

In Vitro Activities of Rifabutin, Azithromycin, Ciprofloxacin, Clarithromycin, Clofazimine, Ethambutol, and Amikacin in Combinations of Two, Three, and Four Drugs against *Mycobacterium avium*

DAVID M. YAJKO,* CYNTHIA A. SANDERS, JANUSZ J. MADEJ, V. LOUISE CAWTHON,
AND W. KEITH HADLEY

Department of Laboratory Medicine, University of California and San Francisco General Hospital,
San Francisco, California 94110

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Multidrug therapy is recommended for treatment of *Mycobacterium avium* complex (MAC) bacteremia in patients with AIDS. Azithromycin, clarithromycin, rifabutin, ciprofloxacin, ethambutol, clofazimine, and amikacin have all been suggested for use in treating MAC bacteremia, but the most active combinations of these drugs have not been identified, nor has the minimum number of drugs needed for effective therapy been determined. To address the former, the in vitro bactericidal activities of all two-, three-, and four-drug combinations of these seven agents was determined by using 10 blood-derived strains of MAC isolated from patients with AIDS. The activities of the 132 drug combinations were compared by statistical analysis of survival means (analysis of variance) and further evaluated by determining the percentage of strains considered susceptible to each combination. When susceptibility was defined as a decrease in CFU of $\geq 2 \log_{10}$, no two- or three-drug combination and only two four-drug combinations were active against all 10 MAC strains. When a less stringent definition was applied ($\geq 1 \log_{10}$ decrease in CFU), 1 two-drug combination, 9 three-drug combinations, and 31 four-drug combinations showed activity against all 10 strains. Eighteen selected drug combinations were also tested for intracellular activity in MAC-infected J774 cells. Combinations which contained amikacin as a component were considerably less active against intracellular MAC organisms than against organisms in broth. The opposite result was obtained for the combination of clarithromycin plus clofazimine.

The current recommendation for therapy of disseminated *Mycobacterium avium* complex (MAC) infection in patients with human immunodeficiency virus infection (19) states that treatment regimens outside clinical trials should include at least two agents and that every regimen should contain either azithromycin or clarithromycin. The recommendation notes that ethambutol is often preferred as a second drug and that many clinicians add one or more of the following as a second, third, or fourth agent: clofazimine, rifabutin, rifampin, ciprofloxacin, and in some situations amikacin (19). Several of these agents have been tested in combination against MAC in in vitro studies (8, 15, 17, 23) and in clinical trials (3-5, 10, 11, 18), but no study has compared the activities of all possible combinations of these agents.

One of the issues associated with the use of multidrug therapy is the potential for drug interactions. Both synergistic and antagonistic drug interactions have been reported for some of the two-drug combinations that have been suggested for use in treating MAC infection (6, 8, 9, 14, 20, 25). The effects of the addition of a third or fourth drug to these combinations, and the interactive effects of other drug combinations, are unknown. In addition, while MAC strains are known to differ considerably in their susceptibilities to individual antimicrobial

agents (7, 12, 13, 16, 21), it is not known whether drug combinations which are widely active against MAC strains exist.

In the study reported here, 10 strains of MAC were used to determine the in vitro activities of all two-, three-, and four-drug combinations of seven agents commonly used or suggested for use in the treatment of MAC infection. The relative activities of combinations were assessed from the proportion of MAC CFU that survived treatment. Drug combinations were also analyzed to determine which combinations showed activity against 90 or 100% of MAC strains tested.

MATERIALS AND METHODS

Mycobacterial isolates. The isolates used in this study were recovered from blood specimens from patients with AIDS. All isolates were identified as *M. avium* on the basis of reactivity with the Gen-Probe (San Diego, Calif.) *M. avium* probe. All of the isolates were collected early during the AIDS epidemic, prior to the widespread use of antibiotics to treat MAC infection in patients with AIDS. Stock cultures were maintained at -70°C . To avoid bias in the selection of isolates, the first 10 consecutive blood isolates in the frozen stock culture collection at San Francisco General Hospital were used, with the exception of stock isolates 6 and 11, which were not included because of contamination or nonviability. Each isolate was tested with every drug combination.

Activities of drug combinations against *M. avium* in broth. Drug combinations were tested for activity in 7HSF broth, the broth equivalent of Middlebrook 7H11 agar (21), using drug concentrations that are achievable in serum (peak level) at routine dosages: rifabutin, 0.5 $\mu\text{g/ml}$; azithromycin, 0.5 $\mu\text{g/ml}$; ciprofloxacin, 2.6 $\mu\text{g/ml}$; ethambutol, 4 $\mu\text{g/ml}$; clofazimine, 0.7 $\mu\text{g/ml}$; clarithromycin, 2.7 $\mu\text{g/ml}$; and amikacin, 20 $\mu\text{g/ml}$. Azithromycin was also tested at 23 $\mu\text{g/ml}$, a concentration which can be achieved intracellularly (2). The seven drugs yielded a total of 132 different combinations when mixed in combinations containing two, three, or four drugs. Tubes containing drugs in a total of 2 ml of broth were inoculated to yield a final concentration of approximately 3×10^5 MAC CFU/ml (21, 22). To minimize the possible effects of day-to-day variability in the cultures

* Corresponding author. Mailing address: Clinical Laboratories, Rm. 2M35, San Francisco General Hospital, 1001 Potrero Ave., San Francisco, CA 94110. Phone: (415) 206-8578. Fax: (415) 206-3045. Electronic mail address: yajko@labmed.ucsf.edu.

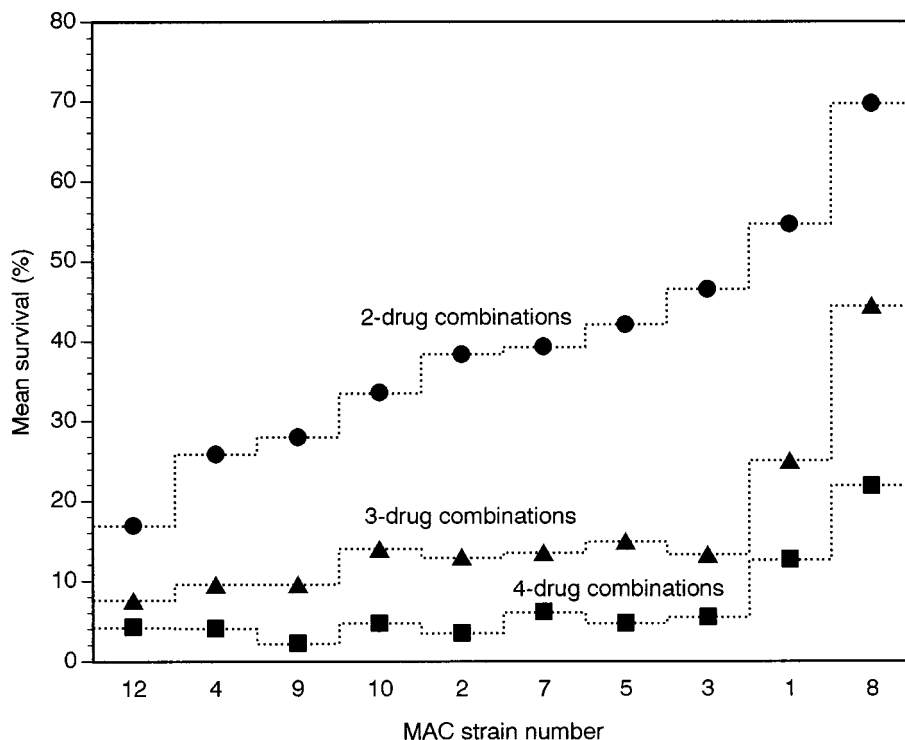


FIG. 1. Differences among 10 strains of *M. avium* in susceptibility to combinations containing two, three, or four drugs in broth. Results are means of organisms surviving treatment with two-drug combinations ($n = 27$), three-drug combinations ($n = 50$), or four-drug combinations ($n = 55$) tested against each strain.

used as inocula, each strain of MAC was tested with all drug combinations on the same day, using the same inoculum culture for all tubes. After incubation in an ambient atmosphere at 35°C for 7 days, tube contents were subcultured on Middlebrook 7H10 agar and colonies were counted with the aid of a dissecting microscope after 7 days of incubation at 35°C (22).

Activities of drug combinations against MAC organisms in J774 cells. Selected drug combinations were tested for activity against MAC organisms inside cells of the mouse macrophage-derived cell line J774, using methods described previously (24). Briefly, MAC was added to tissue culture flasks containing approximately 10^5 J774 cells at a ratio of approximately 3 MAC CFU per macrophage. After overnight incubation, monolayers were washed and drugs were added to each flask except control flasks. At this time (time zero), J774 cells in one set of control flasks were lysed, and the number of MAC CFU in the lysates was determined by plating aliquots onto 7H10 agar. All remaining flasks were incubated at 35°C for 7 days, at which time the J774 cells were washed and lysed and the lysates were plated. The effect of drugs on the survival of intracellular MAC organisms was determined by the following formula: (number of MAC CFU in lysates of drug-treated J774 cells at 7 days/number of MAC CFU in lysates at time zero) \times 100. Control flasks (no drugs) at 7 days always showed an increase in the number of MAC CFU compared with time zero flasks. The mean increase at 7 days was 18-fold.

Statistical analysis. Data analysis was performed with SuperANOVA computer software (Abacus Concepts Inc., Berkeley, Calif.). Data were analyzed by analysis of variance after arcsine \sqrt{p} transformation (1), where p is the proportion of the initial MAC inoculum that survived exposure to drugs for 7 days. Differences among proportion means for the 132 combinations were determined by post hoc analysis of type III sums of squares, using Fisher's protected least significant difference.

RESULTS

Relative activities of drug combinations tested in broth. The proportion of MAC CFU that survived treatment was determined for each drug combination tested against each strain. The mean survival \pm the standard error (SE) for each combination was calculated from the data for the 10 strains. Drug combinations were then ranked in the order of their survival means (Table 1). Statistical analysis by analysis of variance showed that the survival means for combinations ranked 1

through 92 were not significantly different ($P > 0.05$). Amikacin was a component in 18 (72%) of the 25 highest-ranked combinations.

In addition to the overall ranking of drug combinations, combinations were also ranked after being divided into categories containing only two, three, or four drugs. Mean survival \pm SE values for these categories were $40.3\% \pm 2.7\%$, $13.5\% \pm 1.2\%$, and $3.9\% \pm 0.6\%$, respectively. Analysis of the data within each category showed that means were not significantly different for the two-drug combinations ranked 1 through 12, the three-drug combinations ranked 1 through 38, and the four-drug combinations ranked 1 through 45.

Contributions of specific drugs in combinations tested in broth. To determine the relative contributions of each of the drugs included in the study, combinations were grouped into seven categories by drug: all combinations which included rifabutin, all combinations which included ciprofloxacin, etc. The mean survival for each category (drug) was then calculated and the means were compared. A statistical analysis of the means showed the following rankings: amikacin < rifabutin = clarithromycin < azithromycin (intracellular concentration) = ciprofloxacin = ethambutol < clofazimine = azithromycin (concentration in serum), where "<" signifies that the difference in means was statistically significant.

Variability among MAC strains in susceptibility to combinations of drugs. An analysis of the data in Table 1 indicated that many of the drug combinations tested against MAC were statistically equivalent. However, a lack of statistical difference could be a consequence of wide differences in susceptibility among the MAC strains included in the study. An analysis of variance of differences among strains was therefore performed; it showed that the 10 MAC strains differed significantly ($P = 0.0001$) in their susceptibilities to the 132 drug combinations.

TABLE 1. Ranks of 132 combinations of two, three, or four drugs by mean survival of 10 strains of *M. avium* in broth

| Overall rank | Drug combination ^a | Mean survival ± SE (%) ^b | Category rank for combinations containing: | | |
|--------------|--|-------------------------------------|--|---------|---------|
| | | | 2 drugs | 3 drugs | 4 drugs |
| 1 | CLAR + RBT + EMB + AMIK ^c | 0.03 ± 0.02 | | | 1 |
| 2 | EMB + CIPRO + AMIK ^c | 0.2 ± 0.2 | | 1 | |
| 3 | RBT + EMB + CIPRO + AMIK | 0.2 ± 0.1 | | | 2 |
| 4 | EMB + AZI low + CIPRO + AMIK | 0.2 ± 0.1 | | | 3 |
| 5 | CLAR + RBT + AZI high + AMIK | 0.4 ± 0.2 | | | 4 |
| 6 | CLAR + RBT + CIPRO + AMIK | 0.4 ± 0.3 | | | 5 |
| 7 | CLAR + RBT + EMB + AZI low | 0.4 ± 0.2 | | | 6 |
| 8 | CLAR + RBT + CLOF + EMB ^c | 0.4 ± 0.3 | | | 7 |
| 9 | CLAR + RBT + EMB + AZI high | 0.5 ± 0.3 | | | 8 |
| 10 | CLAR + RBT + AZI high + CIPRO | 0.5 ± 0.4 | | | 9 |
| 11 | CLAR + CLOF + EMB + AMIK | 0.6 ± 0.4 | | | 10 |
| 12 | CIPRO + AMIK ^c | 0.6 ± 0.3 | 1 | | |
| 13 | RBT + EMB + AZI high + AMIK | 0.6 ± 0.4 | | | 11 |
| 14 | RBT + AZI low + CIPRO + AMIK | 0.7 ± 0.4 | | | 12 |
| 15 | RBT + CLOF + EMB + AZI high ^c | 0.7 ± 0.3 | | | 13 |
| 16 | CLAR + RBT + AMIK | 0.8 ± 0.4 | | 2 | |
| 17 | CLAR + RBT + AZI low + AMIK | 0.9 ± 0.4 | | | 14 |
| 18 | CLAR + RBT + EMB + CIPRO | 1.1 ± 0.9 | | | 15 |
| 19 | RBT + EMB + AZI low + AMIK | 1.1 ± 0.8 | | | 16 |
| 20 | RBT + CLOF + CIPRO + AMIK | 1.2 ± 0.7 | | | 17 |
| 21 | RBT + AZI high + CIPRO + AMIK | 1.2 ± 0.7 | | | 18 |
| 22 | CLAR + RBT + EMB ^c | 1.2 ± 1.1 | | 3 | |
| 23 | AZI low + CIPRO + AMIK | 1.2 ± 0.5 | | 4 | |
| 24 | EMB + AZI high + CIPRO + AMIK | 1.3 ± 0.7 | | | 19 |
| 25 | CLAR + EMB + AZI low + AMIK | 1.4 ± 1.2 | | | 20 |
| 26 | CLAR + AZI high + CIPRO + AMIK | 1.4 ± 1.1 | | | 21 |
| 27 | CLAR + EMB + AZI high + AMIK | 1.4 ± 1.0 | | | 22 |
| 28 | CLAR + CIPRO + AMIK | 1.6 ± 1.0 | | 5 | |
| 29 | CLAR + CLOF + AZI high + AMIK | 1.6 ± 0.6 | | | 23 |
| 30 | CLAR + CLOF + EMB + AZI high | 1.7 ± 0.8 | | | 24 |
| 31 | CLAR + CLOF + CIPRO + AMIK | 1.7 ± 0.7 | | | 25 |
| 32 | CLAR + EMB + AMIK | 1.8 ± 1.0 | | 6 | |
| 33 | CLOF + EMB + AZI high + AMIK | 1.8 ± 0.7 | | | 26 |
| 34 | CLAR + RBT + CLOF + AMIK | 1.9 ± 0.8 | | | 27 |
| 35 | CLAR + RBT + CLOF + AZI high | 2.0 ± 0.8 | | | 28 |
| 36 | RBT + EMB + AMIK | 2.1 ± 1.2 | | 7 | |
| 37 | CLAR + RBT + CLOF + CIPRO | 2.1 ± 1.0 | | | 29 |
| 38 | RBT + CLOF + EMB + AMIK | 2.1 ± 1.2 | | | 30 |
| 39 | CLAR + AZI high + AMIK | 2.2 ± 1.0 | | 8 | |
| 40 | CLAR + RBT + AZI high | 2.4 ± 1.2 | | 9 | |
| 41 | AZI high + CIPRO + AMIK | 2.4 ± 1.0 | | 10 | |
| 42 | CLOF + AZI low + CIPRO + AMIK | 2.5 ± 1.4 | | | 31 |
| 43 | CLOF + EMB + AMIK | 2.7 ± 1.5 | | 11 | |
| 44 | CLAR + CLOF + AZI low + AMIK | 3.1 ± 1.0 | | | 32 |
| 45 | RBT + CIPRO + AMIK | 3.1 ± 2.2 | | 12 | |
| 46 | CLAR + CLOF + AZI high + CIPRO | 3.2 ± 1.4 | | | 33 |
| 47 | CLAR + CLOF + EMB + CIPRO | 3.5 ± 2.0 | | | 34 |
| 48 | RBT + AZI low + AMIK | 3.6 ± 1.5 | | 13 | |
| 49 | CLOF + AZI high + CIPRO + AMIK | 3.6 ± 1.8 | | | 35 |
| 50 | CLAR + CLOF + AMIK | 3.7 ± 1.1 | | 14 | |
| 51 | RBT + CLOF + AZI high + AMIK | 3.8 ± 1.1 | | | 36 |
| 52 | CLAR + EMB + CIPRO + AMIK | 4.1 ± 2.8 | | | 37 |
| 53 | CLOF + EMB + CIPRO + AMIK | 4.3 ± 3.9 | | | 38 |
| 54 | RBT + EMB + AZI high + CIPRO | 4.3 ± 4.2 | | | 39 |
| 55 | CLOF + CIPRO + AMIK | 4.5 ± 1.7 | | 15 | |
| 56 | CLAR + AZI low + AMIK | 4.5 ± 1.5 | | 16 | |
| 57 | RBT + AMIK | 4.5 ± 1.7 | 2 | | |
| 58 | CLAR + RBT + CIPRO ^c | 4.7 ± 4.0 | | 17 | |
| 59 | CLAR + EMB + AZI high + CIPRO | 4.7 ± 2.7 | | | 40 |
| 60 | CLAR + RBT + AZI low + CIPRO | 4.8 ± 3.9 | | | 41 |
| 61 | CLOF + EMB + AZI low + AMIK | 4.8 ± 2.3 | | | 42 |
| 62 | CLAR + CLOF + EMB + AZI low | 5.0 ± 2.5 | | | 43 |
| 63 | RBT + CLOF + EMB + AZI low | 5.1 ± 3.0 | | | 44 |
| 64 | RBT + EMB + AZI high ^c | 5.1 ± 4.2 | | 18 | |
| 65 | CLAR + CLOF + AZI high | 5.7 ± 1.6 | | 19 | |
| 66 | CLAR + RBT + CLOF ^c | 6.3 ± 2.3 | | 20 | |
| 67 | CLAR + RBT + CLOF + AZI low | 6.6 ± 2.6 | | | 45 |

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TABLE 1—Continued

| Overall rank | Drug combination ^a | Mean survival ± SE (%) ^b | Category rank for combinations containing: | | |
|--------------|--------------------------------|-------------------------------------|--|---------|---------|
| | | | 2 drugs | 3 drugs | 4 drugs |
| 68 | RBT + CLOF + AMIK | 6.9 ± 2.2 | | 21 | |
| 69 | CLAR + CLOF + EMB ^c | 7.0 ± 3.5 | | 22 | |
| 70 | RBT + CLOF + AZI low + AMIK | 7.2 ± 1.8 | | | 46 |
| 71 | RBT + CLOF + AZI high + CIPRO | 7.2 ± 5.8 | | | 47 |
| 72 | RBT + AZI high + CIPRO | 7.9 ± 4.4 | | 23 | |
| 73 | CLAR + RBT + AZI low | 9.1 ± 4.0 | | 24 | |
| 74 | CLOF + AZI high + AMIK | 9.5 ± 2.6 | | 25 | |
| 75 | CLOF + AZI low + AMIK | 10.8 ± 2.8 | | 26 | |
| 76 | RBT + CLOF + EMB + CIPRO | 11.1 ± 9.9 | | | 48 |
| 77 | RBT + AZI high + AMIK | 11.1 ± 8.6 | | 27 | |
| 78 | RBT + CLOF + AZI high | 11.3 ± 3.9 | | 28 | |
| 79 | CLAR + RBT ^c | 11.5 ± 4.9 | 3 | | |
| 80 | CLAR + AZI low + CIPRO + AMIK | 11.8 ± 9.9 | | | 49 |
| 81 | CLAR + CLOF + CIPRO | 12.1 ± 5.5 | | 29 | |
| 82 | RBT + EMB + CIPRO | 12.4 ± 9.9 | | 30 | |
| 83 | RBT + EMB + AZI low + CIPRO | 12.5 ± 9.9 | | | 50 |
| 84 | CLOF + EMB + AZI low + CIPRO | 12.6 ± 9.8 | | | 51 |
| 85 | EMB + AZI high + AMIK | 12.8 ± 9.5 | | 31 | |
| 86 | CLOF + EMB + AZI high + CIPRO | 12.8 ± 9.7 | | | 52 |
| 87 | CLOF + EMB + CIPRO | 13.2 ± 9.8 | | 32 | |
| 88 | CLAR + EMB + AZI high | 13.5 ± 9.8 | | 33 | |
| 89 | CLAR + EMB + AZI low + CIPRO | 13.5 ± 9.8 | | | 53 |
| 90 | CLAR + AMIK | 13.6 ± 9.7 | 4 | | |
| 91 | RBT + EMB ^c | 13.9 ± 9.8 | 5 | | |
| 92 | CLAR + EMB + CIPRO | 14.0 ± 9.8 | | 34 | |
| 93 | RBT + CLOF + AZI low + CIPRO | 14.2 ± 9.6* | | | 54 |
| 94 | CLOF + AMIK | 14.7 ± 3.6* | 6 | | |
| 95 | CLAR + EMB + AZI low | 14.9 ± 9.7* | | 35 | |
| 96 | EMB + AZI low + CIPRO | 15.2 ± 9.8 | | 36 | |
| 97 | CLAR + CLOF + AZI low + CIPRO | 16.0 ± 9.7* | | | 55 |
| 98 | RBT + CLOF + EMB | 16.6 ± 10.1* | | 37 | |
| 99 | RBT + CLOF + CIPRO | 17.6 ± 9.5* | | 38 | |
| 100 | CLOF + AZI high + CIPRO | 18.1 ± 9.6* | | 39 | |
| 101 | EMB + AZI low + AMIK | 18.3 ± 11.5 | | 40 | |
| 102 | EMB + CIPRO ^c | 18.5 ± 10.9 | 7 | | |
| 103 | CLAR + CLOF + AZI low | 19.8 ± 7.3* | | 41 | |
| 104 | RBT + EMB + AZI low | 20.8 ± 13.2* | | 42 | |
| 105 | EMB + AMIK | 20.8 ± 11.2* | 8 | | |
| 106 | AZI high + AMIK | 21.5 ± 9.5* | 9 | | |
| 107 | CLAR + AZI high + CIPRO | 21.6 ± 13.1* | | 43 | |
| 108 | RBT + AZI low + CIPRO | 21.8 ± 13.1* | | 44 | |
| 109 | RBT + CIPRO ^c | 23.3 ± 12.9* | 10 | | |
| 110 | CLAR + CLOF ^c | 23.6 ± 7.1* | 11 | | |
| 111 | EMB + AZI high + CIPRO | 25.0 ± 12.7* | | 45 | |
| 112 | CLOF + EMB + AZI high | 26.3 ± 12.4* | | 46 | |
| 113 | CLAR + EMB ^c | 28.9 ± 13.4* | 12 | | |
| 114 | RBT + AZI high ^c | 33.4 ± 11.8* | 13 | | |
| 115 | CLAR + AZI high | 34.8 ± 13.8* | 14 | | |
| 116 | RBT + CLOF + AZI low | 35.8 ± 11.6* | | 47 | |
| 117 | AZI low + AMIK | 40.8 ± 14.2* | 15 | | |
| 118 | CLAR + CIPRO ^c | 42.9 ± 15.6* | 16 | | |
| 119 | CLOF + CIPRO | 45.2 ± 15.0* | 17 | | |
| 120 | CLAR + AZI low + CIPRO | 47.1 ± 15.1* | | 48 | |
| 121 | CLOF + AZI low + CIPRO | 49.2 ± 14.4* | | 49 | |
| 122 | AZI high + CIPRO | 50.6 ± 16.5* | 18 | | |
| 123 | RBT + CLOF | 51.5 ± 12.5* | 19 | | |
| 124 | CLOF + AZI high | 57.5 ± 14.1* | 20 | | |
| 125 | EMB + AZI high | 57.6 ± 14.4* | 21 | | |
| 126 | CLAR + AZI low | 59.6 ± 15.7* | 22 | | |
| 127 | AZI low + CIPRO | 60.2 ± 15.9* | 23 | | |
| 128 | RBT + AZI low | 64.1 ± 14.8* | 24 | | |
| 129 | CLOF + EMB + AZI low | 96.0 ± 4.0* | | 50 | |
| 130 | CLOF + EMB | 99.9 ± 0.1* | 25 | | |
| 131 | CLOF + AZI low | 100 ± 0.0* | 26 | | |
| 132 | EMB + AZI low | 100 ± 0.0* | 27 | | |

^a Abbreviations: AMIK, amikacin; AZI high, azithromycin (intracellular level); AZI low, azithromycin (level in serum); CIPRO, ciprofloxacin; CLAR, clarithromycin; CLOF, clofazimine; EMB, ethambutol; RBT, rifabutin.

^b *, Statistically significant value ($P < 0.05$).

^c Combination was tested in the J774 cell line.

TABLE 2. Drug combinations which demonstrated a high or modest level of activity against 90 or 100% of *M. avium* strains in broth^a

| Combination | Drug combination ^b yielding result | | | |
|-------------|---|----------------------------|--|--|
| | Decrease in CFU of $\geq 2 \log_{10}$ with: | | Decrease in CFU of $\geq 1 \log_{10}$ with: | |
| | 100% of strains | 90% of strains | 100% of strains | 90% of strains |
| Two drugs | None | None | 12 | None |
| Three drugs | None | 2, 22 | 2, 16, 23, 28, 32, 36, 39, 41, 50 | 22, 40, 43, 45, 48, 58, 64, 77 |
| Four drugs | 1, 4 | 3, 5, 6, 8, 10, 11, 18, 54 | 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 17, 18, 19, 20, 21, 24, 26, 27, 29, 30, 31, 33, 34, 35, 37, 38, 44, 51 | 25, 42, 46, 49, 53, 54, 60, 71, 76, 80, 84 |

^a A high level is a decrease of $\geq 2 \log_{10}$ CFU; a modest level is a decrease of $\geq 1 \log_{10}$ CFU.

^b Drug combinations are represented by their overall rank numbers, given in Table 1.

Figure 1 shows the relative susceptibilities of the 10 MAC strains, listed in order of increasing mean survival, to combinations containing two, three, or four drugs. The 10 strains exhibited a wide range of survival means (16.9 to 69.7%) when exposed to combinations containing two drugs, and there was a gradual increase in resistance from the most susceptible strain to the most resistant strain. In contrast, when strains were exposed to combinations containing three or four drugs, variability in susceptibility was confined primarily to the two most resistant strains (strains 1 and 8). The survival means for strains 1 and 8 were significantly higher than the means for all other strains tested with combinations containing three or four drugs.

The earlier finding (Table 1) that the means for most drug combinations were not significantly different is understandable in light of the observation that there is significant variability among MAC strains. This observation indicated that the combinations in Table 1 should be reevaluated by taking into account differences among strains. All drug combinations were therefore evaluated on the basis of the percentage of strains considered susceptible to each combination. To accomplish this, it was necessary to define the term "susceptible."

Interpretation of broth susceptibility test results. Since interpretative criteria for antimicrobial susceptibility test results have not yet been established for MAC, the proportion of MAC strains considered susceptible to a drug combination is dependent upon the definition of susceptibility applied. Table 2 shows that if a relatively stringent definition is applied (a decrease in CFU of $\geq 2 \log_{10}$), no two- or three-drug combination was active against all 10 strains of MAC in broth and only two four-drug combinations were active against all 10 strains. If a less stringent requirement is applied (CFU decrease of $\geq 1 \log_{10}$), 1 two-drug combination, 9 three-drug combinations, and 31 four-drug combinations could be considered active against all 10 strains.

Intracellular activities of drug combinations. An additional factor in testing the activities of combinations of drugs is their behavior against intracellular MAC organisms. A limited number of the most active two-, three-, and four-drug combinations was therefore selected for testing against MAC organisms inside cells of the macrophage-derived cell line J774. Several factors were taken into consideration in deciding which drug combinations to test in J774 cells: (i) on the basis of the assumption that an oral regimen for treatment of MAC is the most desirable, a minimum number of combinations containing amikacin were tested in J774 cells (only the single most active two-, three-, and four-drug combinations which contained amikacin as a component were tested); (ii) drug combinations which contained both of the macrolide antibiotics clarithromycin and azithromycin were excluded; and (iii) be-

cause of therapeutic considerations, such as drug toxicity, adverse side effects, and patient compliance, a greater number of two-drug combinations than three- or four-drug combinations were tested in J774 cells.

With the application of the above-listed criteria and exclusions, the nine most active two-drug combinations, the six most active three-drug combinations, and the three most active four-drug combinations in Table 1 were tested for activity against each of the 10 MAC strains in J774 cells. The results of these tests are shown in Table 3. For comparison, the relative rank for the same combination tested in broth is also shown. When combinations were ranked in order of mean survival in J774 cells, means for the top 11 combinations were not significantly different ($P > 0.05$). The top 11 combinations included all of the four-drug combinations, all of the three-drug combinations, and two of the nine two-drug combinations tested.

The rank of drug combinations tested in J774 cells was usually close to the rank of the same combination tested in broth. Exceptions to this were combinations which contained either amikacin or clofazimine. All combinations which contained amikacin had a poorer rank in J774 cells than in broth, and mean survival values were 78 to 97 times higher in J774

TABLE 3. Activities and ranks of 18 drug combinations tested against 10 strains of *M. avium* in J774 cells

| Drug combination ^a | Mean survival \pm SE (%) ^b | Rank in J774 cells | Rank in broth ^c |
|-------------------------------|---|--------------------|----------------------------|
| CLAR + RBT + CLOF + EMB | 2.8 \pm 1.4 A | 1 | 3 |
| CLAR + RBT + EMB + AMIK | 2.9 \pm 0.9 A | 2 | 1 |
| CLAR + RBT + CLOF | 4.9 \pm 1.7 A | 3 | 8 |
| RBT + CLOF + EMB + AZI | 5.0 \pm 1.7 A | 4 | 7 |
| CLAR + RBT + CIPRO | 6.7 \pm 1.9 AB | 5 | 6 |
| CLAR + RBT + EMB | 6.8 \pm 2.5 AB | 6 | 5 |
| CLAR + CLOF + EMB | 7.7 \pm 2.4 AB | 7 | 9 |
| CLAR + CLOF | 8.9 \pm 3.0 AB | 8 | 15 |
| CLAR + RBT | 9.2 \pm 3.1 AB | 9 | 10 |
| RBT + EMB + AZI | 15.6 \pm 7.0 ABC | 10 | 13 |
| EMB + CIPRO + AMIK | 16.1 \pm 6.7 ABC | 11 | 2 |
| CLAR + EMB | 21.0 \pm 4.6 BC | 12 | 16 |
| CLAR + CIPRO | 28.1 \pm 8.3 C | 13 | 17 |
| RBT + EMB | 31.1 \pm 4.5 CD | 14 | 11 |
| RBT + AZI | 32.3 \pm 7.3 CD | 15 | 18 |
| EMB + CIPRO | 46.0 \pm 14.5 DE | 16 | 12 |
| CIPRO + AMIK | 46.7 \pm 10.1 DE | 17 | 4 |
| RBT + CIPRO | 51.3 \pm 10.6 E | 18 | 14 |

^a Abbreviations are the same as in Table 1.

^b The same letter is placed next to combinations whose means were not significantly different ($P > 0.05$).

^c Rank in this subset of 18 combinations. See Table 1 for overall rank in broth.

cells than in broth (Table 1). In contrast, all combinations which contained clofazimine had a more favorable rank in J774 cells than in broth, with the combination of clarithromycin plus clofazimine showing the greatest difference.

DISCUSSION

A statistical analysis of 132 different combinations of drugs commonly suggested for use in the treatment of disseminated MAC infection revealed that 95 combinations were statistically equivalent whereas 37 combinations yielded survival means that were significantly higher. Although most of the poorest combinations (21 of the 37) were two-drug combinations, 14 were three-drug combinations and 2 were four-drug combinations.

Of interest was the finding that amikacin was significantly more active than each of the other drugs when tested against MAC organisms in broth but was poorly active against intracellular organisms. On the other hand, combinations containing clofazimine were more active against MAC organisms in macrophages than in broth. The clinical significance of these findings is unknown. However, since it is unclear whether MAC is exclusively or only intermittently intracellular in vivo, these findings may have clinical implications.

The finding that MAC strains differ significantly in their susceptibilities to drug combinations complicated the analysis of the mean survival data. Although it is well documented that MAC strains differ in susceptibility to single drugs, it was hoped that combinations of drugs which were active against all MAC strains could be identified. Significant differences among strains were observed most commonly when combinations containing two drugs were tested. However, even with three- and four-drug combinations, two of the MAC strains were found to be significantly more resistant ($P < 0.05$) than all other strains. These findings could have clinical implications for therapy of MAC infections.

One of the difficulties of assessing the potential clinical utility of drug combinations against MAC is the lack of interpretive criteria for in vitro susceptibility tests. When a relatively stringent definition (≥ 2 -log decrease in CFU) was applied, only two (four-drug) combinations were considered active against all 10 strains of MAC tested in broth. At present, it is not known whether such a high level of in vitro activity is necessary to achieve microbiologic efficacy in MAC-infected patients with AIDS. If a less stringent definition of activity is applied (≥ 1 -log decrease in CFU), one two-drug combination, several three-drug combinations, and numerous four-drug combinations could be considered active against all of the MAC strains included in the study. Overall, with the exclusion of drug combinations that contained amikacin, combinations (numbered by ranks shown in Table 1) 22 (clarithromycin plus rifabutin plus ethambutol) and 8 (clarithromycin plus rifabutin plus clofazimine plus ethambutol) were the most widely active three- and four-drug combinations in broth.

This study demonstrated that MAC strains differ significantly in their susceptibilities to drug combinations, and it identified combinations which are widely active against MAC strains in vitro. In vitro drug combination susceptibility tests can be used to identify MAC isolates that are relatively susceptible or relatively resistant to drug combinations. If supported by clinical data, such an approach could aid in minimizing the number of drugs used against susceptible isolates, thereby helping to reduce adverse drug reactions, improve patient compliance, and reduce the cost of therapy. Con-

versely, the identification of isolates that are highly resistant may signal the need for more aggressive therapy.

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