NOTES

In Vitro Bactericidal Synergy of Gentamicin Combined with Penicillin G, Vancomycin, or Cefotaxime Against Group G Streptococci

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Serious infections due to Lancefield group G streptococci (GGS) have been increasingly reported in the recent literature (4, 5, 7, 10). Despite exquisite in vitro susceptibility of GGS to penicillin G (1, 5, 12), the in vivo responses have often been suboptimal, particularly in cases of GGS endocarditis and septic arthritis (4, 5, 7, 10). Empirical addition of aminoglycosides to penicillin G therapy has effected a favorable clinical response in certain patients with severe GGS infections (5); however, there has been no systematic in vitro evaluation of the potential for bactericidal synergy between cell wall-active agents and aminoglycosides versus GGS. In this regard, we evaluated the synergistic activity of three cell wall-active agents (penicillin G, cefotaxime, and vancomycin) in combination with gentamicin against 20 recent clinical GGS isolates.

(This study was presented in part previously [K. Lam and A. S. Bayer, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 24, 1983].)

Twenty recent clinical beta-hemolytic streptococcal isolates, identified as GGS by the Lancefield technique (6), were utilized in this study. The GGS were isolated from the following sources: blood (eight isolates), synovial fluid (six isolates), wound (four isolates), cerebrospinal fluid (one isolate), and arterial catheter (one isolate). The spectrum of GGS infections represented by these isolates included septicemia without an obvious primary focus (six cases), septic arthritis (six cases), soft tissue sepsis (four cases), infective endocarditis (two cases), arthritis (one case), and meningitis (one case). There were no duplicate isolates from individual patients. The GGS were maintained on Todd-Hewitt agar slants (Difco Laboratories, Detroit, Mich.) until susceptibility studies were performed.

Penicillin G and vancomycin were purchased from U.S. Pharmacopoeial Standard, Rockville, Md.; cefotaxime was provided by Hoechst-Roussel Pharmaceuticals, Somerville, N.J.; and gentamicin was provided by Schering Corp., Kenilworth, N.J. Stock solutions of each antibiotic were stored at −70°C after reconstitution at 10 mg/ml until the day of susceptibility testing.

The MICs and MBCs of the three cell wall-active agents and gentamicin, used in subsequent synergy testing, for the 20 GGS strains were determined. The microtiter broth dilution technique was employed (1, 12). Volumes of 50 μl of each antibiotic in twofold serial dilutions were added to a separate microtiter row of wells. A 50-μl sample of each GGS isolate grown in Todd-Hewitt broth to the logarithmic phase of growth was added to each antibiotic-containing well to achieve a final concentration of 10^3 CFU/ml. The final drug concentrations (in micrograms per millilitre) in the wells were as follows: penicillin G, 0.0025 to 2.5; cefotaxime, 0.005 to 5; vancomycin, 0.01 to 10; gentamicin, 0.01 to 10. These concentrations were chosen to encompass levels of each agent readily attainable in serum at standard clinical dosages. For each isolate tested, one microtiter well contained only GGS in antibiotic-free Todd-Hewitt broth as a growth control. After inoculation, all plates were incubated for 24 h at 37°C. The MIC was then read as the lowest antibiotic concentration yielding no visible turbidity. At this time, 25-μl portions were taken from all visibly clear wells and subcultured onto antibiotic-free Todd-Hewitt agar. After 24 h of incubation at 37°C, the MBC was read as the lowest antibiotic concentration which yielded no visible GGS colonies on subculture.

The timed kill curve technique was utilized in synergy studies (8). Logarithmic-phase cells of each GGS isolate in Todd-Hewitt broth were added to antibiotic-containing tubes to achieve a final inoculum of ∼5 × 10^6 CFU/ml. The relatively high in vitro inoculum was chosen to approximate the in vivo inocula seen in endocarditis and septic arthritis (3, 9), the most problematic clinical syndromes caused by GGS (4, 5, 7, 10). Penicillin G (0.02 μg/ml), cefotaxime (0.02 μg/ml), and vancomycin (1.25 μg/ml) were tested alone or in combination with gentamicin (1.25 μg/ml) against each GGS isolate. These final drug concentrations were chosen to represent levels of each agent readily achievable in serum; in addition, the single agents at these drug concentrations did not effect a rapid kill of the GGS within 4 to 24 h in pilot studies in our laboratory. For each GGS isolate tested, an antibiotic-free broth tube was inoculated as described above as a growth control. All inoculated tubes were incubated at 37°C in a water bath. Samples from each tube were quantitatively subcultured into antibiotic-free Todd-Hewitt agar at 0, 4, and 24 h of incubation. After 24 h of incubation of each
subculture plate at 37°C, the log_{10} CFU of surviving GGS per milliliter was calculated for each sampling time.

In vitro bactericidal synergy was considered present when at least a 2-log_{10} decline in CFU per milliliter was achieved at 24 h by the drug combination, as compared with that obtained by the most active single drug constituent (8). Indifference was defined as a <2-log_{10} change in CFU per milliliter at 24 h achieved by the drug combination versus both single drug constituents. Antagonism was defined as a ≥2-log_{10} increase in CFU per milliliter at 24 h by the drug combination versus the single agents.

The MICs and MBCs of the individual drugs, used in subsequent synergy testing, for the 20 GGS isolates are shown in Table 1. All 20 isolates were very susceptible to the in vitro inhibitory and killing action of both penicillin G and cefotaxime; as an example, the MBCs of penicillin and cefotaxime for 90% of the isolates (MBC_{90}) were 0.018 and 0.04 μg/ml, respectively. Vancomycin exhibited less of an inhibitory and bactericidal effect on the 20 GGS isolates, having both an MIC and an MBC for 90% of the isolates of 1.13 μg/ml, a level readily achievable in serum. The MBC of gentamicin for 90% of the isolates was 5 μg/ml, within levels attainable in serum; however, only 50% of the strains were highly susceptible to the killing action of this agent (MBCs, ≤1.25 μg/ml). Of note is that there were no instances of in vitro GGS tolerance to the three cell wall-active agents noted in these studies. This finding is in contrast to those of other reports (11).

Bactericidal synergy was commonly achieved by combinations of cell wall-active agents and gentamicin in our study at the following frequencies: penicillin plus gentamicin, 16 of 20 strains (80%); cefotaxime plus gentamicin, 17 of 20 strains (85%); vancomycin plus gentamicin, 18 of 20 strains (90%) (Fig. 1 and 2).

Antibiotic susceptibility studies from our laboratory and others have delineated the exquisite in vitro susceptibility of GGS to a variety of cell wall-active agents, particularly penicillin G and cefotaxime (1, 5, 12). Despite these in vitro findings, the in vivo outcome in many patients with GGS septic arthritis and endocarditis has been suboptimal (2, 4, 3, 7, 10). These in vitro-in vivo disparities prompted us to examine the potential for enhanced bactericidal effect of combinations of cell wall-active agents plus aminoglycosides against GGS in vitro. We studied the bactericidal interactions at a relatively high inoculum (~10^6 CFU/ml) to reflect in vivo inocula likely to be encountered clinically in endocar-

![FIG. 1. In vitro bactericidal interactions of gentamicin (G) in combination with penicillin G (P), cefotaxime (C), or vancomycin (V) against a GGS isolate from our study. Note complete bactericidal effect produced by the drug combinations at 24 h versus that produced by the single agents.](image1)

![FIG. 2. In vitro bactericidal interactions of gentamicin (G) in combination with penicillin G (P), cefotaxime (C), or vancomycin (V) against a different GGS isolate. Note the rapid and complete bactericidal effect produced by penicillin G plus gentamicin at 4 h of incubation.](image2)

**TABLE 1. In vitro susceptibilities of 20 GGS**

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (μg/ml)*</th>
<th>50%</th>
<th>90%</th>
<th>MBC (μg/ml)*</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>0.0025–0.04</td>
<td>0.0045</td>
<td>0.017</td>
<td>0.0025–0.04</td>
<td>0.007</td>
<td>0.018</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.005–0.04</td>
<td>0.008</td>
<td>0.027</td>
<td>0.005–0.08</td>
<td>0.010</td>
<td>0.04</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.312–2.5</td>
<td>0.31</td>
<td>1.13</td>
<td>0.312–2.5</td>
<td>0.47</td>
<td>1.13</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.156–5</td>
<td>0.73</td>
<td>2.5</td>
<td>0.156–10</td>
<td>1.25</td>
<td>5</td>
</tr>
</tbody>
</table>

* 50% and 90% MIC inhibiting 50 and 90% of the strains, respectively.

* 50% and 90% MBC killing 50 and 90% of the strains, respectively.
dritis and septic arthritis, the most recalcitrant GGS syndromes (2, 4, 5, 7, 10). The three cell wall-active agents commonly exhibited bactericidal synergy when combined with gentamicin at a frequency of 80 to 90%. There was no antagonism observed.

The exact explanation for the in vitro-in vivo disparities in regard to clinical-bacteriological failure of penicillin G therapy despite "susceptible" GGS isolates is not known. It is clear from our studies and others that penicillin tolerance is not a major feature of GGS (1, 5, 11, 12). We have recently shown in detailed in vitro killing curve studies that there appears to be an important combined growth phase-inoculum effect operative in impairing penicillin-induced killing of GGS (5). When GGS strains were tested at high inocula (10⁶ CFU/ml) of stationary-phase cells, there was a marked reduction of killing by penicillin G; this impaired bactericidal effect was not seen at either high inocula of logarithmic-phase cells or at low inocula of stationary-phase cells. Of note, this impaired killing at high inocula plus stationary growth phase was not reversible by pH normalization of the medium (5). These in vitro phenomena may partially explain the relatively poor clinical outcome seen in cases of GGS endocarditis, as this syndrome is prototypic of the high inoculum-stationary phase condition (3).

LITERATURE CITED