Comparison of Bactericidal Activities of Streptomycin, Amikacin, Kanamycin, and Capreomycin against *Mycobacterium avium* and *M. tuberculosis*

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The bactericidal activities of four injectable antituberculosis drugs, streptomycin, amikacin, kanamycin, and capreomycin, against *Mycobacterium avium* and *M. tuberculosis* were tested. All four drugs were highly bactericidal against *M. tuberculosis*, with low MBC/MIC ratios and MBCs significantly lower than the maximum achievable concentrations in serum (Cmax). In contrast, all four drugs had very low bactericidal activities against *M. avium*: the broth-determined MBCs were significantly higher than the Cmax. On a basis of comparisons with the broth-determined MICs found for susceptible *M. tuberculosis* strains and with the Cmax, about one-third of 100 *M. avium* strains tested can be tentatively considered as susceptible to three aminoglycosides (streptomycin, amikacin, and kanamycin) but not to capreomycin. In regard to the MBCs and MICs, the three aminoglycosides tested have about identical potentials as drugs of choice in combination with other drugs for chemotherapy of *M. avium* disease. The low bactericidal activities of these drugs against *M. avium* in vitro do not exclude their therapeutic usefulness, because they may produce a synergistic effect in combination with other drugs. Such an option is especially promising for patients whose isolates can be considered susceptible on the basis of the MIC. We found no differences in susceptibility to the four drugs tested for *M. avium* strains (identified by Gen-Probe) isolated from 50 patients with and 50 patients without acquired immune deficiency syndrome.

In designing the drug regimen for treatment of a patient with a *Mycobacterium avium* infection, a physician often faces the problem of having to choose among the four injectable drugs to be combined with other antitubercular drugs. Sometimes, differences in the susceptibilities of a strain from a patient to these four drugs and in the tolerance of the patient may be helpful in making such a selection. The difference in drug susceptibility is easier to detect if the degree of susceptibility is quantitated by determining the MIC (7,8), but sometimes even this approach does not clearly indicate the drug of choice. Therefore, general information about the relative potentials of the four injectable drugs against *M. avium* is necessary. Particularly important is comparison of the bactericidal activities of these drugs. It has been reported that streptomycin (3) and other aminoglycosides (23) are highly bactericidal in vitro against *M. tuberculosis*, but the MBC of this or any other injectable antituberculosis drug has not previously been studied systematically in in vitro experiments with *M. avium*. There were reports about the in vitro inhibitory activities of these drugs against *M. avium* (2,16,18,21,23), but it is imperative that the MBC/MIC ratios be derived from a test in which MICs and MBCs are determined simultaneously in liquid medium and with the same strains.

In previous studies (7,8), the highest broth-determined MIC found for wild *M. tuberculosis* strains susceptible to all drugs was considered to be the first breakpoint which indicates the susceptibility of *M. avium*. The second breakpoint, which indicates that an *M. avium* strain is moderately susceptible, is an MIC substantially lower than the peak levels in serum (Cmax) known for these drugs (13,17,20).

The strains were tentatively considered moderately resistant if the MIC was at about the Cmax level, usually twofold higher than at the previous breakpoint. The fourth category was designated very resistant, with the MIC significantly higher than the Cmax. This interpretation of MICs was a tentative suggestion, because with *M. avium* disease there have been no clinical trials to provide correlations between in vitro laboratory findings and responses of patients to chemotherapy which, in turn, would justify establishment of critical concentrations of drugs to be used in in vitro drug susceptibility tests. We considered it important to evaluate further the comparison of the inhibitory activities of four injectable drugs against *M. avium*. The aims of this study were (i) to evaluate the degrees of susceptibility of *M. avium* strains to four injectable drugs, (ii) to compare the bactericidal activities in vitro by determining the MBCs of these four drugs, and (iii) to determine the MBC/MIC ratios. Both MIC and MBC determinations for *M. avium* were done in comparison with wild susceptible *M. tuberculosis* strains under the same experimental conditions.

MATERIALS AND METHODS

Test strains. Of 100 *M. avium* strains included in this study, 50 were isolated from the blood of patients with acquired immune deficiency syndrome (AIDS) and 50 were isolated from the sputa of patients with pulmonary disease. These 100 strains were selected on the basis of their identification by the Gen-Probe method as *M. avium* only. For each strain, smooth transparent colonies were picked from agar plates and subcultivated in 7H9 broth for 3 to 5 days. Aliquots were preserved at −70°C until needed for testing. Only strains found to produce mostly transparent colonies during subsequent subcultivations were included in our *M.
avium culture collection. Drug-susceptible M. tuberculosis strains isolated from nontreated patients and used for the comparison study with M. avium were also preserved in frozen samples.

Antimicrobial agents. Streptomycin was obtained from GIBCO Laboratories (Grand Island, N.Y.), amikacin and kanamycin were from Bristol Laboratories (Syracuse, N.Y.), and capreomycin was from Eli Lilly & Co. (Indianapolis, Ind.). Stock solutions were prepared in accordance with manufacturer instructions and kept in aliquots at −70°C. From these stock solutions, working solutions were made in distilled water.

MIC determination. The MICs of the four drugs, determined by two methods, agar and broth dilution, were reported previously (8) for a limited number of M. avium-M. intracellulare strains. The main purpose of determining the MIC in this study with M. avium strains only was to provide a sufficient background for comparison with the MBC and for finding MBC/MIC ratios. Since the MBC can be determined only in broth culture, we have included in this report the results of only broth-determined MICs. The MIC was defined as the lowest concentration of a drug that inhibited more than 99% of the bacterial population. MICs were determined radiometrically in the BACTEC 460-TB system (Johnston Laboratories, Inc., Towson, Md.) on the basis of comparisons of growth in drug-containing vials with growth in the drug-free vial that represents 1% of the bacterial population (the 1:100 control). Our previous studies (7–9, 11) indicated that the radiometric method of MIC determination is as accurate as the conventional technique based on the plating of samples from broth cultures and determination of the number of CFU per milliliter. We also stated previously (8) that the turbidometric method, including that performed in microtiter plates, should be less accurate than either plating of samples or radiometry. The drug concentration in the presence of which the daily increases in the growth index (GI) and the final GI reading were lower than those in the 1:100 control was considered to have inhibited more than 99% of the bacterial population and was therefore defined as the MIC. More technical details about the MIC determination are given in our previous publication (8).

MBC determination. The definition of MBC most often used in clinical microbiology designates it as the lowest concentration that kills 99.9% of the bacterial population within a limited cultivation period in a liquid medium (19, 24). We suggested previously (10) that in the field of mycobacteriology the 99% killing criterion is more accurate and reproducible than the 99.9% criterion. The MBCs of streptomycin, amikacin, and kanamycin were determined in 7H12 broth. Beginning with an initial concentration of 105 to 106 CFU/ml, the cultures were allowed to incubate until growth was in the exponential phase and there were about 108 CFU/ml. Previous studies (9, 11, 12) showed that for M. avium this bacterial concentration occurred when the radiometric GI was 20 to 80 and for M. tuberculosis when the GI was approximately 500. In the current study, with five M. avium and four M. tuberculosis strains, the number of viable organisms in 7H12 broth at the moment when the drugs were added appeared to be in accordance with these expectations (see Table 3). The drugs were added to achieve 1, 2, 4, 8, 16, 32, and 64 times the previously determined MIC. Samples were taken from alternate vials at various time points and diluted 10−1 to 10−6 on the basis of the GI reading, and 0.5-ml volumes of the dilution were inoculated into duplicate 7H11 agar plates. At the end of an experiment, the 10−5 dilution reduced the concentration of drugs below the previously determined MIC. Plates seeded with such samples taken from vials containing concentrations subsequently found to be MBCs showed no growth. The number of CFU per milliliter was calculated from the colony counts on plates inoculated with 10−1 dilutions on the final day of plating. The fact that there was no growth from 10−3 diluted samples but that growth consistently appeared in plates seeded with 10−1 diluted samples convinced us that there was no drug carry-over (22).

RESULTS

The distribution of the 100 M. avium strains, based on their susceptibilities to different drug concentrations in 7H12 broth, indicated that these strains were not inhibited by capreomycin at relatively low concentrations (<3.0 μg/ml), while MICs of streptomycin, amikacin, and kanamycin were found within this range for a significant number of strains (Table 1). Higher MICs (>8.0 μg/ml) of capreomycin were found substantially more frequently (58 to 60%) than with the other three drugs. These findings are in agreement with our previous report about the higher activities of streptomycin and kanamycin than capreomycin when 31 M. avium-M. intracellulare strains were tested (8).

In addition, there was no difference in the degree of susceptibility between strains isolated from patients with or without AIDS (Table 1). These data do not support a suggestion about some differences in susceptibility and resistance between strains isolated from patients with or without AIDS. (14). Our conclusion that there are no differences between these two groups of clinical isolates is based on the larger number of strains tested and is derived from experiments methodologically different from those in the publication cited above (14). (i) We tested only M. avium strains instead of M. avium and M. intracellulare, (ii) all strains represented subcultures from transparent-type colonies, and (iii) we tested each strain with multiple concentrations of each drug in 7H12 broth radiometrically and expressed the results quantitatively as a degree of susceptibility or resistance in MICs rather than testing by the proportion method with critical concentrations in agar plates.

Comparisons of the MICs of the four injectable drugs for M. avium strains showed that the lowest MICs for 50 and 90% of the strains studied, as well as the lowest range of MICs, were found for streptomycin and amikacin and the highest values were found for capreomycin (Table 2). This places the activity of kanamycin between those of streptomycin and amikacin on the one hand and that of capreomycin on the other. But in terms of the highest MICs found for

![Table 1. Distribution of M. avium strains isolated from 50 patients with AIDS and 50 patients without AIDS by susceptibility or resistance to four injectable antituberculosis drugs](http://aac.asm.org/)
TABLE 2. Comparison of broth-determined MICs of four injectable antituberculosis drugs against 100 M. avium strains

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>% of strains tentatively considered susceptible in regard to chosen breakpoint (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1.0-8.0</td>
<td>2.53</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1.0-8.0</td>
<td>2.65</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>3.0-12.0</td>
<td>3.95</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>5.0-20.0</td>
<td>5.93</td>
</tr>
</tbody>
</table>

*50% and 90%, MICs for 50 and 90%, respectively, of the strains tested.

M. tuberculosis as a breakpoint, the percentage of M. avium strains that could have been tentatively called susceptible to streptomycin and kanamycin was higher than the percentage of strains that could have been called susceptible to amikacin, and none could have been called susceptible to capreomycin.

Evaluation of bactericidal activities (Table 3) indicated that all four drugs were highly bactericidal for M. tuberculosis and had the same very low MBC/MIC ratios, from 1 to 4. The MBCs of these drugs for M. tuberculosis were lower than the known Cmax (13, 17, 20), with some advantages for streptomycin and amikacin over kanamycin and capreomycin. The bactericidal activities against M. avium were significantly lower, with MBCs much higher than the known Cmax for these drugs. The MBC/MIC ratios for M. avium strains were also substantially higher: 32 to 128 for streptomycin; 16 to 24 for amikacin; 16 to 32 for kanamycin; and 8 to 32 for capreomycin. By this criterion, none of the four drugs tested has any advantage over other drugs, and they should be considered to have low bactericidal activities against M. avium in contrast to their bactericidal activities against M. tuberculosis.

DISCUSSION

M. avium clinical isolates, 50 from patients with AIDS and 50 from patients without AIDS, were found to be more susceptible in vitro to streptomycin, amikacin, and kanamycin than to capreomycin. This conclusion confirms our previous suggestion made in studies with 31 M. avium-M. intracellulare strains (8). About one-third of the M. avium strains tested were susceptible to 3.0 µg or less of streptomycin, amikacin, and kanamycin per ml, and there were no substantial differences among these three drugs. In regard to the MIC ranges and the calculated MICs for 90% of the strains tested, streptomycin and amikacin seemed to be more active than kanamycin, but in terms of the highest MIC found for susceptible M. tuberculosis strains, the percentage of strains that could have been tentatively called susceptible was higher for streptomycin and kanamycin than for amikacin. We found no significant difference in the bactericidal activities of all four drugs for M. avium in regard to the MBCs and the MBC/MIC ratios. Therefore, we conclude that, overall, based on in vitro evaluation of inhibitory and bactericidal activities against M. avium, three drugs, streptomycin, amikacin, and kanamycin, have about equivalent potentials and are clearly more promising than capreomycin. It was reported that amikacin seems to be effective in accordance with clinical observations in the treatment of M. avium infections (1) and in experiments on animals (6, 15). These and other studies did not compare the efficacy of amikacin with those of other aminoglycosides, and it is not known whether streptomycin or kanamycin would have been more or less active in the treatment of M. avium disease. Our in vitro findings showed no significant advantages of amikacin over streptomycin and kanamycin. Results of testing in 7H12 broth, but by a method different from ours, indicated that a higher percentage of M. avium strains (isolated only from patients with AIDS) was susceptible to amikacin than to streptomycin at 4.0 µg or less per ml (16). We could not confirm this conclusion by our method of determining the MICs of those drugs for M. avium strains, either from patients with AIDS or from patients without AIDS. Comparison of in vitro activities also showed no significant differences among streptomycin, amikacin, and kanamycin by the agar dilution method (2), in Dubos broth (18), and in experiments with intracellular (macrophage) bacterial populations (21).

All four drugs were found to be highly bactericidal against M. tuberculosis, with low MBC/MIC ratios and MBCs below the Cmax, which is in agreement with other findings (22). At the same time, their bactericidal activities against M. avium were very low, with extremely high MBC/MIC ratios and MBCs above the Cmax. In light of these data, the findings about the efficacy of one of these drugs, amikacin, in the treatment of M. avium disease require further study. We can see at least two possible explanations for the controversy over the low bactericidal activity of amikacin in vitro and its efficacy in a clinical situation. One such interpretation is the possibility that the bactericidal effect is not mandatory to achieve clinical efficacy. It is possible that, owing to the high variability of M. avium strains, the bactericidal effects of...

TABLE 3. Bactericidal activities of four injectable antituberculosis drugs against M. avium and M. tuberculosis

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of CFU/ml when drug added</th>
<th>MBC (µg/ml) and no. of CFU/ml at end of cultivation (no. of survivors) with drug at MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. avium</td>
<td></td>
<td>Streptomycin</td>
</tr>
<tr>
<td>211</td>
<td>1.5 x 10^4</td>
<td>256.0; 9.0 x 10^2</td>
</tr>
<tr>
<td>3011</td>
<td>1.6 x 10^4</td>
<td>64.0; 1.3 x 10^2</td>
</tr>
<tr>
<td>1854</td>
<td>2.6 x 10^4</td>
<td>64.0; 2.0 x 10^2</td>
</tr>
<tr>
<td>101</td>
<td>4.2 x 10^4</td>
<td>32.0; 3.4 x 10^2</td>
</tr>
<tr>
<td>168</td>
<td>6.2 x 10^4</td>
<td>256.0; 2.5 x 10^3</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td></td>
<td>Streptomycin</td>
</tr>
<tr>
<td>H37Rv</td>
<td>2.2 x 10^4</td>
<td>2.0; 5.2 x 10^2</td>
</tr>
<tr>
<td>1620</td>
<td>2.5 x 10^4</td>
<td>2.0; 9.0 x 10^2</td>
</tr>
<tr>
<td>3181</td>
<td>7.6 x 10^3</td>
<td>0.5; 0.6 x 10^2</td>
</tr>
<tr>
<td>131</td>
<td>2.4 x 10^4</td>
<td>2.0; 0.9 x 10^2</td>
</tr>
</tbody>
</table>
aminoglycosides can be greater when susceptible strains are used (5, 23). Another is the possibility that amikacin, when used in combination with other drugs, produced a synergistic bactericidal effect, which would lower the MBCs below the \( C_{\text{max}} \) of amikacin, as we found previously for combinations of rifampin or rifabutin with ethambutol (10). The possibility that the aminoglycosides have a synergistic bactericidal effect in combination with other drugs is supported by findings that amikacin in combination with rifampin and ethambutol produced a synergistic inhibitory effect in vitro and in patients (1) and by data showing that amikacin can enhance a therapeutic effect in beige mice treated with amikacin plus imipenem and ciprofloxacin (15) or in combination with clofazimine (6). Besides this option of combining one of the aminoglycosides with other drugs, liposome-based delivery of these drugs is also promising in enhancing their effects in vitro (4). We plan to test the validity of these speculations in further studies, particularly by determining not only the combined inhibitory effects but also the combined bactericidal effects by using a previously developed method (10).

ACKNOWLEDGMENTS

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LITERATURE CITED


