Bacteriostatic and Bactericidal Activities of Gentamicin Alone and in Combination with Clarithromycin against *Mycobacterium avium*

LEONID B. HEIFETS,[1,2]* PAMELA J. LINDHOLM-LEVY,[1] AND R. DAWN COMSTOCK[1]

National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, Colorado 80206,[1] and Departments of Microbiology and Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80262*

The inhibitory activity of gentamicin against *Mycobacterium avium* depended on the pH of the medium, and the broth-determined MICs for 90% of strains were 5.0 μg/ml at pH 7.4, 9.5 μg/ml at pH 6.8, and >16.0 μg/ml at pH 5.0. The MBCs were two- to eightfold higher than the MICs. The combined effect of gentamicin and clarithromycin was additive, and the MICs and MBCs of each drug were either the same as those in the single-drug tests or reduced twofold.

Gentamicin has been known to be an active antimicrobial agent since 1963, but there have been only a few reports analyzing its activity against mycobacterial species. One of the first reports (13) indicated that gentamicin at a concentration of 10 μg/ml inhibited all 15 *Mycobacterium tuberculosis* strains in a tube dilution test. The authors of another earlier publication (11) claimed that gentamicin may be active against streptomycin-resistant *M. tuberculosis* strains, but the limited data provided in this short communication are not sufficient for a definite conclusion. In another study, all 9 gentamicin-resistant strains were resistant to streptomycin, but of 54 streptomycin-resistant strains, only 15% were resistant to gentamicin (15).

In the first systematic study of gentamicin activity against mycobacteria, Sanders et al. (14) determined the MICs of the drug in Proskauer-Beck liquid medium and found that the MICs for 13 *M. tuberculosis* strains were 1.6 to 3.1 μg/ml, while the MICs of streptomycin were >25 μg/ml for 3 of these strains and ranged from 0.8 to 6.2 μg/ml for the remaining 10 strains. In experiments with 10 strains of *M. kansasii*, the MICs of gentamicin ranged from 1.6 to 6.2 μg/ml and the MICs of streptomycin ranged from 3.1 to 25.0 μg/ml. Gentamicin was also more active than streptomycin in experiments with 10 *M. intracellulare* strains: the MICs ranged from 1.6 to 6.2 μg/ml and from 1.6 to >25.0 μg/ml, respectively. The MBC/MIC ratios ranged from two to eight in experiments with two strains each of *M. tuberculosis*, *M. kansasii*, and *M. intracellulare*. Experiments with mice infected with *M. tuberculosis* H$_{37}$Rv indicated that gentamicin was only weakly bacteriostatic and was less effective than was streptomycin.

Gangadharam and Candler (4) tested the activities of gentamicin, amikacin, tobramycin, and sisomycin against various mycobacterial species. In experiments with *M. tuberculosis*, the agar-determined MICs were 3.2 to 6.2 μg/ml for 99 of 100 strains tested. The authors stated that gentamicin showed activity against *M. tuberculosis* only, but the actual data for this drug were not tabulated. Activity against nontuberculous mycobacteria was found only for amikacin. Unfortunately, the authors combined the results

of experiments with other mycobacteria (*M. scrofulaceum*, *M. intracellulare*, *M. fortuitum*, and *M. chelonae*) in one group and concluded that the agar-determined MICs of amikacin were higher than 12.8 μg/ml. The results obtained with other aminoglycosides against any of the nontuberculous mycobacteria were not shown. Davis et al. (3) tested the susceptibilities of *M. avium* complex clinical isolates to various drugs in 7H10 agar plates. The difference in the numbers of the 20 tested strains inhibited by ≤4.0 μg/ml of each of four aminoglycosides was not significant: gentamicin, 13; amikacin, 16; streptomycin, 15; kanamycin, 17.

Klemens et al. (12) evaluated the activity of free and liposome-encapsulated gentamicin in beige mice infected with *M. avium* and reported that both forms of the drug significantly reduced the number of bacteria in the spleen and liver but that the liposome-encapsulated form was substantially more effective than was the free form. This effect was dose related for the encapsulated form. Bermudez et al. (1) tested the activities of two aminoglycosides, gentamicin and amikacin, against *M. avium* in the beige mouse model and reported that only liposome-encapsulated drugs led to a dramatic decrease in viable counts in the blood, liver, and spleen. The aminoglycosides had an equal effect, although amikacin had some advantage in reducing viable counts in the blood, but only when low doses (0.2 mg) were administered.

Our previous studies of aminoglycosides (5, 6) revealed little difference in either the inhibitory or the bactericidal activities of streptomycin, kanamycin, and amikacin against a large number of *M. avium* strains. These studies did not include gentamicin. The aim of our present study was to determine the bacteriostatic and bactericidal activities (MICs and MBCs) of gentamicin alone and in combination with clarithromycin for *M. avium* strains isolated from AIDS patients.

MATERIALS AND METHODS

Antimicrobial agents. Gentamicin was obtained from Sigma Chemical Co. (St. Louis, Mo.). Solutions of 2,560.0 to 40.0 μg/ml were made in sterile deionized water and stored as 1.0-ml samples at −70°C until needed for an experiment. Clarithromycin was obtained from Abbott Laboratories (Ab-

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* Corresponding author.

bott Park, Ill.). This drug was first dissolved in methanol and then diluted fivefold in a phosphate buffer solution (pH 6.5) to make a stock solution of 2 mg/ml. Appropriate working solutions were made in the phosphate buffer.

Test strains. Twenty strains of *M. avium* identified to the species level by RNA-DNA hybridization (Gen-Probe, San Diego, Calif.) were used in this study. Transparent colonies were picked from 7H10 agar plates and subcultivated for 3 to 5 days at 37°C in 7H9 broth until the turbidity equalled that of a no. 1 McFarland standard. Samples of the broth cultures were stored at −70°C until needed for an experiment.

MIC determinations. MICs were determined in three types of 7H12 broth (Becton Dickinson, Sparks, Md.) that differed only in pH (7.4, 6.8, and 5.0) and in Mueller-Hinton agar (Difco, Detroit, Mich.) supplemented with 10% oleate-albumin-dextrose-catalase enrichment (pH 7.4).

For broth-determined MICs, gentamicin was added to respective 7H12 vials in concentrations ranging from 0.25 to 16.0 μg/ml. Each drug-containing vial was inoculated with approximately 10^4 CFU/ml as described previously (7). At each pH for each strain, two control vials were prepared, one containing the same inoculum as the drug-containing vials and one containing a 1:100 dilution of the inoculum, representing 1% of the bacterial population. The vials were incubated at 37°C and read daily in a BACTEC 460 instrument, and the radiometric growth indices (GI) were recorded. The test was complete when the GI of the 1:100-diluted control was greater than 20 for three consecutive days during an 8-day period of cultivation, while at the same time the undiluted control reached the maximum GI, 999, no sooner than the fourth day of cultivation. The MIC was considered the lowest concentration of the drug in the presence of which the GI did not exceed 50 for the duration of the test. Details of these criteria are described in our previous publications and were summarized in a recently published review (7). As in our previous studies, the observance of these standards ensured that the radiometrically determined MIC was the lowest drug concentration that indeed inhibited more than 99% of the bacterial population.

For agar-determined MICs, gentamicin was added to 100-ml batches of sterile cooled agar to yield concentrations of 1.0 to 64.0 μg/ml. Drug-containing and drug-free agar preparations were poured into quadrant petri plates. The plates were inoculated with approximately 10^3 CFU/ml and incubated for 12 to 14 days in 5% CO_2 at 37°C. Incubation in the presence of CO_2 may have lowered the pH slightly (not by more than 0.1 or 0.2, as determined by our observations), but it was necessary to obtain sufficient growth within 2 weeks to avoid possible degradation of the drug. Growth on the drug-containing and drug-free control quandrants was compared, and the gentamicin concentration inhibiting more than 99% of the growth seen on the drug-free agar was considered the MIC.

Bactericidal activity. Bactericidal activity was measured as the MBC, the lowest concentration that killed more than 99% of the bacterial population present at the beginning of the experiment. Gentamicin was added to duplicate 7H12 broth vials to yield final concentrations of the drug at the MIC and two-, four-, and eightfold higher than the MIC. The inoculum was as described above for the MIC determinations. One control vial was sampled, and the sample was plated on 7H10 agar to determine the initial number of CFU per milliliter. On days 5, 10, and 15, the test vials were sampled and the samples were diluted on the basis of the radiometric GI and plated on duplicate 7H10 agar plates at 0.5 ml per plate. The plates were incubated at 37°C for 14

TABLE 1. Distribution of strains by MICs of gentamicin, determined in 7H12 broth at three pHs[a]

| pH | % of strains for which the MIC (μg/ml) was: | | | | | | | MIC_{50} (μg/ml) | MIC_{90} (μg/ml) |
	≤0.5	1.0	2.0	4.0	8.0	16.0	≥16.0		
5.0	0	0	5	15	20	45	15	9.8	>16.0
6.8	0	0	0	40	45	15	0	4.7	9.5
7.4	10	15	35	25	15	0	0	1.6	5.0

[a] MIC_{50} and MIC_{90}, MICs for 50 and 90% of strains, respectively.

days in the presence of 5% CO_2, and the colonies were counted. The bactericidal activity of gentamicin was determined at pH 6.8 so that the results would be comparable to the data on the bactericidal activities of other aminoglycosides tested previously (6). The MBCs for conditions other than pH 6.8 can be projected from the MBC/MIC ratio found at pH 6.8 and specific MICs determined at pH 7.4 or 5.0. The lowest concentration of the drug that killed more than 99% of the initial bacterial population in the 7H12 broth cultures was designated the MBC.

Gentamicin in combination with clarithromycin. The interaction of gentamicin and clarithromycin against *M. avium* was evaluated by a separate determination of the inhibitory and bactericidal effects, expressed accordingly as MICs and MBCs. Appropriate solutions of each drug were added to the 7H12 broth vials to represent 1/2, 1/4, and 1/8 fractions of the MICs or MBCs of each drug alone determined previously for a particular strain. The techniques were the same as those described above for determining MICs and MBCs. In each experiment, determination of the MICs or MBCs for a combination was accompanied by a repeated determination of the same values for each drug singly. The fractional inhibitory and bactericidal concentrations were calculated to determine whether the inhibitory and bactericidal interactions represented additivity, synergism, or antagonism. More details about the principles and actual methods used in determining the drug interaction in experiments with *M. avium* are given in a recent review (8). The selection of concentrations of clarithromycin was based on our previous data on the MICs and MBCs of this drug tested singly with the same *M. avium* strains (9).
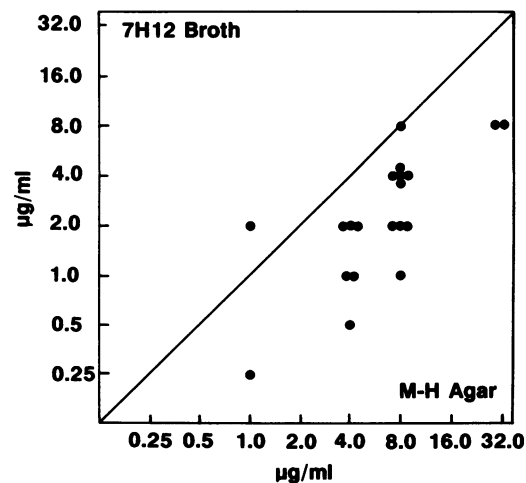

FIG. 1. Comparison of broth- and agar-determined MICs of gentamicin for 20 *M. avium* strains. M-H, Mueller-Hinton.

TABLE 2. Bactericidal activity of gentamicin in 7H12 broth at pH 6.8

Strain	MIC (μg/ml)	MBC (μg/ml)	MBC/MIC ratio
AH	8.0	64.0	8
1854	4.0	16.0	4
NA	4.0	32.0	8
3350	8.0	64.0	8
211	8.0	32.0	4

TABLE 4. MBCs of gentamicin and clarithromycin singly and in combination at pH 6.8

Strain	MBC (μg/ml) of:				Fractional bactericidal concn
	Gentamicin		Clarithromycin		
	Singly	In combination	Singly	In combination	
AH	64.0	32.0	32.0	16.0	1.0
1854	16.0	8.0	8.0	4.0	1.0
3350	64.0	16.0	32.0	8.0	0.5

RESULTS

The inhibitory activity of gentamicin in vitro depended on the pH of the medium (7H12 broth), being highest at pH 7.4, lowest at pH 5.0, and intermediate at pH 6.8 (Table 1). The MICs for 90% of strains were 5.0 μg/ml at pH 7.4, 9.5 μg/ml at pH 6.8, and >16.0 μg/ml at pH 5.0. A comparison of MICs obtained in solid (Mueller-Hinton agar) and liquid (7H12 broth) media at the same pH (7.4) indicated that the agar-determined MICs were two- to eightfold higher than the broth-determined MICs for most of the strains (Fig. 1). This result is in agreement with our previous findings regarding other aminoglycosides (5, 6).

The MBCs of gentamicin were ≥32.0 μg/ml for four of five tested strains and 16.0 μg/ml for one strain, resulting in an MBC/MIC ratio of four or eight, respectively (Table 2).

The inhibitory interactions of gentamicin and clarithromycin were determined in 7H12 broth at pH 7.4 for 10 strains. The MICs for four strains were reduced twofold in comparison with the MICs of each drug singly and remained the same for six strains (Table 3), resulting in a fractional inhibitory concentration of 1.0 or 2.0, representing an additive interaction.

The bactericidal interactions of gentamicin and clarithromycin were determined for three strains and indicated an additive effect for two strains and a synergistic effect for one strain (Table 4).

DISCUSSION

The broth-determined MICs of gentamicin against *M. avium* were substantially lower than the agar-determined MICs, in agreement with the results of our previous studies of other aminoglycosides (5, 6). The range of the MICs found in our study was comparable to the data obtained in other reports for gentamicin (3, 13, 14), some of which have

indicated an advantage of this drug over other agents of this class. An important finding of our studies is that the MBC/MIC ratios for gentamicin were lower than those for other aminoglycosides (6), possibly suggesting a higher bactericidal activity of gentamicin. Data on the effectiveness of gentamicin in beige mice infected with *M. avium* (1, 12) may be considered supportive of the possibility of a bactericidal effect in vivo, especially when the drug is in an encapsulated form, which may lead to concentrations sufficient for such an effect in sites of bacterial persistence and multiplication. Reduced activity at pHs below the neutral pH has been observed for other drugs in our work (9) and that of other groups (2, 10) and is also in agreement with the finding that a low pH may affect the activities of streptomycin and other aminoglycosides by interfering with the binding of the drugs to the surface of the bacterial cell (16).

The combined inhibitory effect of gentamicin and clarithromycin can be considered additive, resulting in either the same MICs and MBCs of each drug singly or a twofold reduction in these values. The combined bactericidal effect is strain dependent, being either additive or synergistic.

TABLE 3. MICs of gentamicin and clarithromycin singly and in combination in 7H12 broth at pH 7.4

Strain	MIC (μg/ml) of:				Fractional inhibitory concn
	Gentamicin		Clarithromycin		
	Singly	In combination	Singly	In combination	
211	2.0	2.0	1.0	1.0	2.0
3354	4.0	4.0	1.0	1.0	2.0
800	2.0	1.0	2.0	1.0	1.0
3344	2.0	1.0	2.0	1.0	1.0
1854	2.0	2.0	0.5	0.5	2.0
1102	2.0	2.0	0.5	0.5	2.0
1017	8.0	4.0	1.0	0.5	1.0
3351	1.0	0.5	0.5	0.25	1.0
3090	4.0	4.0	0.5	0.5	2.0
1625	8.0	8.0	1.0	1.0	2.0

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