In Vitro Synergism Between Carbenicillin and Aminoglycosidic Aminocyclitols Against *Acinetobacter calcoaceticus* var. *anitratus*

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*Acinetobacter calcoaceticus* var. *anitratus* is a nonfermentative, gram-negative bacillus that has been demonstrated to cause severe infections, usually in hospitalized patients. Since mild to moderate resistance of *A. calcoaceticus* to one or more aminoglycosidic aminocyclitols has been noted to occur, a study was undertaken to evaluate the activity of combinations of carbenicillin with either kanamycin, tobramycin, or gentamicin against 28 isolates of *A. calcoaceticus* obtained from clinical sources. Synergism (defined as at least 100-fold-increased killing at 24 h by the combination as compared with the most efficacious of the individual antibiotics) was demonstrated against 26 of 28 strains of *A. calcoaceticus* with carbenicillin plus kanamycin and carbenicillin plus tobramycin and against 25 of 28 strains with carbenicillin plus gentamicin. The median increased killing for the 28 strains was 4.2 log₁₀ with carbenicillin plus kanamycin and with carbenicillin plus tobramycin and 3.1 log₁₀ with carbenicillin plus gentamicin. The most important determinant of synergistic potential of each combination was the level of resistance of each strain of *A. calcoaceticus* to the aminoglycoside component of the combination.

*Acinetobacter calcoaceticus* var. *anitratus* (*Herellea vaginicola*) is a nonfermentative, gram-negative bacillus that has been associated with infrequent but often serious and life-threatening infections in man (7, 9, 24, 25). This organism usually demonstrates in vitro resistance to ampicillin, cephalothin, and chloramphenicol (7, 9, 17, 21). Although it is usually susceptible to commonly used aminoglycosidic aminocyclitol antibiotics, resistance to gentamicin has been noted to occur (9). Furthermore, infections involving *A. calcoaceticus* are often mixed and commonly involve additional organisms, particularly other gram-negative bacteria (9). Since infections due to acinetobacter are usually nosocomial and frequently occur in patients who have received antecedent therapy with broad-spectrum antibiotics (7, 9), one or more of the infecting organisms is likely to be resistant to commonly used antibiotics. Accordingly, treatment regimens in such cases often involve combination antimicrobial therapy.

Preliminary in vitro studies in our laboratories suggested that combinations of carbenicillin plus one of several aminoglycosides possess synergistic bactericidal activity against certain strains of *A. calcoaceticus* (9). This report describes the results of extensive in vitro studies on the interaction between kanamycin, gentamicin, and tobramycin with carbenicillin against 28 strains of acinetobacter.

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**MATERIALS AND METHODS**

Gram-negative coccobacilli isolated from clinical specimens submitted to the Bacteriology Laboratories of the Massachusetts General Hospital were identified as *A. calcoaceticus* var. *anitratus* according to standard criteria (9). All strains failed to ferment lactose (1%) on MacConkey agar plates. Oxidative utilization of dextrose but not maltose was demonstrated. Acid was produced from 10% lactose, nitrates were not reduced, oxidase was absent, and the organisms were nonmotile.

Antibiotics used in these studies included disodium carbenicillin (Roerig, New York, N.Y.), kanamycin sulfate (Bristol Laboratories, Syracuse, N.Y.), gentamicin sulfate (Schering Corp., Bloomfield, N.J.), and tobramycin sulfate (Eli Lilly and Co., Indianapolis, Ind.).

Antimicrobial susceptibility data were obtained...
from earlier studies (9) and had been determined by a microdilution technique in Mueller-Hinton broth (BBL, Baltimore, Md.). Of 102 strains of *A. calcoaceticus* tested, 96% were inhibited by carbenicillin at a concentration of 62.5 μg/ml, 95% were inhibited by kanamycin at a concentration of 8 μg/ml, 81% were inhibited by tobramycin at a concentration of 2 μg/ml, and 57% were inhibited by gentamicin at a concentration of 2 μg/ml.

The in vitro interaction between carbenicillin and three aminoglycosides was evaluated by the method of "time-kill" curves in Mueller-Hinton broth as described previously (9, 20). An inoculum of 10^8 organisms/ml was prepared by appropriate dilution of an overnight culture grown without agitation at 37°C, and samples (0.5 ml) were removed at 0, 4, and 24 h for determination of colony counts by a series of 10-fold serial dilutions. Synergism is herein defined as a decrease of 100-fold or more in the number of viable organisms after 24 h of incubation in the presence of the combination as compared with the most effective of the antibiotics alone. "Killing curves" were determined for a total of 28 selected isolates of *A. calcoaceticus*. Approximately one-half of the isolates were selected for study because of nonsusceptibility to either carbenicillin or one or more of the aminoglycosides; preliminary results of studies in seven of these organisms have been reported previously (9). The remainder of the strains were selected at random from the rest of the 102 clinical isolates. To ensure that the 28 strains selected for study represented unique isolates, organisms were selected from different patients at various times and from diverse clinical areas. Furthermore, the patterns of susceptibility (MICs) to eight antibiotics were distinctly different among these 28 strains.

RESULTS

The interaction of carbenicillin with the three aminoglycosides was evaluated against 28 strains of *A. calcoaceticus* (Table 1). Synergism, defined as at least 100-fold (2 log10)-increased killing at 24 h by the combination as compared with the most efficacious of individual drugs, was obtained against 26 of 28 strains with carbenicillin plus kanamycin and with carbenicillin plus tobramycin and in 25 of 28 strains with carbenicillin plus gentamicin (Fig. 1). Overall, the bactericidal effect was slightly greater with the combination of either kanamycin or tobramycin plus carbenicillin than with gentamicin plus carbenicillin: the median increased killing was 4.2 log10 with kanamycin-carbenicillin and with tobramycin-carbenicillin and 3.1 log10 with gentamicin-carbenicillin. Furthermore, increased killing of at least 5

### Table 1. Effectiveness of carbenicillin plus aminoglycosides against 28 strains of *Acinetobacter calcoaceticus*

<table>
<thead>
<tr>
<th>Drug combination</th>
<th>Magnitude of increased killing by combination (cumulative no. of strains)</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanamycin + carbenicillin</td>
<td>1 2 4 9 13 17 25 28</td>
<td>4.2</td>
</tr>
<tr>
<td>Tobramycin + carbenicillin</td>
<td>1 2 3 8 13 19 26 28</td>
<td>4.2</td>
</tr>
<tr>
<td>Gentamicin + carbenicillin</td>
<td>1 3 5 13 20 24 27 28</td>
<td>3.1</td>
</tr>
</tbody>
</table>

![Graph showing effect of carbenicillin and aminoglycosides](http://aac.asm.org/)

**Fig. 1.** Effect of three aminoglycosides alone and in combination with carbenicillin against *A. calcoaceticus* strain H-7, which is susceptible to carbenicillin (MIC = 31 μg/ml), kanamycin (MIC = 8 μg/ml), tobramycin (MIC = 1 μg/ml), and gentamicin (MIC = 0.5 μg/ml). The starting concentration of each antibiotic (alone and in combination) is listed on the right side of the figure. Abbreviations: CB, carbenicillin; KM, kanamycin; TM, tobramycin; GM, gentamicin.
ence of gentamicin-carbenicillin. Antagonism was not seen with any of the combinations against any of the 28 strains of A. calcoaceticus.

Synergism could be demonstrated even against most strains of A. calcoaceticus that were relatively resistant to the antibiotics used (Table 2). For example, of the 13 strains with kanamycin MICs >8 \( \mu \)g/ml, synergistic killing was obtained with kanamycin-carbenicillin in 11; of 11 strains with tobramycin MICs >4 \( \mu \)g/ml, synergism with tobramycin-carbenicillin was seen in 9; of 14 strains with gentamicin MICs >4 \( \mu \)g/ml, synergism was obtained with gentamicin-carbenicillin in 11 (Fig. 2). However, high-level resistance to an aminoglycoside generally was associated with poor bactericidal activity of the respective aminoglycoside-carbenicillin combination against the isolate. Thus, synergistic killing did not occur with kanamycin-carbenicillin against either of the two strains with kanamycin MICs of 62.5 \( \mu \)g/ml; synergism was not demonstrated with tobramycin-carbenicillin against either of the two strains with tobramycin MICs of 31 \( \mu \)g/ml; synergism occurred with gentamicin-carbenicillin against only two of four strains with gentamicin MICs of >6.25 \( \mu \)g/ml (Fig. 3). These represented the strains having the highest MICs for kanamycin, tobramycin, and gentamicin.

The susceptibility of A. calcoaceticus strains to carbenicillin was not a determinant of the bactericidal interaction of this drug with the three aminoglycosides. In all seven strains with carbenicillin MICs >62.5 \( \mu \)g/ml, synergism was seen with all three combinations. Similarly, examination of the susceptibility patterns of the five strains in which synergism failed to occur in the presence of various combinations shows that in all but one instance the strain exhibited moderate resistance (i.e., MICs = 31 \( \mu \)g/ml for gentamicin and tobramycin, 62.5 \( \mu \)g/ml for kanamycin) to the aminoglycoside (Table 3). Except for the aminoglycoside-resistant strains, there was no relationship between the degree of synergistic bactericidal activity and the degree of susceptibility to carbenicillin or the aminoglycoside.

**DISCUSSION**

Synergistic interaction of several antibiotic combinations has been demonstrated against a variety of gram-negative organisms. In 1962, Plotz and Davis demonstrated synergism between penicillin and streptomycin for Escherichia coli, and presented evidence that the basis of this interaction was the increased penetration into the bacterial cell by the aminoglycoside as a result of penicillin-induced, sublethal damage to the bacterial membrane (23). Other investigators have demonstrated synergistic interaction between penicillin, ampicillin, and carbenicillin or other semisynthetic penicillins plus various aminoglycosides against several Enterobacteriaceae, including species of Proteus, Klebsiella, Enterobacter, and Serratia (5, 6, 10, 14, 29, 30).

Clinically, however, combination therapy of

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**Table 2. Effectiveness of carbenicillin plus aminoglycosides against resistant strains of Acinetobacter calcoaceticus**

<table>
<thead>
<tr>
<th>Strains resistant to:</th>
<th>Genta-micin + Carbenicillin</th>
<th>Tobramycin + Carbenicillin</th>
<th>Kanamycin + Carbenicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin (≥4)(^a)</td>
<td>11/14(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobramycin (≥4)</td>
<td></td>
<td>9/11</td>
<td></td>
</tr>
<tr>
<td>Kanamycin (≥8)</td>
<td></td>
<td></td>
<td>11/13</td>
</tr>
<tr>
<td>Carbenicillin (≥62.5)</td>
<td>7/7</td>
<td>7/7</td>
<td>7/7</td>
</tr>
</tbody>
</table>

\(^a\) Numbers in parentheses indicate MIC (in micrograms per milliliter).

\(^b\) Number of strains showing synergism/total number of strains tested.
gram-negative infections is most commonly used in patients infected with *Pseudomonas aeruginosa* (18). In vitro studies have demonstrated synergism between carbenicillin or ticarcillin and either gentamicin, tobramycin, or amikacin against many strains of *P. aeruginosa* (4, 13, 15, 16, 22, 26–28). In addition, animal studies have demonstrated in vivo synergistic efficacy of these combinations against lethal experimental *Pseudomonas* infections in rodents (2, 3). Furthermore, several reports by Klustersky and associates have demonstrated apparent clinical efficacy of such combinations in the therapy of life-threatening infections due to *P. aeruginosa* in immunocompromised patients; concomitant in vitro studies with the organisms from these cases suggested that clinical success was significantly more likely to occur when the combination of drugs was synergistic in vitro for the infecting organism (11–13).

Of the many indications proposed for administration of multiple antibiotics to patients with infections (18), several can be invoked in cases of infection due to *A. calcoaceticus*. First, infections with this organism are often mixed and involve a variety of gram-negative bacteria (9).

Since these infections are most often acquired in-hospital, these organisms frequently demonstrate resistance to commonly used antibiotics. Second, infections with acinetobacter frequently occur in patients with significant underlying medical and surgical disorders and carry a mortality rate of up to 35% (9). Finally, in nosocomial gram-negative infections, the most commonly used agents that would be active against *A. calcoaceticus* are the aminoglycosides, which possess a relatively small therapeutic/toxic ratio.

In light of these factors, we felt it was important to evaluate the interaction between carbenicillin and aminoglycosides against *A. calcoaceticus*. Studies with 28 strains of *A. calcoaceticus* obtained from clinical sources indicated that combinations of carbenicillin with either kanamycin, tobramycin, or gentamicin were bactericidal in a synergistic fashion against most strains of this organism. Only among strains exhibiting aminoglycoside resistance (MIC $\geq 31 \mu g/ml$ for gentamicin or tobramycin and MIC $\geq 62.5 \mu g/ml$ for kanamycin) was synergism not demonstrable. The combination of carbenicillin with either kanamycin or tobramycin was slightly more active than the combination of carbenicillin plus gentamicin.

With our strains of *A. calcoaceticus*, the level of resistance to the aminoglycoside component of each combination was the primary determinant of synergistic interaction between carbenicillin and the aminoglycosides. Thus, synergistic killing was unlikely to occur with the respective combination if the MIC was $\geq 31 \mu g/ml$ in the case of gentamicin or tobramycin and $\geq 62.5 \mu g/ml$ in the case of kanamycin. In other laboratories, similar studies on the interaction of carbenicillin and aminoglycosides against *P. aeruginosa* have produced conflicting results. Kluge and associates concluded that strains of *P. aeruginosa* with "high-level resistance" to

### Table 3. Antibiotic susceptibility of strains of Acinetobacter calcoaceticus not killed synergistically by carbenicillin plus aminoglycosides

<table>
<thead>
<tr>
<th>Drug combination</th>
<th>Strain</th>
<th>MIC (µg/ml)</th>
<th>Aminoglycoside</th>
<th>Carbenicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin +</td>
<td>H-13</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>carbenicillin</td>
<td>H-130</td>
<td>31</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>lin</td>
<td>H-136</td>
<td>31</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Tobramycin +</td>
<td>H-20</td>
<td>31</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>carbenicillin</td>
<td>H-88</td>
<td>31</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Kanamycin +</td>
<td>H-20</td>
<td>62.5</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>carbenicillin</td>
<td>H-88</td>
<td>62.5</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Effect of three aminoglycosides alone and in combination with carbenicillin against *A. calcoaceticus* strain H-88, which is sensitive to carbenicillin (MIC = 8 µg/ml), resistant to kanamycin (MIC = 62.5 µg/ml) and tobramycin (MIC = 31 µg/ml), but susceptible to gentamicin (MIC = 1 µg/ml).
gentamicin or tobramycin (MICs $\geq 80 \mu g/ml$) were not synergistically inhibited by carbenicillin plus the respective aminoglycoside (15, 16). However, Anderson and co-workers (1) and Marks and associates (19) found no correlation between the level of aminoglycoside resistance and synergistic potential of carbenicillin-aminoglycoside combinations against their isolates of \textit{P. aeruginosa}. Although there were slight differences in experimental techniques between these groups, these variant results are not easily reconciled. However, in some of these studies, criteria for synergism were based on changes in inhibitory activity rather than on bactericidal data such as are provided by killing curves. Although Weinstein and associates have demonstrated good qualitative correlation between inhibitory and bactericidal methods for evaluating antibiotic interaction (30), more standardized studies in this area are needed. Because killing curves are too laborious and time consuming to be used in most clinical microbiology laboratories, it is important to determine the reliability of using levels of resistance to components in antibiotic combinations as predictors of the synergistic killing potential of such combinations.

ACKNOWLEDGMENTS

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