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In Vitro and Ex Vivo Activities of Antimicrobial Agents Used in Combination with Clarithromycin, with or without Amikacin, against *Mycobacterium avium*

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MICs of clarithromycin, amikacin, isoniazid, rifabutin, ciprofloxacin, sparfloxacin, ethambutol, and clofazimine were determined for six isolates of *Mycobacterium avium* complex (MAC) from AIDS patients both by the radiometric method and by an ex vivo model of infection in human macrophages. The median MICs in macrophages were similar or slightly lower than values found in broth, except for amikacin, which had slightly higher MICs inside the cells. Combinations of clarithromycin with other antimicrobial agents showed that clarithromycin-clofazimine and clarithromycin-rifabutin were synergistic on five of six strains while clarithromycin-amikacin and clarithromycin-isoniazid were antagonistic on one and two strains, respectively. The addition of amikacin made the combinations of clarithromycin-clofazimine and clarithromycin-ethambutol synergistic against all the MAC strains. In the macrophage model, the combination of clarithromycin-clofazimine (mean survival, 21%) and clarithromycin-rifabutin (mean survival, 29%) showed a strong reduction in viable counts compared with single drugs, while clarithromycin-amikacin was less active than single drugs alone. In general, the addition of amikacin did not improve the activity of the combinations, except for clarithromycin-isoniazid-amikacin (mean survival, 19%), which was significantly more active than either clarithromycin-isoniazid or clarithromycin-amikacin. The use of the macrophage model can suggest new combinations of antimicrobial agents with anti-MAC activity which, on the basis of their in vitro effectiveness, would probably be disregarded for assay in animal models.

Mycobacterium avium complex (MAC) infections are very common in AIDS patients, with an estimated prevalence of 43% in the 2 years following the diagnosis of AIDS (25). Therapy for MAC diseases usually involves the combination of several drugs, not only to obtain a greater antimycobacterial effect but also to decrease the probability of selecting resistant mutants (13, 15). In some studies, the efficacy of different antimicrobial combinations has been evaluated in vitro (12, 34), while in others the activity has been investigated with human macrophages (MP) (33, 35, 37) or in the murine MP cell line J774 (33, 35, 36). The Public Health Service Task Force on Prophylaxis and Therapy for MAC recently recommended clarithromycin (CLA)-containing regimens for therapy for disseminated infections by these organisms in AIDS patients (20). At the present time, only for a few reports has the activity of CLA-containing combinations been studied, by in vitro and ex vivo methods, on the same strains (27, 28).

In this study, we assessed the susceptibility of MAC strains isolated in Italy from AIDS patients (7) by determining the MICs of eight antimicrobial agents both by the radiometric method (11) and by a model of human monocyte-derived MP infection (22, 23). This was adopted to evaluate the relationship between the extracellular and intracellular activities of drugs with different capacities for accumulating within MP (31). Furthermore, we studied by the radiometric method the possible synergistic, additive, or antagonistic effects of combinations in which CLA was added to six different drugs, with or without further addition of amikacin (AMI), an antimicrobial agent known to be bactericidal in vitro against *M. avium* and used in therapy for MAC infections (17).

Finally, to verify whether further information on drug combination activity could be obtained in a model closer to that of intramacrophagic human infection, we tested in the MP model the same combinations assayed by the radiometric method by using drugs at concentrations achievable in serum.

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MATERIALS AND METHODS

Microorganisms. Six MAC strains (MAC 485, 486, 901, 902, 905, and 911, belonging to types 21, 21, 4, 3, 1, and 2, respectively) (7), isolated from the blood of AIDS patients and identified to the species level by RNA-DNA hybridization (Accuprobe; Gen Probe, San Diego, Calif.), were used throughout this study.

Only smooth transparent colonies, which are known to be associated with high virulence and resistance to antimicrobial agents and chemicals (21, 30, 32), were employed throughout this study. Colonies grown on Middlebrook 7H10 agar medium (Difco Laboratories, Detroit, Mich.) for 1 week were suspended in Middlebrook 7H9 broth (Difco) and stored at −40°C until use. A colony of MAC 485, representative of the morphology of the virulent colonial variant used for susceptibility testing, is shown in Fig. 1.

Antimicrobial agents. CLA (Abbott Laboratories, North Chicago, Ill.) and rifabutin (RFB) (Farmitalia-Carlo Erba, Milan, Italy) were dissolved in methanol; clofazimine (CLO) (Ciba-Geigy, Basel, Switzerland) was dissolved in acetic acid-dimethyl sulfoxide-distilled water (1:3:6); sparfloxacin (SPA) (Rhone Poulenc Rorer, Vitry-Alfortville, France) was dissolved in NaOH (0.1 N). The other agents, ciprofloxacin (CIP), isoniazid (INH), AMI, and ethambutol (EMB) were purchased from Sigma Chemical (St. Louis, Mo.) and dissolved in distilled water. Stock solutions (1 mg/ml) of these agents were stored at −80°C until use. From the stock solutions, working solutions were made by dilution with distilled water.

MIC determination by the radiometric method. The radiometric method used was essentially that of Heifets (11) with 7H12 liquid medium containing

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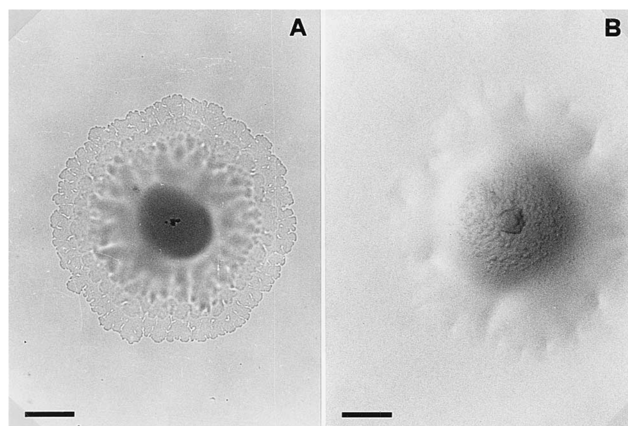

FIG. 1. Photomicrographs of an *M. avium* (strain 485) transparent colony grown on Middlebrook 7H10 agar, observed by inverted microscope (A) and by stereomicroscope (B). Bar = 0.1 mm.

[^{14}C]palmitate as carbon source. Bacterial growth resulting in the release of $^{14}CO_2$ was monitored by an automated BACTEC 460-TB apparatus (Johnston Laboratories, Towson, Md.) and expressed as a numerical value called growth index (GI) ranging from 0 to 999.

Each drug was added to the vials containing 4 ml of 7H12 medium in a volume of 0.1 ml per vial to achieve doubling concentrations from 0.004 to 16 μg/ml. A 7H12 broth vial was inoculated with bacteria from transparent colonies, and growth was recorded daily until it reached the maximum GI of 999. The culture was diluted 1:100, and 0.1 ml of this dilution was inoculated in the test vials and in one of the drug-free control vials. The inoculum provided an initial concentration of bacteria of 10^4 to 10^5 CFU/ml. Another drug-free control vial, the 1:100 control, was inoculated with a bacterial suspension 100 times lower to provide 10^2 to 10^3 CFU/ml, representing 1% of the bacterial population. The vials were incubated at 37°C, and the GI readings were recorded daily. The test was complete when the GI of 1:100 diluted control was greater than 20 for three consecutive days, while the growth in the undiluted control reached the maximum GI reading of 999, not earlier than the fourth day of cultivation. The MICs were defined as the lowest drug concentrations in the presence of which the final GI readings were no greater than 50 after 8 days of incubation.

Combined drug action by the radiometric method. The activity of two- and three-drug combinations was evaluated by adding the antimicrobial agents to the 7H12 broth vials so that each drug was present at concentrations corresponding to 1, 1/2, 1/4, 1/8, or 1/16 MIC in the two-drug combinations and to 1/2, 1/3, 1/4, 1/6, or 1/12 MIC in the three-drug combinations (Table 1). The fractional inhibitory concentration (FIC) (3, 12) was calculated as the ratio between the MIC of the drug in combination and the MIC of the drug alone.

The FIC index was defined as the sum of the FIC for each drug in combination. The breakpoints of FIC indices were as follows: for two drugs, ≤0.5 for synergism, 1 for additivity, and ≥2 for antagonism; for three drugs, ≤0.75 for synergism and ≤1.5 for additivity (3, 12, 34).

Preparation of human MP. Leukocyte buffy coats obtained from healthy donors were diluted 1:1.5 in 0.9% sodium chloride, and peripheral blood mononuclear cells were isolated by centrifugation with Ficoll-Hypaque (Histopaque 1077, Sigma) (5). Peripheral blood mononuclear cells were washed twice and resuspended in RPMI 1640 medium containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (Gibco Laboratories, Grand Island, N.Y.) supplemented with 2 mM L-glutamine and 10% heat-inactivated (56°C, 30 min) fetal calf serum (complete medium). Cell suspension was adjusted to 10^7/ml and distributed in 0.5-ml aliquots in 24-well tissue culture plates (Costar 3524; Costar, Cambridge, Mass.). After 2 h of incubation at 37°C under humidified 5% CO_2, the nonadherent cells were aspirated and the monocytes were washed extensively with warmed medium without antibiotics. After 4 to 5 days of incubation, the cultured cells showed MP morphology and more than 98% of them were able to ingest neutral red. MP viability, as determined by the trypan blue exclusion method, and numbers of MP per well, as assessed by the method of Nakagawara and Nathan (24), were recorded throughout the experimental period.

Establishment of MP infection. Bacteria used for MP infection were prepared by suspending transparent colonies in complete medium to an optical density of 0.2 at 500 nm. To decrease bacterial clumping, the suspensions were ultrasonicated for 10 s (Ultrasonicator Soniprep; MSE Scientific Instruments, Crawley, United Kingdom; 8-μm amplitude); after this treatment, CFU were about 5×10^8/ml. MP monolayers (2×10^5 to 5×10^5 cells per well) in duplicate wells were infected with bacteria at a ratio of 100:1 (MAC to MP) for 2 h at 37°C in humidified 5% CO_2 atmosphere and washed three times to remove the extracellular bacteria.

MIC determination in human monocyte-derived MP. MICs in MP were determined by counting the viable bacteria in infected MP monolayers incubated with 1 ml of drug-containing complete medium, after both 3 and 7 days of treatment. The final drug concentrations were as follows: CLA, AMI, SPA, CIP, EMB, and INH, from 0.25 to 32 μg/ml, respectively, and CLO and RFB, from 0.03 to 4 μg/ml, respectively. MP in control wells received no drugs. Both control (untreated) MP on day 0 and control and drug-treated MP on days 3 and 7 were washed and lysed as previously described (4). Briefly, 0.5 ml of distilled water was added to each well, and after 10 min of incubation at room temperature, 0.5 ml of a lysing solution (110 ml of 7H9 medium [Difco] plus 40 ml of a 0.25% sodium dodecyl sulfate solution in 0.1 M phosphate buffer, pH 7) was added to each well for an additional 10 min. Wells were vigorously scraped with a pipette, and 0.5 ml of a 20% bovine serum albumin solution was added to MP lysate. For CFU counting, MP lysates were serially diluted 10-fold and 0.5-ml aliquots of each dilution were plated in triplicate on 7H10 agar. CFU were counted after incubation of the plates for 10 to 14 days at 37°C in humidified 5% CO_2. MAC survival in drug-treated MP was expressed as a percentage of the CFU per well in untreated MP monolayers at time zero, as previously described (33, 35, 37). The MICs in MP on days 3 and 7 (MP3 and MP7, respectively) were defined as the lowest drug concentrations in the culture medium in the presence of which survival of 100% ± 10% occurred.

Combined drug action in human MP. The activity of single agents or drug combinations in MP was assessed by treating the infected cells with the concentrations of drugs achievable in serum, as follows: CLA, 3 μg/ml; EMB, 4 μg/ml; CIP, 2 μg/ml; RFB, 0.5 μg/ml; AMI, 10 μg/ml; INH, 4 μg/ml; CLO, 0.5 μg/ml; SPA, 1 μg/ml. The same concentrations were used to assay both single drugs and combinations. MP in control wells received no drugs. Monolayers were incubated for 3 days without change of the medium. Both control (untreated) MP on day 0 (time [T] = 0) and control and drug-treated MP on day 3 were washed and lysed as described above. MAC survival in drug-treated MP on day 3 was expressed as a percentage of the CFU per well in untreated MP monolayers at time zero (T = 0). Inhibition inside MP was defined as a survival of 100% ± 10% of the T = 0 control. The significance of the differences of the survival values of two-drug combinations versus each constituent drug and those of three-drug combinations versus two-drug combinations was assessed by Student's t test.

TABLE 1. MICs of combinations of drugs acting against six strains of *M. avium*

Combination	MIC[a] (μg/ml) for strain:					
	485	486	901	902	905	911
CLA-AMI	4, 2	4, 4	0.5, 0.25	1, 1	4, 2	0.25, 1
CLA-INH	4, 2	4, 1	1, 2	1, 4	4, 2	1, 1
CLA-SPA	4, 4	2, 0.25	0.5, 0.5	0.5, 2	4, 2	0.25, 0.12
CLA-EMB	4, 2	2, 1	1, 2	0.25, 0.12	4, 2	0.25, 0.12
CLA-CLO	4, 0.12	1, 0.03	0.5, 0.06	0.5, 0.03	2, 0.12	0.12, 0.03
CLA-RFB	4, 0.25	1, 0.06	0.5, 0.001	0.25, 0.03	1, 0.25	0.06, 0.002
CLA-INH-AMI	2.67, 1.33, 1.33	2, 0.5, 2	0.67, 1.33, 0.33	1, 4, 1	2.67, 1.33, 1.33	0.5, 0.5, 2
CLA-SPA-AMI	2.67, 2.67, 1.33	2, 0.25, 2	0.67, 0.67, 0.33	0.5, 2, 0.5	2.67, 1.33, 1.33	0.17, 0.08, 0.67
CLA-EMB-AMI	1.33, 0.67, 0.67	1, 0.5, 1	0.5, 1, 0.25	0.5, 0.25, 0.5	2, 1, 1	0.25, 0.12, 1
CLA-CLO-AMI	2, 0.06, 1	0.67, 0.02, 0.67	0.33, 0.04, 0.17	0.33, 0.02, 0.33	1.33, 0.08, 0.67	0.16, 0.04, 0.67
CLA-RFB-AMI	2.67, 0.17, 1.33	0.67, 0.04, 0.67	0.67, 0.001, 0.33	0.33, 0.04, 0.33	1.33, 0.33, 0.67	0.16, 0.005, 0.67

[a] MICs reported refer to the values of a drug in combination. For MICs of each drug alone, please see Table 2. MICs for each drug in a combination are given in the same order as the drugs.

TABLE 2. MICs of various drugs against six strains of *M. avium*

Strain no.	MIC (μg/ml) of:																							
	CLA			AMI			SPA			CIP			EMB			INH			CLO			RFB		
	MP3[a]	MP7[a]	B[b]	MP3	MP7	B	MP3	MP7	B	MP3	MP7	B	MP3	MP7	B	MP3	MP7	B	MP3	MP7	B	MP3	MP7	B
485	2	2	8	8	8	4	4	4	8	8	8	8	2	4	4	2	2	4	0.25	0.25	0.25	0.25	0.25	0.5
486	1	1	4	4	8	4	0.5	1	0.5	1	2	8	2	2	2	1	1	1	0.25	0.5	0.125	0.06	0.06	0.25
901	4	4	2	1	2	1	0.25	0.5	2	0.5	1	2	4	4	4	0.5	1	4	0.125	0.25	0.25	<0.03	<0.03	0.004
902	0.5	0.5	2	0.5	1	2	0.5	0.5	8	1	1	1	1	2	1	0.5	0.5	8	0.125	0.25	0.125	0.03	0.03	0.25
905	2	2	8	32	32	4	0.5	1	4	2	2	8	2	2	4	2	4	4	0.25	0.5	0.5	0.5	0.5	2
911	2	2	1	8	8	4	0.5	1	0.5	1	1	1	4	4	0.5	2	4	1	0.25	0.25	0.25	0.125	0.125	0.03
Median MIC	2	2	3	6	8	4	0.5	1	3	1	1.5	5	2	3	3	1.5	1.5	4	0.25	0.25	0.25	0.09	0.09	0.25
No. of strains inhibited[c]	5/6	5/6	3/6	5/6	5/6	6/6	5/6	5/6	2/6	5/6	5/6	3/6	6/6	6/6	6/6	6/6	6/6	5/6	6/6	6/6	6/6	6/6	6/6	5/6

[a] MICs in MP, as determined at days 3 (MP3) and 7 (MP7).
[b] MICs in 7H12 broth.
[c] Ratio of number of strains inhibited at concentrations achievable in serum to total number of strains. See Materials and Methods.

RESULTS

Growth of MAC in human MP. Each MAC strain was grown in MP cultures obtained from a different donor. Over a total of 23 experiments, all the six MAC strains tested were able to grow inside the phagocytic cells; the average increase in bacterial cell count was 5.7 ± 0.8 (standard error of the mean) on day 3 and 21 ± 5 times on day 7. Either uninfected or infected MP retained more than 95% viability and did not appreciably detach from the surface of the plates.

Comparison between MICs in human MP and those in 7H12 broth. Table 2 shows the MICs of CLA, AMI, SPA, CIP, EMB, INH, CLO, and RFB determined in the MP model on days 3 and 7 (MP3 and MP7, respectively) and in 7H12 broth. Despite some strain-related differences, the median MICs in MP were similar to or slightly lower than those found in 7H12 broth, with the exception of those of SPA and CIP, which were lower in MP than in broth, and those of AMI, which were slightly higher in MP. Median MICs in MP on day 7 were the same as or 1 dilution higher than MICs in MP on day 3. On day 7, all the strains tested were still inhibited inside the MP at concentrations achievable in serum of EMB, INH, CLO, and RFB, while CLA, AMI, SPA, and CIP failed to so inhibit one strain each. In this respect, there was a general agreement between results obtained by the two methods except for SPA, CIP, and CLA, which showed a higher activity in MP.

Combined drug activity determined by the radiometric method. To measure the activity of the combinations, FIC indices were determined (Table 3). In general, the antimicrobial combinations had additive or synergistic effects on the strains tested except for CLA-AMI, which showed antagonistic effect on one strain, and CLA-INH, which showed antagonistic effect on two strains. CLA-CLO and CLA-RFB had a clear synergistic effect on five of six strains, while CLA-EMB and CLA-SPA were synergistic on two and three strains, respectively. No synergistic effect of the combination CLA-INH was found.

In general, the addition of AMI to the two-drug combinations enhanced the synergistic activity of CLA-EMB and CLA-CLO in all the strains. No substantial modification by AMI was observed with all other two-drug combinations.

Combined drug activity determined in MP. Figure 2 shows the intramacrophagic survival of the six MAC strains after 3 days of incubation in the presence of drugs, alone and in combination, at concentrations achievable in serum. Each strain was tested by using MP obtained from a different donor. For each strain, the survival in drug-treated MP was expressed as percentage of the CFU per well of untreated MP at $T = 0$. Bars indicate the mean survival values of the strains tested. EMB, RFB, CLO, INH, and SPA (mean survival, 80, 58, 51, 49, and 26%, respectively) inhibited the growth of all the MAC strains inside MP, while CIP, AMI, and CLA (mean survival, 74, 66, and 56%, respectively) failed to inhibit the multiplication of one strain each.

Notably, the strains showing resistance to the drugs above were not the same for each drug. Rather, they corresponded to the strains having higher MICs in MP such as, for instance, strain 485, which was not inhibited by CIP, and strains 901 and 905, not inhibited by CLA and AMI, respectively (Table 2). Among the combinations, CLA-CLO (mean survival, 21%) significantly reduced CFU in comparison with each combina-

TABLE 3. FIC indices of combinations of drugs acting against six strains of *M. avium*

Strain no.	FIC index[a] for the combination:										
	CLA-AMI	CLA-INH	CLA-SPA	CLA-EMB	CLA-CLO	CLA-RFB	CLA-INH-AMI	CLA-SPA-AMI	CLA-EMB-AMI	CLA-CLO-AMI	CLA-RFB-AMI
485	1	1	1	1	1	1	1	1	0.5	0.75	1
486	2	2	1	1	0.5	0.5	1.5	1.5	0.75	0.5	0.5
901	0.5	1	0.5	1	0.5	0.5	1	1	0.75	0.5	1
902	1	1	0.5	0.25	0.5	0.25	1.5	0.75	0.75	0.5	0.5
905	1	1	1	1	0.5	0.25	1	1	0.75	0.5	0.5
911	0.5	2	0.5	0.5	0.25	0.125	1.5	0.5	0.75	0.5	0.5

[a] See Materials and Methods. The breakpoints of FIC indices are as follows: for two drugs, ≤0.5 for synergism, 1 for additivity, and ≥2 for antagonism; for three drugs, ≤0.75 for synergism and ≤1.5 for additivity.

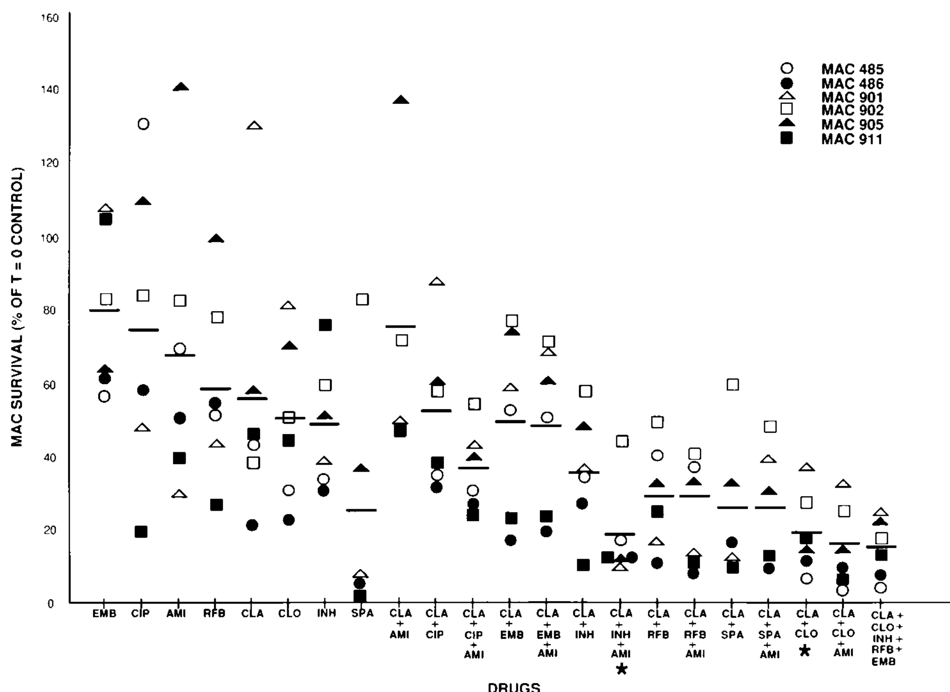

FIG. 2. Activity of EMB, CIP, AMI, RFB, CLA, CLO, INH, and SPA, used alone and in combination against MAC inside MP, after 3 days of incubation. MAC survival in drug-treated MP was expressed as a percentage of CFU per well in untreated MP at time zero (T = 0 control). For each drug, concentrations achievable in serum were used (see Materials and Methods). Bars show mean survival for strains tested. *, $P < 0.05$, Student's t test (see Results).

torial drug (P = 0.007, compared with CLO, and P = 0.036, compared with CLA). CLA-RFB (mean survival, 29%) was significantly more active than RFB (P = 0.024) but not more active than CLA alone (P = 0.18). The combinations of CLA with CIP, EMB, INH, and SPA (mean survival, 53, 50, 36, and 26%, respectively) were not significantly more active than each combinatorial drug.

CLA-AMI was less effective than either CLA or AMI alone. The addition of AMI to the two-drug combinations did not significantly improve the effectiveness of any combination with the exception of the combination CLA-INH-AMI (mean survival, 19%; P = 0.012 compared with CLA-INH, and P = 0.029, compared with CLA-AMI).

Of interest is that the five-drug combination CLA-CLO-INH-RFB-EMB (mean survival, 17%) was as active as CLA-INH-AMI, CLA-CLO, or CLA-CLO-AMI and that any drug combination with SPA (which was the most effective single drug) was no more effective than SPA itself (Fig. 2).

DISCUSSION

Treatment of MAC infections in AIDS patients is often unsuccessful not only because of the severe impairment of the normal host defenses but also because of the emergence of mutant strains resistant to antimicrobial agents. To overcome these difficulties, combinations of different antimicrobial agents have been widely investigated in vitro to identify favorable interactions among drugs (10, 12, 27, 28, 34).

In this study, we systematically investigated the activity of several antimicrobial agents, alone or combined with CLA, or CLA-AMI, against MAC strains from AIDS patients, both by the radiometric method and in a model of infection in human MP. Since opaque colonial variants of MAC are reported to be more susceptible to antimicrobial agents (30, 32), only transparent colonies from 7H10 plates were used throughout this study. The six strains grew in MP to a different level, in general about 6 times the original inoculum at day 3 and about 21 times at day 7. These results are in keeping with previously reported data on intracellular MAC growth (6, 26, 27, 37). Thus, we determined MICs for eight antimicrobial agents used in MAC infections by both methods. The results obtained warrant particular attention, as very few studies on MICs in MP for mycobacteria, and those obtaining MICs by testing one or two drugs only, have been published previously (22, 23). Our results show that, regardless of strain-to-strain variability, there is a general accordance between the two methods, the median MICs being similar or only slightly lower (in particular with SPA and CIP) in MP than in 7H12 broth. The results with AMI, which showed MICs in five of six cases higher in MP than in broth, are possibly due to the reported poor penetration of the drug in the MP (31).

It remains somewhat surprising that antimicrobial agents known to be appreciably concentrated inside the MP, such as CLO (16), RFB (9), or CLA (2), had MICs very similar to those found in broth. Our results with CLA are in keeping with those of others (22), and the same phenomenon has been recently described for quinolones on *Mycobacterium tuberculosis* (23). Of interest is also that some *M. avium* strains failed to be inhibited inside phagocytes by CLA, AMI, SPA, and CIP, at concentrations achievable in serum.

The activity of a large panel of CLA-containing combinations, with or without AMI, was evaluated by the radiometric method by the calculation of the FIC indices. Only combinations of CLA-AMI on one strain and CLA-INH on two strains showed antagonistic effects. CLA-CLO and CLA-RFB showed a remarkable synergistic effect on five of six strains, but other combinations such as CLA-EMB and CLA-SPA also showed a synergistic effect on two and three strains, respectively. The addition of AMI to the combinations further improved the

activity of only CLA-EMB and CLA-CLO. Differences in methods and combinations used, or in the mode of reporting the results, make particularly hard a direct comparison between our data and those of others. Nevertheless, enhanced activity for CLA-EMB combination in vitro has already been reported (10, 27), while CLA-SPA (used at a sublethal fixed concentration) showed enhancement of activity only on 1 of 10 strains (28).

Single drugs and combinations, used at concentrations achievable in serum, were also assayed in the MP model to obtain a general insight into the inhibitory or killing capacity of each drug or combination in this model. No single drug or combination could be strictly defined as bactericidal at the time used (3 days) because in no cases did we obtain a reduction of 2 logs in the viable counts, in comparison with controls at time zero. Nevertheless, only a few strains failed to be inhibited by single drugs and all but one were inhibited by all the combinations tested.

Moreover, differences in the activity of various combinations could be demonstrated, showing that not only did further growth of initial MAC inoculum generally not occur but also that the antibiotics caused a significant decrease in the CFU number, in comparison with single drugs. In particular, CLA-CLO and CLA-RFB showed a strong reduction in viable counts compared with single drugs.

The same MP model has been used by others to assess the activity of some of our combinations, such as CLA-EMB (27, 35), CLA-RFB (26), CLA-AMI (33), and CLA-SPA (28). In these cases, too, no reduction in viable counts higher than 1 log in 3 to 7 days was usually obtained, in keeping with our results.

In general, the addition of AMI did not improve the effect of combinations except for CLA-INH-AMI, which significantly reduced the viable counts, in comparison with either CLA-INH or CLA-AMI. Combinations of CLA-AMI were no more active than single drugs in MP, nor more effective in animal models than CLA alone (8, 18, 19). The combination CLA-INH-AMI has also been evaluated with the MP model despite the fact that, on the basis of published evidence (14), a negative result was expected. It was therefore surprising to see that either INH itself or its addition to CLA or CLA-AMI resulted in a consistent anti-MAC activity in the MP model, contrasting with the results in vitro.

Even if there are rare reports on the use of INH in human MAC infections (2, 29), its administration as a therapeutic adjunct is discouraged (11, 20) and no animal models have been extensively evaluated. Considering that the mechanisms of action of this drug are not yet fully elucidated (1), it could be interesting to investigate the activity of INH, in combination with other antimicrobial agents, such as CLA-AMI, in appropriate animal models.

Each experimental model used here to assess the effectiveness of anti-MAC drugs has its own limitations; the MP model, for instance, has several limits, such as the differences from donor to donor in supporting the intramacrophagic growth and the time required to have the results. Nevertheless, our data emphasize that, for such difficult-to-treat infections as those caused by MAC, a number of approaches should be used in testing for antimicrobial susceptibility, so as to facilitate further in vivo experimentation with drug combinations. Among these approaches, the comparative analysis of which we have given an example here may offer useful information for designing drug interaction experiments in animal models.

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