

Amikacin, Ciprofloxacin, and Imipenem Treatment for Disseminated *Mycobacterium avium* Complex Infection of Beige Mice

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The *Mycobacterium avium* complex (MAC) is a common cause of disseminated infection in patients with acquired immunodeficiency syndrome and is increasingly seen as a cause of infection in other immunocompromised patients. Traditional antimycobacterial therapy often is ineffective, and there is a clear need for antibiotics with proven activity against the MAC. Three agents, amikacin, ciprofloxacin, and imipenem, were tested *in vitro* for activity against MAC strain 101. Amikacin was bacteriostatic, with an MIC of 4.8 µg/ml, which is significantly lower than the concentration in serum obtained with standard dosing. Imipenem and ciprofloxacin had little or no activity alone (MICs, >16 and 4.7 µg/ml, respectively), but when they were combined with amikacin there was bactericidal activity. Each agent was tested individually and in combination by using the beige mouse model of disseminated MAC infection. There was no mortality in a group of animals infected with MAC 101 and treated with amikacin alone; also, there was a significant decrease in the infection of the blood, liver, and spleen. There was no apparent improvement in therapeutic effectiveness when amikacin was combined with the other agents. Neither ciprofloxacin nor imipenem was active as a single agent, which was consistent with the *in vitro* activities of these agents. Amikacin in combination with traditional antimycobacterial agents warrants further study as potential therapy for disseminated MAC infections.

Disseminated *Mycobacterium avium* complex (MAC) infections are the most common bacterial infections seen in patients with the acquired immunodeficiency syndrome (AIDS) (1, 12, 20). Remarkably, the increased incidence of MAC infections associated with AIDS is paralleled by an increased incidence of infection in non-AIDS patients (1, 10; R. C. Good, Clin. Microbiol. Newsl. 1:1-4, 1979). Traditional antimycobacterial therapy for *M. tuberculosis*-caused disease is not effective for the treatment of MAC infections; certainly, no single agent, such as isoniazid or rifampin, is as potent against the MAC as against *M. tuberculosis* (16; R. C. Good, V. A. Silcox, J. C. Kilburn, and B. D. Plikaytis, Clin. Microbiol. Newsl. 7:133-136, 1986). When therapy for MAC infection is attempted, a combination of five or six antimycobacterial drugs is de rigueur (4, 6). Indeed, there is some evidence that combinations of antimycobacterial agents are synergistic against the MAC (21); however, in most cases the MAC isolates are resistant *in vitro* and the response to therapy is poor (14). There is a clear need for antimicrobial agents with proven effectiveness for treating MAC infections, especially in immunocompromised patients (18).

Amikacin has been shown previously to have *in vitro* activity against the MAC (2, 5, 15), and there is even some clinical evidence for improved therapy for MAC disease in AIDS patients when amikacin was used in combination with ethambutol and rifampin (2). Ciprofloxacin is a new fluorinated quinolone with broad-spectrum antimicrobial activity which has been reported to have good activity against *M. tuberculosis* (11, 19) but variable activity against the MAC (8, 13, 14, 17). Imipenem also is a broad-spectrum antimicrobial agent (carbapenem) with variable *in vitro* activity against the MAC (17). Here we report on the *in vitro* activity against the MAC and the *in vivo* effectiveness of these

agents alone and in combination using the beige mouse model of disseminated MAC infection (3, 9).

MATERIALS AND METHODS

Mice. Specific-pathogen-free, randomly selected female mice (4 to 6 weeks old) were used for all studies. These mice have the murine analog of the Chediak-Higashi syndrome in humans and are commonly referred to as beige mice (C57BL/6J-*bg^J/bg^J*; Jackson Laboratory, Bar Harbor, Maine). In certain pharmacokinetic experiments, coisogenic C57BL/6J-*bg^J* black mice were used (Jackson Laboratory).

Organism and culture conditions. MAC strain 101 (serotype 1), which was used in all of the experiments, was isolated from the blood of a patient with AIDS at the University of California at Los Angeles Medical Center. Mycobacteria were grown on Middlebrook 7H11 agar medium (Difco Laboratories, Detroit, Mich.) supplemented with oleic acid, albumin, dextrose, and catalase (OADC; Difco Laboratories). Only irregularly shaped, flat, and transparent colonies were collected and frozen at -70°C in Middlebrook 7H9 broth (Difco Laboratories) supplemented with 10% (wt/vol) glycerol and stored as a primary stock. The virulence of MAC 101 decreases with time, but periodic (every 3 months) passage through beige mice restored virulence.

Antimicrobial agents. Amikacin (Bristol Laboratories, Syracuse, N.Y.), ciprofloxacin (Miles Laboratories, Inc., Elkhart, Ind.), imipenem (Merck & Co., Inc., Rahway, N.J.), and cefamandole (Eli Lilly & Co., Indianapolis, Ind.) were provided by the manufacturers. The antimicrobial agents were dissolved and diluted in sterile distilled water.

***In vitro* susceptibility testing.** Discrete MICs were determined by using a broth radiometric procedure and the T100 method of datum analysis (13). A standard inoculum of 10⁴ CFU was used for determination of MICs. Samples (0.1 ml) of the broth cultures (BACTEC vials) were inoculated onto

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Middlebrook 7H11 agar plates and incubated for 2 to 3 weeks at 37°C in air to determine bactericidal activity; the endpoint was no growth. The practical sensitivity of this assay limits the bactericidal determination to a 99% endpoint rather than the more conventional 99.9% endpoint used for MBC determinations.

Levels of agents tested in serum. Peak levels of antimicrobial agents in the sera of uninfected C57BL/6J-*bg*^j black mice were determined by bioassay (imipenem and ciprofloxacin) or polarized fluorescence immunoassay (amikacin) by standard procedures (7).

Therapeutic trials. The beige mouse model of disseminated MAC infection has been described in detail previously (3, 9). Mice were infected by tail vein inoculation of 10⁷ CFU, and treatment with the various regimens was initiated 7 days after infection. Adjustments were made in the dosing schedule to account for mouse pharmacokinetics in an effort to parallel the pharmacokinetics of these agents in humans. Amikacin was administered with an osmotic infusion pump (Alza Corp., Palo Alto, Calif.); cefamandole, ciprofloxacin, and imipenem were given as subcutaneous injections. Control animals were infected and treated with only cefamandole to prevent gram-negative sepsis and to parallel the somewhat traumatic subcutaneous therapy with ciprofloxacin and imipenem. Treatment was continued until the death of the animal or until the end of the experiment (4 weeks). The cefamandole control group represents the results of three separate experiments, and the three-agent treatment group represents the results of two experiments. All three treatment groups and both control groups were tested simultaneously, except for the aforementioned repeat experiments. The total number of animals in each group was at least 20 (see Results).

Quantities of mycobacteria in organs and blood. Livers and spleens of mice were removed aseptically at the time of death or at the end of the experiment. Organs were weighed, and Middlebrook 7H9 broth was added to yield an approximately 10% (wt/vol) suspension. After homogenization with a handheld glass homogenizer, serial 10-fold dilutions were plated in duplicate on Middlebrook 7H11 agar supplemented with OADC. After incubation for 7 to 14 days at 37°C in air, colonies were counted and the number of CFU per gram of tissue was calculated for each organ. The organ weight was calculated as a function of total body weight. Blood was collected twice (1 week after infection and at the end of the experiment or on the death of the animal) by tail vein bleeding, and 0.1 ml was inoculated into 4 ml of BACTEC 12B radiometric medium (Johnston Laboratories, Inc., Towson, Md.). The quantity of bacteria present in the blood was determined by the T100 method as previously described (13). Briefly, 0.1 ml of blood was inoculated directly into BACTEC vials, incubated, and read daily with the BACTEC 460 instrument. A T100 value was determined for the blood sample and related to a standard curve of CFU per milliliter versus T100; extrapolation from the curve yielded a CFU per milliliter value. Blood was sampled 1 week after infection and at the end of the experiment; an attempt was made to culture the blood at the time of death of the animal, but often this was not feasible.

Statistical analysis. The paired *t* test and Fisher's exact test were used to evaluate the data.

RESULTS

In vitro antibiotic susceptibility. The MICs for MAC 101 were as follows: amikacin, 4.8 µg/ml; ciprofloxacin, 4.7

TABLE 1. Doses and peak concentrations of amikacin, ciprofloxacin, and imipenem in the sera of C57 black mice

Agent	Dose (mg/kg)	Route ^a	Level in serum (µg/ml)
Amikacin	220	Infusion	22
Ciprofloxacin	40	Subcutaneous injection	4
Imipenem	75	Subcutaneous injection	48

^a Infusion was continuous, and injections were given every 8 h.

µg/ml; imipenem, >16 µg/ml; cefamandole, >32 µg/ml. The activities of two- and three-agent combinations were tested radiometrically and by plating onto agar medium. The effect of all three agents in combination was assessed by measuring the bactericidal activity. A combination of amikacin (4 µg/ml), ciprofloxacin (4 µg/ml), and imipenem (8 µg/ml) killed 87% of a population of 10⁴ CFU of MAC 101. The concentrations of agents used in the bactericidal assay were based on achievable levels in serum or MICs.

Doses and peak levels in serum. The doses and peak levels in serum of amikacin, ciprofloxacin, and imipenem with cilastatin administered to C57BL/6J-*bg*^j black mice are shown in Table 1. Amikacin was maintained at a constant level of 22 µg/ml by an osmotic infusion pump implanted under the skin of the mice.

Effect of therapy on infection of beige mice. The three previously described treatment regimens were evaluated by using the dosing schedules shown in Table 1. Therapeutic effectiveness was assessed by mortality and quantitative culture of the blood, spleen, and liver for mycobacteria. The results were compared with those of two types of control groups. One group was infected and treated only with cefamandole for the duration of the experiment or until the death of the animal. A second group of control animals was infected, and the blood, spleen, and liver were cultured 1 week after infection. This latter group received no antimicrobial therapy, and the average CFU per gram or milliliter in these mice represented the level of infection when therapy was initiated.

The mortality, in terms of percent survival, for each treatment regimen is shown in Table 2 and Fig. 1. In the amikacin-treated group, there was no mortality and in the combined-therapy (amikacin-ciprofloxacin-imipenem)-treated group there was 18% mortality; these differences were statistically significant (*P* < 0.001 and *P* < 0.01, respectively) for both groups when compared with the 4-week control group. There was no significant difference between the mortality in the group treated with ciprofloxacin (*P* > 0.1) or imipenem (*P* = 0.056) alone and that in the untreated control group.

From 1 to 4 weeks after infection, there was an increase that was significant but less than 1 order of magnitude in the

TABLE 2. Effects of various treatment regimens on the mortality of beige mice infected (10⁷ CFU per animal) with MAC 101

Regimen	No. of animals	% Survival	<i>P</i> value ^a
Amikacin	23	100	<0.001
Ciprofloxacin	20	45	>0.1
Imipenem	20	30	>0.05
Amikacin-ciprofloxacin-imipenem	44	82	<0.01
Control	52	63	NA

^a *P* values are relative to the untreated 4-week control group. NA, Not applicable.

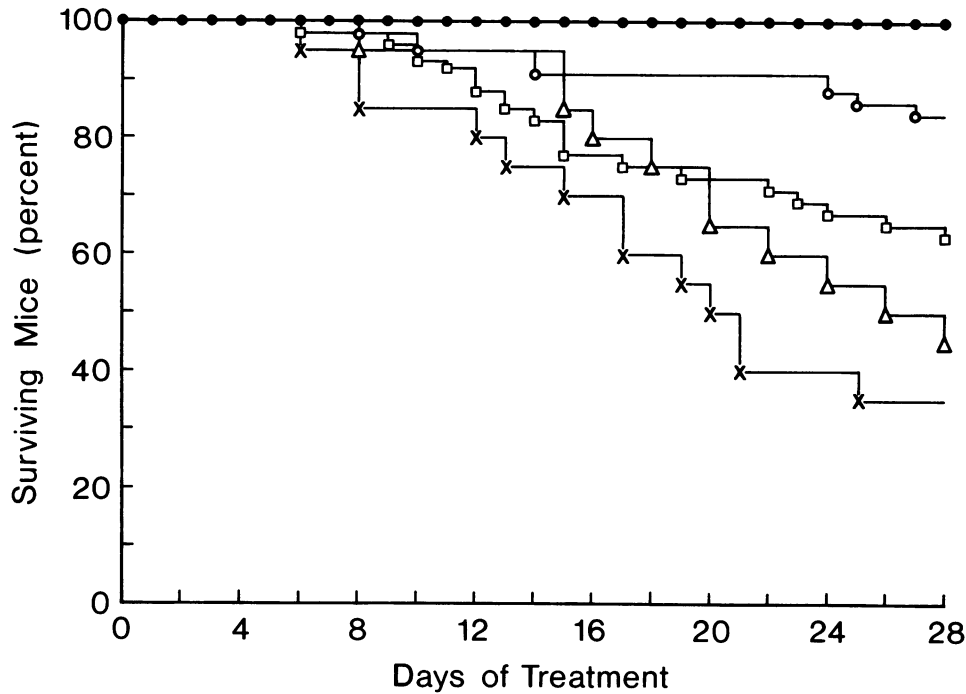


FIG. 1. Percent survival of beige mice infected with the MAC (10^7 CFU per mouse) over a 4-week period without treatment (□) or with treatment with amikacin (●), amikacin-ciprofloxacin-imipenem (○), ciprofloxacin (△), or imipenem (×).

average level of infection of liver and spleen (Fig. 2 and Table 3). Treatment with amikacin resulted in a decrease of nearly 3 orders of magnitude in liver CFU per gram and a decrease of 2 orders of magnitude in spleen CFU per gram. Amikacin-imipenem-ciprofloxacin resulted in a similar decrease in CFU per gram. There was little or no difference between treatment with imipenem or ciprofloxacin alone and no treatment of tissue infection (Fig. 2). The effects of the various treatment regimens on organomegaly were similar to

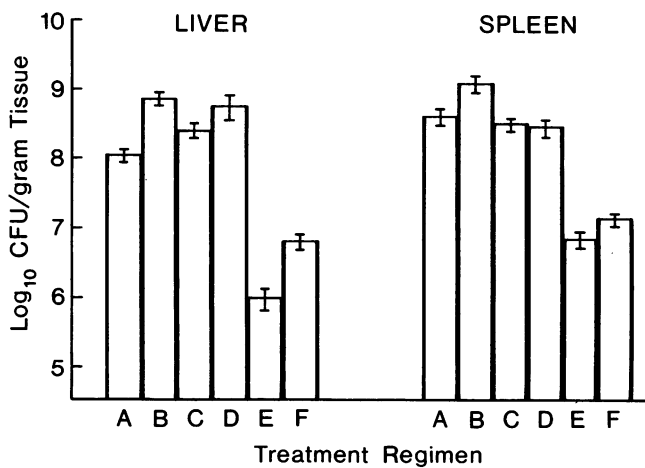


FIG. 2. Effects of various therapeutic regimens on the mean numbers of mycobacteria in the livers and spleens of beige mice infected with the MAC (10^7 CFU per mouse) over a 4-week period. The bars show the levels of infection at the start of therapy (A), with no treatment (B), with imipenem (C), with ciprofloxacin (D), with amikacin (E), and with amikacin-ciprofloxacin-imipenem (F). Two-directional error bars indicate the standard errors of the means.

the effects on CFU per gram (Fig. 3). Amikacin, alone or in combination, resulted in a significant decrease in hepatosplenomegaly, and there was little or no difference between untreated animals and those treated with imipenem or ciprofloxacin alone. The average change in the log CFU of mycobacteria per milliliter of blood is shown in Fig. 4. In the untreated group of animals, bacteremia increased by 0.5 order of magnitude. There was no significant effect of imipenem alone, whereas there was a significant decrease in septicemia with either ciprofloxacin or amikacin alone, and the effects of these two regimens were not significantly different from one another. Bacteremia decreased by 1.5 orders of magnitude in mice treated with amikacin-imipenem-ciprofloxacin, and this decrease was significantly different from the level of bacteremia in untreated animals and animals treated with any of the other regimens.

TABLE 3. Effects of various treatment regimens on the numbers of mycobacteria in the livers and spleens of beige mice infected (10^7 CFU per animal) with MAC 101

Regimen	CFU/g in:		P value (liver/spleen) ^a
	Liver	Spleen	
1-wk control	1.1×10^8	4.1×10^8	NA/NA
Untreated 4-wk control	7.4×10^8	1.2×10^9	NA/NA
Amikacin	1.0×10^6	6.9×10^6	<0.001/<0.001
Ciprofloxacin	5.6×10^8	8.8×10^8	NS/NS
Imipenem	2.6×10^8	3.1×10^8	<0.01/<0.01
Amikacin-ciprofloxacin-imipenem	6.5×10^6	1.4×10^7	<0.001/<0.001

^a The P values are relative to the untreated 4-week control group. NA, Not applicable; NS, not significant.

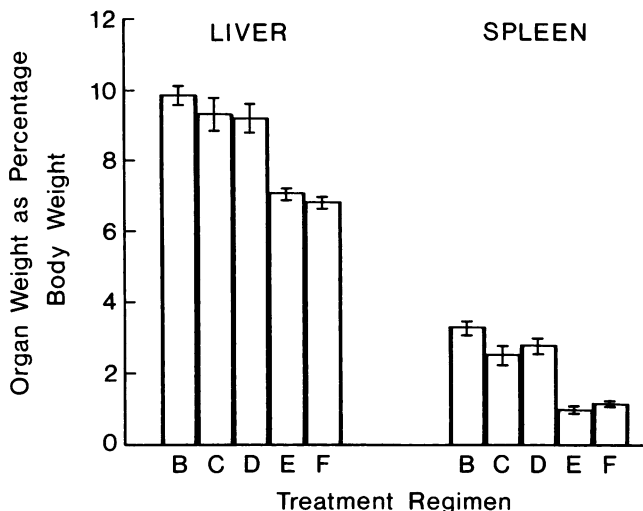


FIG. 3. Effects of various therapeutic regimens on hepatosplenomegaly expressed as the mean organ weight as a percentage of total body weight in beige mice infected with the MAC (10^7 CFU per mouse) over a 4-week period. The bars show results obtained with no treatment (B), imipenem (C), ciprofloxacin (D), amikacin (E), and amikacin-ciprofloxacin-imipenem (F). Two-directional error bars indicate the standard errors of the means.

DISCUSSION

Several interesting observations emerged from this study. (i) The nature of the infection in this animal model of disseminated MAC disease can be characterized as more chronic than acute. Indeed, the level of infection in terms of the number of mycobacteria per gram of liver or spleen and per milliliter of blood did not appreciably increase from 1 to 4 weeks after infection. As shown here, an effective regimen, such as amikacin, resulted in improved survival, which correlated with a significant decrease in the number of mycobacteria per milliliter of blood or gram of tissue. (ii) In this study, there was good correlation of in vitro and in vivo

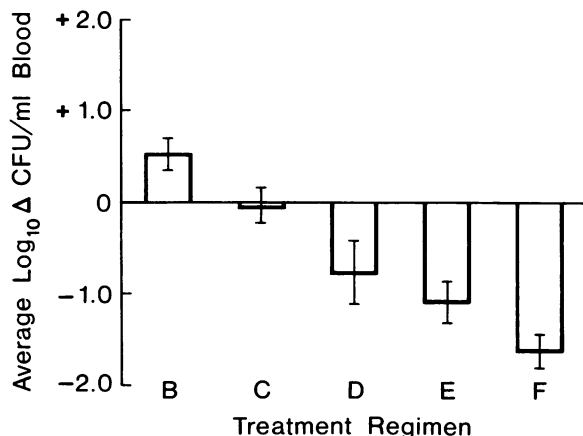


FIG. 4. Effects of various therapeutic regimens on bacteremia expressed as the mean log change in CFU per milliliter of blood in beige mice infected with the MAC (10^7 CFU per mouse) over a 4-week period. The bars show results obtained with no treatment (B), imipenem (C), ciprofloxacin (D), amikacin (E), and amikacin-ciprofloxacin-imipenem (F). Two-directional error bars indicate the standard errors of the means.

results. MAC 101 was resistant to imipenem and moderately susceptible to ciprofloxacin. Single-drug trials with these agents failed to decrease the number of mycobacteria in blood or tissues, and mortality was high in these treatment groups. (iii) Amikacin alone was an effective therapeutic agent. When amikacin was combined with ciprofloxacin and imipenem, there was no clear improvement in therapeutic effectiveness, although there was a significant decrease in bacteremia in the three-drug-treated group compared with the groups treated by the other regimens. It may be significant that amikacin was administered in a manner (osmotic infusion pump) that maintained high peak levels in serum continuously. Although this mode of administration is uncommon for treating humans, continuous infusion is feasible. Recently, we have shown that once-a-day dosing with amikacin by subcutaneous injection is equally efficacious (P. T. Kolonoski, M. L. Petrofsky, J. Cogger, M. Wu, C. B. Inderlied, L. S. Young, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1416, 1988). These results support our earlier observations that amikacin can contribute to the therapeutic effectiveness of a traditional antimycobacterial regimen, such as ethambutol and rifampin, for disseminated MAC disease (2, 18). The objective of antimicrobial therapy for a chronic infection such as disseminated MAC infection in a severely immunocompromised host would be to achieve bactericidal activity and long-term suppression. Therefore, it is important to identify synergism between amikacin and other antimicrobial agents which results in significant bactericidal activity both in vitro and in vivo.

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