NOTES

Antistaphylococcal Activity of Ceforanide and Cefonicid in the Presence of Human Serum

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Tests with 52 strains of Staphylococcus aureus compared ceforanide and cefonicid. Addition of 50% human serum to the test system reduced the bacteriostatic and bactericidal activities of cefonicid, but ceforanide was not affected as greatly.

Cefonicid is a cephalosporin with a prolonged serum half-life of approximately 3.5 to 4 h (1, 7). Ceforanide is another cephalosporin with a similar spectrum of antibacterial activity (5) and a serum half-life of approximately 2.5 to 3 h (12). Protein binding of cefonicid has been estimated to be approximately 98% (8), compared with 81% for ceforanide and 92% for cefazolin (12). Binding to serum proteins affects the pharmacokinetic properties of such drugs, resulting in prolonged half-lives; it may also reduce the bioavailability of free unbound drug during the course of therapy.

The purpose of this study was to determine the extent to which serum proteins might affect the in vitro antistaphylococcal activity of ceforanide and cefonicid. Microdilution susceptibility tests were performed with 52 methicillin-susceptible isolates of Staphylococcus aureus. Doubling dilutions of both drugs (0.12 to 32 μg/ml) were prepared in cation-supplemented Mueller-Hinton broth and in the same broth diluted with equal volumes of human serum. The serum was collected from a normal healthy male volunteer and was heat inactivated at 56°C for 15 min before addition of the system. Growth control wells containing Mueller-Hinton broth and broth with 50% serum were inoculated with each strain. We observed no evidence of inhibition by the serum. The inocula were prepared by diluting logarithmic-phase broth cultures to achieve approximately 10^3 CFU/ml in each well. MICs were recorded after 18 to 20 h of incubation at 35°C.

An estimate of the bactericidal activity of the two drugs against 10 randomly selected strains was also determined. After the MICs were recorded, the trays were vigorously shaken to dislodge the buttons of growth and then reincubated for another 4 h. From wells with no visible evidence of growth, 25-μl samples were subcultured to a quarter of a blood agar plate and spread to minimize the effect of drug carry-over (2, 10). MBCs were not influenced when a concentrated K-1 β-lactamase preparation was added to the system before subculturing. Furthermore, no inhibition could be demonstrated when 25 μl samples from wells containing 32 μg of either drug per ml in uninoculated trays were spotted onto plates seeded with a control strain of S. aureus. Thus, carry-over of a drug was not a problem with the range of concentrations that was tested. The inocula ranged from 3.0 × 10^2 to 4.6 × 10^5 CFU/ml. The MBC was defined as the lowest concentration yielding ≤0.1% of the viable cells in the initial inoculum (99.9% kill). The calculated end points ranged from ≤7 to ≤12 colonies for the MBC, depending on the inoculum density. Most strains demonstrated a paradoxical effect when either drug was tested in broth alone; i.e., heavy growth was often observed when wells containing 16 or 32 μg/ml were subcultured, although lower concentrations were clearly bactericidal.

MIC data were obtained with 27 β-lactamase-negative strains and with 25 β-lactamase-positive strains. The two types of isolates were equally susceptible to ceforanide, but cefonicid was not as active against β-lactamase-producing strains as against β-lactamase-negative strains (MICs for 50% of strains were 4.0 and 2.0 μg/ml, and the MICs for 90% of strains were 8.0 and 4.0 μg/ml for the two types of strains, respectively).

Table 1 summarizes MIC data obtained in cation-supplemented Mueller-Hinton broth with or without 50% serum added. When serum was added to the test system, the ceforanide MICs for 50 and 90% of strains were increased by 1 doubling dilution. The bacteriostatic activity of cefonicid was virtually eliminated by the addition of serum (MICs for 50 and 90% of strains were ≥32 μg/ml).

Ten randomly selected strains were further tested to document the bactericidal activity of the study drugs. When tested in broth, ceforanide MICs for the 10 selected strains were 2.0 or 4.0 μg/ml, and in the presence of serum the MICs ranged from 2.0 to 8.0 μg/ml. When tested in broth, 7 of the 10 MBCs were no more than 2 doubling dilutions greater than the MIC, and three strains had an MBC of >32 μg/ml. Although seven strains had MBCs of ≤8.0 μg/ml, all but one produced significant growth when wells containing 32 μg/ml were subcultured. This paradoxical effect was not observed when serum was added to the test system: MBCs for all 10 strains were 8.0 or 16 μg/ml. The three strains with MBCs of >32 μg/ml in broth were killed by 16 and by 32 μg/ml in the presence of serum.

The 10 strains tested against cefonicid diluted in broth had MICs of 2.0 or 4.0 μg/ml. The MBCs for two strains were >32 μg/ml, and the remaining eight strains had MBCs of 4.0 or 8.0 μg/ml (no more than twice the MIC for each strain). The paradoxical effect was also observed when cefonicid was tested in Mueller-Hinton broth; i.e., only 1 of the 10 strains was killed by 32 μg/ml, 16 μg/ml was bactericidal.

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against 5 of the 10 strains, and 8 of 10 strains were killed by 8.0 μg/ml. When serum was added to the test system, cefonicid MICs ranged from 8.0 to 32 μg/ml, and all MBCs were ≥32 μg/ml.

One strain which demonstrated a paradoxical effect with both drugs was tested to determine whether resistant mutants were being selected. Colonies that recovered from 32 μg/ml of either drug demonstrated no evidence of increased resistance to the two cephalosporins.

To further evaluate the bactericidal activity of the two drugs, killing rates were determined. Each drug was added to tubes containing 5.0-ml volumes of Mueller-Hinton broth or the same broth diluted with equal amounts of human serum. Each tube was inoculated with 8.0 \times 10^6 CFU/ml, and viable cell counts were determined after 0, 2, 4, 6, 24, and 48 h of incubation at 35°C. Control tubes with both types of test media but no antibiotics were also tested. Satisfactory growth was obtained in both types of media, but in the broth diluted with serum, the rate of growth and total cell yield were diminished (Fig. 1). The strain selected for these studies had cefonicid microdilution MICs of 2.0 and 8.0 μg/ml in broth and in serum, respectively. In broth, the cefonicid MBC was 4.0 μg/ml (with no killing at 32 μg/ml), and in serum the cefonicid MBC was >32 μg/ml. In both types of media, ceforanide MICs were 2.0 μg/ml, and MBCs were 8.0 μg/ml. When that strain was retested on 3 separate days, the MICs and MBCs cited above varied no more than 1 doubling dilution.

Figure 1 presents the results of such tests with 16 μg of both drugs per ml. In broth, cefonicid was bactericidal after 24 h (99.9% kill). In the presence of 50% serum, cefonicid produced an early decrease (6 h) in the number of viable cells, but with continued incubation, the cells regrew, approaching the concentrations in the initial inoculum after 48 h. Ceforanide was bactericidal for the tested strain, with <10 CFU/ml after 24 h. In the presence of serum, the rate of killing was accelerated: 99.9% of the cells were killed by 4 h.

Fairly heavy growth was frequently observed when wells containing 16 or 32 μg/ml were subcultured, even though lower concentrations were often bactericidal. Additional cefonicid killing rates were determined with the S. aureus tested in Fig. 1. Cefonicid was tested at 16, 32, and 64 μg/ml in 50% serum and at 4.0, 8.0, and 16 μg/ml in Mueller-Hinton broth (two, four, and eight times the microdilution MIC for that strain). A concentration of 16 μg/ml was not bactericidal in the presence of serum (Fig. 2). At 32 μg/ml, cefonicid was bactericidal, but when the concentration was increased to 64 μg/ml, the rate of killing was reduced. In broth, all three concentrations of cefonicid were bactericidal, but the rate of killing was reduced with each increase in concentration of drug.

Cefonicid and ceforanide are not as active as some cephalosporins against staphylococci (1, 4). With the addition of 50% serum to the in vitro test system, the antistaphylococcal activity of cefonicid was compromised. Ceforanide was not affected as dramatically by the addition of serum. In fact, the rate of killing appeared to be enhanced by the presence of

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**TABLE 1.** In vitro activity of cefonicid and ceforanide against 52 S. aureus isolates* tested in Mueller-Hinton broth with or without 50% human serum

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>Test medium</th>
<th>MIC range (μg/ml)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
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<tr>
<td>Cefonicid</td>
<td>Mueller-Hinton broth</td>
<td>1.0-8.0</td>
<td>4.0</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>50% Serum</td>
<td>8.0-32</td>
<td>32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ceforanide</td>
<td>Mueller-Hinton broth</td>
<td>2.0-8.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>50% Serum</td>
<td>2.0-16</td>
<td>8.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

* Includes 27 β-lactamase-negative strains and 25 β-lactamase-positive strains; all isolates were methicillin susceptible.

<sup>b</sup> MIC<sub>50</sub> and MIC<sub>90</sub>, MICs for 50 and 90% of the strains tested, respectively (in micrograms per milliliter).
human serum. Diminished activity that may be expected as a result of protein binding may not explain all of the observations reported here. It is difficult to explain why ceforanide appears to be more rapidly bactericidal in the presence of human serum, whereas cefonicid is bactericidal only in fairly high concentrations.

It is tempting to extrapolate these in vitro observations to predict the clinical effectiveness of the two drugs. A variety of factors that are not considered in our studies will influence the therapeutic effectiveness of a drug. In treating endocarditis, a bactericidal drug is clearly preferred, and our observations raise the question of whether cefonicid is truly bactericidal against S. aureus. Chambers et al. (3) reported that cefonicid failed to effectively treat staphylococcal endocarditis in humans. However, twice daily intramuscular injections of ceforanide have been shown to be effective in treating staphylococcal endocarditis (4). On the other hand, daily administration of cefonicid has been found to be effective in treating staphylococcal osteomyelitis (6) and soft-tissue infections (11). In the latter infections, a bacteriostatic agent may be sufficient.

There appears to be a favorable interaction with human serum proteins which enhance bactericidal activity of ceforanide against staphylococci. Favorable interactions of other cephalosporins with serum factors have been described previously, i.e., cefotaxime-like drugs against enterococci (9). Cefonicid, on the other hand, had very limited antistaphylococcal activity when tested in the presence of human serum. We conclude that ceforanide might have some advantage over cefonicid for treatment of staphylococcal infections in humans.

LITERATURE CITED

FIG. 2. Bactericidal activity of increasing concentrations of cefonicid tested in Mueller-Hinton broth or in broth with 50% serum. Microdilution MICs for the S. aureus strain were 2.0 μg/ml in broth and 8.0 μg/ml in 50% serum.