40 mg/kg intramuscularly four times daily.

Serum antibiotic levels were measured by an agar diffusion method (16) utilizing a strain of Bacillus globigii as the test organism. Serum half-lives were established for each antibiotic, with serum antibiotic levels determined at 0.5, 1, 2, 4, and 6 hours after a dose.

RESULTS

Synergism was demonstrated in vitro for the combinations of penicillin-gentamicin, cephalothin-gentamicin, and cefazolin-gentamicin against the test strain (Fig. 1). In all cases the combination produced a greater than 100-fold increase in killing when compared to the effect of penicillin or the cephalosporin alone.

Enterococcal endocarditis was a rapidly fatal infection in the untreated rabbits (Table 1). All animals in the control group succumbed with a mean survival of 4.25 days after the initiation of the infection. Blood cultures throughout this period were continually positive, and at autopsy extensive vegetations were noted on the aortic valves. Renal cortical and splenic abscesses were frequently present. Between 10^8 and 10^11 colonies of enterococci/g of valve vegetation were present.

Administration of gentamicin at a dose of 3.5 mg/kg produced peak levels of 10 \mu g/ml (Fig. 2). The half-life of this agent was 60 minutes and essentially no antibiotic was present 4 hours after a dose. Neither the peak level nor half-life of the gentamicin was influenced by concomitant administration of other antibiotics.

The use of low-dose penicillin (10^4 U/kg) and gentamicin (3.5 mg/kg) thrice daily in group 2 resulted in cure of three of the eight animals treated (37.5%) (Table 1). The three animals that were cured had positive blood cultures for an average of 2.3 days. Those animals that died survived an average of 21 days after initiation of infection and had intermittently positive blood cultures throughout this period. In these animals, autopsy studies revealed extensive aortic valve vegetations with colony counts ranging between 10^10 and 10^10/g of vegetation. The aortic valves of cured animals demonstrated healed sterile subintimal plaques. Peak penicillin levels in this group averaged 11 U/ml with a half-life of 55 min (Fig. 2), and exceeded the MIC of the organism by a factor of 2.75 (Table 1).

![Figure 1. The effect of combinations of penicillin-gentamicin, cephalothin-gentamicin and cefazolin-gentamicin against the test strain. Abbreviations: PCN, penicillin G; CP, cephalothin; CZ, cefazolin; GM, gentamicin.](http://aac.asm.org/Downloadedfrom)
All eight animals in group 3, who received high-dose penicillin (2.5 x 10⁸ U/kg three times daily) and gentamicin, were rapidly cured (Table 1). Blood cultures were positive for a mean of 0.6 day after initiation of therapy, and remained negative thereafter. At autopsy, 21 days later, healed sterile plaques were noted on the aortic valves. Three of the eight animals had sterile renal cortical scars. With the penicillin dose used in group 3, peak serum penicillin levels averaged 62 U/ml 0.5 h after a dose and the serum half-life for penicillin was 55 min (Fig. 2). At peak levels, the MIC of penicillin for the organism was exceeded by a factor of 15 (Table 1).

Treatment of the eight animals in group 4 with low-dose cephalothin (40 mg/kg thrice daily) and gentamicin significantly prolonged survival when compared with the control group, but did not result in cure (Table 1). Animals survived an average of 9.6 days after initiation of therapy, with blood cultures almost continually positive during this period. All animals died spontaneously, and 10⁶ to 10⁹ colonies of enterococci/g of vegetation were recovered from the extensive aortic valve vegetations found at autopsy. Peak serum cephalothin levels in this group averaged 31 μg/ml with a half-life of 45 min (Fig. 2). At peak levels, the MIC of the organism was exceeded by a factor of 1.2 (Table 1).

Of the nine animals in group 5, treated with high-dose cephalothin (80 mg/kg four times daily) and gentamicin, five were cured of their infection (55%) (Table 1). Those animals dying of infection survived an average of 17 days and had intermittently positive blood cultures throughout this period. The five surviving animals had bacteremia for an average of 2.4 days. Autopsies demonstrated the presence of aortic valve enterococcal endocarditis in those animals which died spontaneously. Colony counts ranged between 10⁶ and 10⁹ colonies/g of vegetation. Healed, sterile subintimal aortic valve plaques were noted in cured animals at sacrifice 21 days after bacteremia had ceased. Peak serum cephalothin levels in this group averaged 66 μg/ml and thus exceeded the MIC for the enterococcus by a factor of 2.75 (Table 1).

![FIG. 2. Mean serum concentrations of four antibiotics in the therapy of experimental enterococcal endocarditis.](image)

### Table 1. Results of therapy in experimental enterococcal endocarditis

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Therapeutic regimen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibiotics</th>
<th>Frequency</th>
<th>Survival rate&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean time of death (failures)</th>
<th>Days of bacteremia (cures)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>None</td>
<td></td>
<td></td>
<td>0/8 (0%)</td>
<td>4.25 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>PCN (10⁴ U/kg); GM (3.5 mg/kg)</td>
<td>TID</td>
<td></td>
<td>3/8 (37.5%)</td>
<td>21 days</td>
<td>2.3</td>
<td>2.75</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>PCN (2.5 x 10⁶ U/kg); GM (3.5 mg/kg)</td>
<td>TID</td>
<td></td>
<td>8/8 (100%)</td>
<td>0.6</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>CP (40 mg/kg); GM (3.5 mg/kg)</td>
<td>TID</td>
<td></td>
<td>0/8 (0%)</td>
<td>9.6 days</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>CP (80 mg/kg); GM (3.5 mg/kg)</td>
<td>QID</td>
<td></td>
<td>5/9 (55%)</td>
<td>17 days</td>
<td>2.4</td>
<td>2.75</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>CZ (16 mg/kg); GM (3.5 mg/kg)</td>
<td>TID</td>
<td></td>
<td>2/8 (25%)</td>
<td>11.2 days</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>CZ (40 mg/kg); GM (3.5 mg/kg)</td>
<td>QID</td>
<td></td>
<td>6/9 (67%)</td>
<td>21 days</td>
<td>2.8</td>
<td>4.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> All antibiotics administered intramuscularly. Abbreviations: PCN, penicillin G; TID, three times a day; CP, cephalothin; QID, four times a day; CZ, cefazolin.

<sup>b</sup> Chi-square test with Yates correction.

<sup>c</sup> Ratio of peak serum concentration of penicillin, cephalothin, or cefazolin to minimal inhibitory concentration of that antibiotic for the enterococcus.
The serum half-life of cephalothin was 45 min (Fig. 2).

Eight animals received low-dose cefazolin (16 mg/kg thrice daily) and gentamicin (Table 1). Two (25%) were cured of their infection, with blood cultures becoming sterile an average of 2.5 days after the initiation of therapy. In the six animals who succumbed to infection, death occurred an average of 11.2 days after the development of endocarditis. All had vegetations involving the aortic valve, with valve colony counts ranging between $10^6$ and $10^{11}$ enterococci/g of vegetation. In this group peak serum cefazolin levels averaged 26 $\mu$g/ml with a half-life of 45 min (Fig. 2). Peak serum levels of cefazolin exceeded the MIC by a factor of 1.2 (Table 1).

The final group of nine animals received high-dose cefazolin (40 mg/kg four times daily) and gentamicin (Table 1). Six (67%) were cured, with blood cultures becoming sterile an average of 2.8 days after beginning therapy, and sterile plaques noted on the aortic valve at autopsy 21 days later. The remaining three animals died an average of 21 days after initiation of therapy, had intermittently positive blood cultures, and had extensive aortic valve vegetations at the time of death. Valve colony counts ranged between $10^6$ and $10^{11}$ colonies/g of valve vegetation. Peak serum cefazolin levels in this group were 98 $\mu$g/ml and exceeded the MIC for the enterococcus by a factor of 4.5 (Table 1). The half-life of the cefazolin was 45 min (Fig. 2).

DISCUSSION

Since the studies of Hunter (3), the combination of penicillin and an aminoglycosidic aminocyclitol antibiotic has been accepted as the standard therapeutic regimen for enterococcal endocarditis (4, 14). In those patients with serious penicillin allergy, the combination of vancomycin and an aminoglycoside has been suggested as an effective alternative regimen (15).

Cephalosporin antibiotics resemble penicillin both in structure and mechanism of action. In vitro synergism against the enterococcus, using clinically achievable doses, has been demonstrated for the combination of cephalothin with aminoglycoside antibiotics (1). However, the failure of cephalothin, when combined with either streptomycin or gentamicin, to cure enterococcal endocarditis has been repeatedly observed (10, 12). This failure is not related to cephalosporinase production by enterococci (11). It has been suggested that the lack of in vivo synergism may be due, in part, to the rapid conversion of cephalothin to desacetylcephalothin, a compound with one-fourth to one-sixteenth the antibacterial efficacy of the parent compound (11). Between one-third and 90% of each dose of cephalothin is converted to desacetylcephalothin (5). Although desacetylcephalothin does not directly interfere with the antibacterial effect of cephalothin (11), the rapid conversion of cephalothin to its less active metabolite, along with its rapid excretion, may sufficiently deplete serum levels of the more active material so that soon after administration there is no longer a sufficient concentration of cephalothin to react synergistically with the accompanying aminoglycoside. Thus, it has been proposed that a cephalosporin antibiotic which does not undergo desacetylation might provide more effective synergism in vivo (11).

The observations in the present study do not support this hypothesis. When used in doses similar to those which would be administered to humans with enterococcal endocarditis, the combination of penicillin and gentamicin produced a rapid and complete cure. In contrast, when doses of cephalothin sufficient to produce peak serum antibiotic levels equivalent to those which can be achieved clinically were used, none of the animals were cured, although a significant prolongation of survival was observed. Even with the administration of larger doses of cephalothin, the cure rate (55%) was significantly lower than that achieved with penicillin ($P < 0.05$). These observations reproduce in the animal model the results which have been observed in clinical practice. Cefazolin is a cephalosporin antibiotic which is excreted almost entirely by the kidney and does not undergo desacetylation (9). However, when cefazolin was given in combination with gentamicin, in both the low- and high-dose schedules, neither mortality rate nor length of survival was significantly different from that observed in the cephalothin-treated groups ($P > 0.10$).

An association was observed between the efficacy of the cephalosporin or penicillin regimen and the degree by which the peak serum level of either penicillin or the cephalosporins exceeded the MIC of the organism. Thus, in the very effective high-dose penicillin-gentamicin regimen the peak serum penicillin level exceeded the MIC for the enterococcus by a factor of 15. None of the cephalosporin regimens approached this. In the high-dose cephalothin and cefazolin groups, the MIC for the enterococcus was exceeded by factors of 2.75 and 4.5, respectively. This was associated with cure rates of 55 and 67%. When the dose of penicillin was reduced so that the MIC was exceeded only by a factor of 2.75 (group 2), the cure rate fell to
37.5%. Similarly, when low-dose cephalothin and cefazolin regimens were used, the MIC was exceeded only by a factor of 1.2 and cure rates were correspondingly low (0 and 25%, respectively). Thus, in both the penicillin and cephalosporin regimens, cure rates appeared to be related to the degree by which the MIC of the enterococcus was exceeded in the serum. With the cephalosporins, there was no significant difference between the two agents employed.

The failure of cephalosporin antibiotics, in combination with aminoglycosides, to cure enterococcal endocarditis in humans, may, therefore, be a function not of the metabolism of the antibiotic, but rather of the amount by which the MIC of the organism can be exceeded, in the serum, with clinically achievable doses. Thus, it may be possible that the combination of a cephalosporin antibiotic with an aminoglycoside could be effective in treatment of human enterococcal endocarditis if sufficiently large doses of the cephalosporin could be administered to exceed the MIC of the enterococcus by several orders of magnitude.

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LITERATURE CITED