Antimycobacterial Spectrum of Sparfloxacin and Its Activities Alone and in Association with Other Drugs against *Mycobacterium avium* Complex Growing Extracellularly and Intracellularly in Murine and Human Macrophages

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The MICs and MBCs of the new difluorinated quinolone drug sparfloxacin against type strains belonging to 21 species of mycobacteria were screened. The MICs and MBCs were within the range of 0.1 to 2.0 and 0.1 to 4.0 \( \mu \text{g/mL} \), respectively (with an MBC/MIC ratio of 1 to 2), and against 18 of the 21 species tested, the drug showed significant bactericidal activity (at least 99% killing or more of the initial inoculum added) at concentrations well within the reported peak concentrations in serum (\( C_{\text{max}} \)) in humans. MICs of sparfloxacin for 7 of 10 *Mycobacterium avium* complex strains were below the \( C_{\text{max}} \) with MBC/MIC ratios within the range of 2 to 4. Enhancement of its activity by ethambutol, rifampin, amikacin, and clarithromycin (which were used at sublethal concentrations) assessed by using BACTEC radiometry revealed that its activity was further enhanced in 2 of 10 strains by rifampin and in 7 of 10 strains by ethambutol. The bactericidal effects of various drugs used alone as well as two-drug combinations used at \( C_{\text{max}} \) levels were also screened against four strains of *M. avium* complex growing intracellularly in two different macrophage systems, namely, mouse bone marrow-derived macrophages and peripheral blood monocyte-derived human macrophages. Our results showed a satisfactory correlation between the extracellular and intracellular drug activity data.

Recently, newer fluoroquinolones have been reported to have broad antimicrobial spectra, including mycobacteria (3, 14, 25). Both the N-cyclopropyl analog ciprofloxacin and the tricyclic compound ofloxacin were found to penetrate actively and concentrate inside mammalian cells (6, 13, 35), and were found to be capable of killing intracellularly growing tubercle bacilli (19). A newly synthesized difluorinated quinolone, sparfloxacin [5 amino-1-cyclopropyl-6,8 difluoro-1,4-dihydro-7 cис-3,5-dimethyl-1-piperazinyl]-4-oxoquinolone-3-carboxylic acid], was retained for this study because it is a broad-spectrum drug with in vitro and in vivo efficacies equal to or better than those of ofloxacin and ciprofloxacin (17), and compared with other fluoroquinolones, it has lower MICs against both tubercle bacilli (24) and the multiple-drug-resistant opportunistic pathogen belonging to the *Mycobacterium avium* complex (MAC) (37). Considering the intracellular parasitism of MAC organisms as well as the fact that other difficult-to-treat atypical mycobacteria, it was interesting that sparfloxacin attained levels in mice and rat tissues which were 1 to 11 times higher than its concentrations in plasma (maximum concentration in serum \( [C_{\text{max}}] \), 1.4 \( \mu \text{g/mL} \)) (16).

After we made the observations described above, we decided to perform the present four-point study on sparfloxacin activity (i) to assess its antimycobacterial spectrum against 21 species of mycobacteria by MIC and MBC determinations with *H*9 broth; (ii) by radiometric determination of its MICs and MBCs against 10 MAC strains; (iii) to investigate the enhancement of its action (used at a sublethal concentration of 0.25 \( \mu \text{g/mL} \)) against 10 MAC strains by amikacin, rifampin, ethambutol, and clarithromycin (used at fixed sublethal concentrations of 1 \( \mu \text{g/mL} \)) by the radiometric \( X/Y \) quotient calculation method (see below), which permits assessment of the activity of both the two-drug and three-drug combinations (9, 10, 23, 27, 29); and (iv) to assess its intracellular activity used alone and in combination with the drugs listed above (used at their reported \( C_{\text{max}} \)s in humans) in the case of four MAC strains growing inside murine and human macrophages.

Results of the present study indicate that sparfloxacin has significant mycobactericidal activity and demonstrate a good correlation between the extracellular and intracellular drug activity data against MAC bacteria.

**MATERIALS AND METHODS**

**Bacteria and growth.** The strains representative of 21 mycobacterial species and the 10 strains belonging to MAC used in this study (Tables 1 and 2, respectively) were from our own culture collection. All the species were grown in complete *H*9 broth (containing 0.05% [vol/vol] Tween 80; Difco Laboratories, Detroit, Mich.) to an optical density at 650 nm (\( OD_{650} \)) of 0.15 measured by using a Coleman junior II spectrophotometer; this corresponded to about \( 10^7 \) to \( 10^8 \) viable counts, depending on the species used. All bacteria were grown at 37°C, except *M. xenopi* which was grown at 42°C, and *M. malmoense, M. marinum, M. gordonae,* and *M. chelonae* which were grown at 30°C.

**Drug susceptibility testing.** For MIC determination by the broth dilution method for 21 mycobacterial species, tubes containing 5 mL of complete *H*9 liquid medium alone (control) or supplemented with increasing concentrations of sparfloxacin were inoculated with 0.1 mL of a freshly grown culture containing about \( 10^7 \) to \( 10^8 \) viable counts. The growth was followed until the control tube reached an \( OD_{650} \) of 0.15, which corresponded to \( 9.0 \times 10^6 \) (M. *triviale*) to \( 2.0 \times 10^8 \) (M. *simiae*) CFU/mL, according to the species studied. The MIC was defined as the minimal concentration of the drug...
which resulted in no visible growth in a tube (equal to or less than an OD of 0.015).

Parallel controls showed that a 10-fold dilution of the control tube with an OD of 0.15 correlated well with a 10-fold decrease in the viable counts; however, Tween 80 at 0.05% (vol/vol) was necessary to avoid clumping which, otherwise, could give an erroneous correlation between the viable count and OD data. Moreover, this linear correlation between OD and viable counts reached a plateau from an OD of 0.25 and greater. For the reasons given above, we performed all the MIC determinations in the presence of 0.05% Tween 80 and stopped the experiments when the control tubes reached an OD of 0.15.

For parallel MBC determinations, the exact number of bacteria at time zero in the case described above was determined by plating serial dilutions onto 7H11 agar and enumerating the CFU per milliliter after the counts had stabilized. In the same way, all the tubes showing no visible growth as well as the control tube were titrated at the end of the experiment, and the MBC was defined as the minimal concentration of the drug which effectively reduced the initial bacterial inoculum by at least 99% at the time of drug addition.

Radioisotopic determination of the MIC against MAC organisms (8) was performed essentially as reported earlier, with a few modifications (29). Briefly, the growth of bacteria was monitored in a confined atmosphere, using the BACTEC 460-TB apparatus (Becton Dickinson, Towson, Md.), as a function of their ability to catabolize 14C-labeled palmitic acid in 7H12 broth and by automatically measuring the 14CO2 released (23, 27, 29). The growth of bacteria was represented as a numerical value called the growth index (GI), which ranged from 1 to 999. Mycobacterial growth in this system is dependent on the standardization of the initial bacterial inoculum (26), and because of the more rapid growth of MAC bacteria compared with that of M. tuberculosis in this system, the initial MAC inoculum added to the BACTEC vials was prepared as follows. After a preculture of a strain in an initial BACTEC vial to a GI of 500, this preculture was diluted 1:10 and 1:1,000, and 0.1 ml of the culture diluted 1:10 was used to inoculate the drug-containing vials as well as a 1:10 control (corresponding to about 5 x 106 to 6 x 107 CFU/ml, depending on the MAC strains studied). The daily GI in the drug-containing vials was compared with that of another control vial inoculated with 0.1 ml of the culture diluted 1:1,000, and the results were interpreted once the GI in the control diluted 1:1,000 reached a value of 30 or more (29). The MIC was defined as the minimal concentration which resulted in a lower ΔGI in the drug-containing sample compared with that in the control diluted 1:1,000.

For MBC assessment, the CFU per milliliter for each strain was determined both at the time of inoculation of the BACTEC vials (time zero) and at the end of the experiment, as follows. A total of 0.1 ml of culture from the BACTEC vials was removed and successively diluted 10-fold in sterile double-distilled water to give dilutions of 10−1, 10−2, 10−3, and 10−4. A total of 0.1 ml of each of these dilutions was plated onto 7H11 agar medium, and the resulting bacterial counts were enumerated after 21 days of incubation at 37°C. These experimental conditions allowed the possibility of an accidental loss of bacterial viability because of a carryover of drugs to the solid medium. The MBC was defined as the minimal concentration which effectively reduced by at least 99% the viable counts in the drug-containing sample compared with those in the initial inoculum. In this way, a drug was considered to be efficient against a MAC strain if it effectively had both MICs and MBCs below its Cmax reported in humans, i.e., 1.4 μg/ml for sparfloxacin (16).

Combined drug action. For combined drug action studies, all the drugs were used at their sublethal concentrations, i.e., 0.25 μg/ml for sparfloxacin and 1 μg/ml for the other drugs. The reason for this choice was the fact that at this concentration (below their Cmax values), the drugs used alone were unable to significantly reduce the initial inoculum added to the BACTEC vials. In such a case, any significant drug enhancement observed according to the radiometric X/Y quotient criterion (9, 10, 23, 27, 29) could eventually suggest a reproducible effect in infected host cells, where the drugs are available at much higher concentrations.

Briefly, the combined drug action was equal to X/Y, where X was the BACTEC GI obtained with the combination of two or three drugs and Y was the lowest GI obtained at the same time for any of the drugs used alone. In the case of a two-drug combination, an X/Y value of 1 indicated that there was no interaction between the two drugs, an X/Y quotient of <0.5 indicated an enhanced drug action, and an X/Y quotient of ≥2 indicated the presence of antagonism between the two drugs. In the case of a three-drug combination, an X/Y quotient of <0.3 indicated enhanced drug action.

Enhanced drug action observed by radiometric X/Y quotients was further confirmed by viable count data by enumerating the CFU per milliliter in some of the test vials both at time zero and at the end of the experiment.

Murine macrophage culture. Bone marrow-derived macrophages were cultured essentially as reported earlier for studies involving MAC organisms (5), but with a few modifications, as follows. Briefly, 2 x 10⁵ bone marrow cells (isolated from 6- to 13-week-old C57BL/6 female mice) were seeded in 2 ml (per well) of RPMI 1640 medium containing L-cell conditioned medium (10% [vol/vol]), fetal calf serum (10% [vol/vol]; heat inactivated at 56°C for 60 min), 2 mM L-glutamine, 100 U of penicillin G per ml, and 0.25 μg of amphotericin B per ml were seeded in 12-well tissue culture clusters (Costar, Cambridge, Mass.). The macrophages were grown in the presence of 5% CO2 in a water-jacketed incubator at 37°C. After 5 days of incubation, 1.5 ml of medium per well was replaced with fresh medium that was devoid of antibiotics. After another 3 days (i.e., day 8), the culture medium was completely changed and replaced with 2 ml of new medium that was devoid of antibiotics. Two days later, the 10-day-old macrophage monolayers that were obtained (about 10⁶ cells per well) were used for further experiments. The trypan blue dye exclusion test showed that more than 97% cells were viable at this time point, and neither the number per well nor the proportion of viable cells changed significantly during this study.

Human macrophage culture. Cultures of human macrophages were prepared from adherent peripheral blood monocytes as described previously (1), with slight modifications. Briefly, blood from healthy human donors (Centre National de Transfusion Sanguine, Paris, France) was anticoagulated with one-sixth of its final volume of citric acid-citrate-glucose (7, 93, and 139 mM, respectively [pH 6.4]) containing heparin (20 U/ml; Choay) collected in plastic bottles, mixed with an equal volume of 3% (wt/vol) dextran (0.9% NaCl) solution, and allowed to stand for 30 min. Under sterile conditions, 35 ml of supernatant was transferred to plastic tubes containing 15 ml of Ficoll, and the mixture was centrifuged at 200 x g for 45 min at room temperature. Mononuclear cells were carefully removed and washed twice with 10 ml of prewarmed RPMI 1640 medium. The
viabilities of the isolated cells were greater than 97%, as judged by the trypan blue dye exclusion test.

The cells were suspended in RPMI 1640 medium supplemented with 3% (vol/vol) heat-inactivated fetal calf serum–2 mM l-glutamine–100 U of penicillin G per ml–0.25 μg of amphotericin B per ml, adjusted to a final concentration of 2 × 10⁸ cells per ml, distributed in 2-ml aliquots in 12-well tissue culture clusters, and incubated overnight at 37°C in the presence of 5% CO₂ to permit the adherence of monocytes. Then, 1.5 ml of culture medium was replaced with fresh medium devoid of antibiotics. After 2 days, the cells were washed free of nonadherent cells and 2 ml of new medium devoid of antibiotics was added. By 3 to 4 days later, the 6- to 7-day-old cell monolayer had matured into macrophages (1), with an average of about 10⁶ cells per well. Such 6- to 7-day-old human macrophage monolayers were used for all experiments.

**Intracellular drug activity.** Both murine and human macrophage monolayers were infected by MAC organisms as described above. The bacterial cultures were grown to their exponential phase of growth (to an OD₆₅₀ of 0.15, corresponding to about 10⁶ CFU/ml), and 0.1 ml of the culture was added to 10 ml of RPMI 1640 medium supplemented with appropriate growth factors. For infection, 1 ml of the culture medium was removed from each well and was replaced with 1 ml of the medium described above (initially containing about 10⁶ CFU/ml). The macrophages were allowed to phagocytize the bacteria for 4 h at 37°C, after which all the extracellular bacilli were thoroughly washed away with Hanks balanced salt solution, and the number of bacteria effectively phagocytized was determined by lysing the macrophages by using 0.25% (wt/vol) sodium dodecyl sulfate, doing immediate serial dilutions, and plating the lysates on 7H11 agar medium for viable count determinations. The addition of 0.25% sodium dodecyl sulfate to parallel cultures of MAC organisms, which were immediately serially diluted for viability assessment in parallel control experiments, showed that it did not lower the bacterial viable counts.

After phagocytosis, fresh medium containing the desired antibiotics was refed to macrophage-containing wells, and the bacteria were enumerated after lysing the macrophages at day 5. The results were compared with the growth of bacteria in the control culture (untreated macrophages). A drug alone or in combination with another drug was considered to be bactericidal intracellularly if it effectively reduced the bacterial viable counts in the test samples by more than 1 log unit compared with those of the control at the time of drug addition.

In accordance with our experimental model for determining the intracellular actions of drugs (29, 31), all the drugs were used at their reported Cₘₐₓ in humans, i.e., 1.5 μg/ml for sparfloxacin (17), 2 μg/ml for clarithromycin (12), 6 μg/ml for ethambutol (15), 15 μg/ml for rifampin (15), and 20 μg/ml for amikacin (4).

**Drugs.** Sparfloxacin (Rhône Poulenc-DPC Europe, Antony, France), amikacin (Laboratoire Bristol, Paris, France), ethambutol (Lederle, Oullins, France), and clarithromycin (Abbott Laboratories, North Chicago, Ill.) were kindly provided by their manufacturers. Rifampin, penicillin G, and amphotericin B were purchased from Sigma Chemical Co. (St. Louis, Mo.).

**RESULTS**

**Antimycobacterial spectrum of sparfloxacin.** Our results showing the MICs and MBCs of sparfloxacin against 21 species of mycobacteria are given in Table 1. MICs among the group representing strict pathogens (M. tuberculosis complex) varied from 0.1 to 0.2 μg/ml, with MBC/MIC ratios being between 1 and 2. Among the potential pathogens representing nine species of mycobacteria, MICs varied from 0.1 to 0.5 μg/ml for seven species; for M. malmoense and M. marinum, however, the MICs were 1.5 and 2.0 μg/ml, respectively. MBC/MIC ratios were between 1 and 2, irrespective of the MICs that were obtained. Finally, among eight species representing rare pathogens, MICs were between 0.1 to 0.5 μg/ml for seven species; for M. chelonae the MIC was 1.0 μg/ml. MBC/MIC ratios were within the range of 1 to 2.

Thus, overall sparfloxacin MICs and MBCs were within the range of 0.1 to 2.0 and 0.1 to 4.0 μg/ml, respectively (with a MBC/MIC ratio of 1 to 2), and against 18 of the 21 species tested, the drug showed significant bactericidal activity (at least 99% killing or more of the initial inoculum added) at concentrations well within the reported Cₘₐₓ in humans.

**Extracellular activity of sparfloxacin used alone and in combination with other drugs against 10 MAC strains.** The action of sparfloxacin against 10 MAC strains studied radiometrically (Table 2) showed that the MIC was 0.25 μg/ml for 2 strains, 0.5 μg/ml for 4 strains, 1 μg/ml for 2 strains, and 1.5 and 2.5 μg/ml for 1 strain each. MBC/MIC ratios were within the range of 2 to 4, and significant bactericidal activity (more than 99% killing of the initial bacterial inoculum) was achieved against 5 of 10 strains at concentrations of sparfloxacin below its Cₘₐₓ.
TABLE 2. Activity of sparfloxacin used alone and in various drug combinations against MAC strains

<table>
<thead>
<tr>
<th>MAC strain</th>
<th>Sparfloxacin MIC (µg/ml)</th>
<th>MBC/MIC ratio</th>
<th>SPAR + AMIK</th>
<th>SPAR + RIF</th>
<th>SPAR + EMB</th>
<th>SPAR + AMIK + RIF</th>
<th>SPAR + AMIK + EMB</th>
<th>SPAR + AMIK + CLA</th>
<th>SPAR + RIF + EMB</th>
<th>SPAR + RIF + CLA</th>
<th>SPAR + CLA + EMB</th>
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</thead>
<tbody>
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<td>ATCC 15769</td>
<td>0.25</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>CIPT</td>
<td>1.50</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>140310030</td>
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<tr>
<td>ATCC 13959</td>
<td>0.50</td>
<td>4</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<td>90-0731</td>
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<td>90-0738</td>
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<tr>
<td>90-0779</td>
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<td>2</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90-0827</td>
<td>0.50</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>90-1253</td>
<td>0.50</td>
<td>2</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90-1257</td>
<td>0.25</td>
<td>4</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>90-1295</td>
<td>1.00</td>
<td>2</td>
<td>-</td>
<td>-</td>
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</table>

*Enhancement of drug activity was calculated by determining X/Y quotients radiometrically. X was the BACTEC GI obtained with the combination of drugs, and Y was the minimal GI for any of the drugs used alone. An X/Y quotient of <0.5 (two-drug combinations) and <0.33 (three-drug combinations) indicated enhanced drug action. In enhancement experiments, all the drugs were used at sublethal concentrations (0.25 µg/ml for sparfloxacin and 1 µg/ml for the other drugs). Symbols for two-drug combinations: +, X/Y quotient of <0.5; ++, X/Y quotient of <0.1; ++++, X/Y quotient of <0.05. Symbols for three drug combinations: +, X/Y quotient of <0.33; ++, X/Y quotient of <0.06; ++++, X/Y quotient of <0.03. For strains ATCC 15769, CIPT 140310030, 90-0827, 90-1253, and 90-1257, the enhancement of drug action was also confirmed by viable count determinations (see Table 3 for these data). SPAR, sparfloxacin; AMIK, amikacin; RIF, rifampin; EMB, ethambutol; CLA, clarithromycin.
growth inside macrophages and is bactericidal if it kills some of the initial bacterial inoculum. In macrophage studies, a drug used alone or in combination was considered significantly bactericidal if it effectively reduced the bacterial viable counts in test samples by more than 1 log unit compared with those in the control at the time of drug addition.

As can be seen from our data, both the intracellular growth as well as the bactericidal effect of drugs were more pronounced in human macrophages compared with those in murine macrophages, but in general, there was a good correlation of bacterial growth and drug action in the two macrophage systems used. Contrary to the BACTEC \( X/Y \) radiometric drug enhancement data observed at sublethal concentrations of drugs (Tables 2 and 3), it was more difficult to follow enhancement of drug action at the much higher \( C_{\text{max}} \)s tested in macrophage systems; however, the following observations can be made: (i) Variable intracellular killing of the four MAC strains was observed, and in general, the various drugs used alone, except ethambutol, reduced the initial viable counts in three MAC strains; (ii) strain 90-1253 was not killed by any of the drugs used alone; (iii) although ethambutol was the most inefficient drug used alone, it increased the bactericidal effect of sparfloxacin in all three MAC strains found to be susceptible to the drug, irrespective of the macrophage system used (this was also true for the drug action against strain 90-1253); and (iv) all other drugs except ethambutol were more or less active against the three MAC strains, and therefore, it was difficult to assess whether they really enhanced the intracellular bactericidal action of sparfloxacin. However, on the basis of the data obtained for strain 90-1253, the enhancement of the action of sparfloxacin by ethambutol was successfully reproduced intracellularly. The increased intracellular action of sparfloxacin against strain 90-1295 in the presence of rifampin and clarithromycin in human macrophages was also observed. In conclusion, our results showed a satisfactory correlation between the extracellular and intracellular drug activity data.

### DISCUSSION

MAC organisms are opportunistic pathogens that cause severe pulmonary disease and disseminated disease in immunocompromised hosts (38). Adequate chemotherapy of these organisms is hampered, first, because of the multiple drug resistance, which has been attributed to an exclusion barrier localized in the cell wall (2, 22), and, second, because of their particular mode of intracellular parasitism (20, 21), implying that actively growing \( M. avium \) is not only surrounded by a capsule-like structure (28) but is also further protected inside the usually hostile phagosomal or phagolysosomal environment by its ability to inhibit normal macrophage functions (5, 20, 31).

We attempted to circumvent this problem by using a wide range of strategies, e.g., to screen the anti-MAC activities of newer drugs like fluoroquinolones (25) and clarithromycin (29) by showing the enhancement of drug action by simultaneous cell wall and/or outer layer inhibition by ethambutol and m-fluorophenylalanine (27, 29) and by attaching fatty acid side chains to existing hydrophilic drugs to render them amphipathic (23, 30). Finally, like other investigators (1, 36), we have also attempted to correlate the extracellular drug activities to their intracellular action using in vitro macrophage systems (19, 29, 31).

Following the reports that sparfloxacin was a broad-spectrum drug (17) with in vitro efficacies equal to or better than those of ofloxacin and ciprofloxacin against both tubercle bacilli and MAC (24, 37) and that it attained levels in mouse and rat tissues which were 1 to 11 times higher than its concentrations in plasma (16), we decided to perform a detailed study on both its extracellular and intracellular antimycobacterial activity.

The results obtained in this investigation showed that the new difluorinated quinolone sparfloxacin is highly active against mycobacteria in general. As in the 18 of the 21 species tested in this study, the drug showed significant bactericidal activity (Table 1). Furthermore, the drug showed promising activity against otherwise multiple-drug-resistant MAC organisms, because more than 99% killing of

### TABLE 3. Actions of various drugs alone and in combination against selected MAC strains measured by viable count determinations

<table>
<thead>
<tr>
<th>Drug(s)* (conc [( \mu g/ml )])</th>
<th>ATCC 15769</th>
<th>CIPT 140310030</th>
<th>90-0827</th>
<th>90-1253</th>
<th>90-1257</th>
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<tbody>
<tr>
<td>None (control at day 0)</td>
<td>2.3 ( \times 10^6 )</td>
<td>2.3 ( \times 10^6 )</td>
<td>8.0 ( \times 10^3 )</td>
<td>2.8 ( \times 10^3 )</td>
<td>1.5 ( \times 10^4 )</td>
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<tr>
<td>None (control at day 5)</td>
<td>6.5 ( \times 10^6 )</td>
<td>2.4 ( \times 10^7 )</td>
<td>2.7 ( \times 10^7 )</td>
<td>1.0 ( \times 10^3 )</td>
<td>4.5 ( \times 10^5 )</td>
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<tr>
<td>SPAR (0.25)</td>
<td>1.5 ( \times 10^4 )</td>
<td>1.2 ( \times 10^7 )</td>
<td>1.5 ( \times 10^7 )</td>
<td>4.0 ( \times 10^3 )</td>
<td>1.3 ( \times 10^3 )</td>
</tr>
<tr>
<td>CLA (1.0)</td>
<td>4.7 ( \times 10^7 )</td>
<td>ND*</td>
<td>5.0 ( \times 10^6 )</td>
<td>ND</td>
<td>3.1 ( \times 10^6 )</td>
</tr>
<tr>
<td>AMIK (1.0)</td>
<td>3.9 ( \times 10^4 )</td>
<td>2.3 ( \times 10^7 )</td>
<td>4.5 ( \times 10^4 )</td>
<td>2.0 ( \times 10^6 )</td>
<td>4.2 ( \times 10^6 )</td>
</tr>
<tr>
<td>RIF (1.0)</td>
<td>1.9 ( \times 10^4 )</td>
<td>1.2 ( \times 10^4 )</td>
<td>3.1 ( \times 10^6 )</td>
<td>ND</td>
<td>2.6 ( \times 10^4 )</td>
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<tr>
<td>EMB (1.0)</td>
<td>4.5 ( \times 10^5 )</td>
<td>1.8 ( \times 10^6 )</td>
<td>1.5 ( \times 10^7 )</td>
<td>4.0 ( \times 10^6 )</td>
<td>1.6 ( \times 10^8 )</td>
</tr>
<tr>
<td>SPAR + AMIK</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SPAR + RIF</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SPAR + CLA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SPAR + EMB</td>
<td>2.5 ( \times 10^2 )</td>
<td>9.0 ( \times 10^4 )</td>
<td>2.6 ( \times 10^3 )</td>
<td>2.6 ( \times 10^3 )</td>
<td>1.0 ( \times 10^5 )</td>
</tr>
<tr>
<td>SPAR + AMIK + RIF</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SPAR + AMIK + EMB</td>
<td>5.0 ( \times 10^1 )</td>
<td>5.3 ( \times 10^3 )</td>
<td>8.0 ( \times 10^2 )</td>
<td>1.6 ( \times 10^3 )</td>
<td>4.0 ( \times 10^5 )</td>
</tr>
<tr>
<td>SPAR + AMIK + CLA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SPAR + RIF + CLA</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SPAR + RIF + EMB</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>SPAR + CLA + EMB</td>
<td>2.0 ( \times 10^2 )</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.2 ( \times 10^3 )</td>
</tr>
<tr>
<td>SPAR + CLA + CLA + EMB</td>
<td>1.2 ( \times 10^2 )</td>
<td>ND</td>
<td>2.1 ( \times 10^3 )</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* SPAR, sparfloxacin; CLA, clarithromycin; AMIK, amikacin; RIF, rifampin; EMB, ethambutol.
* ND, not done, because no enhancement was observed by \( X/Y \) quotient calculations (see Table 2).
further details.

Results on the right side indicate bacterial growth after 5 days of incubation at 37°C, and bars on the right side indicate the relative bactericidal effects of the drugs used. Results are means ± standard errors. Refer to the text for further details.

FIG. 1. Activity of sparfloxacin (SPAR) used alone and in combination with clarithromycin (CLA), amikacin (AMIK), rifampin (RIF), and ethambutol (EMB) against four MAC strains growing inside murine macrophages. All the drugs were used at their C_{max}.

In all cases, the initial bacterial inoculum (2 × 10^7 to 1 × 10^8 CFU/ml) was given a value of 1 in the figure. Bars on the left side indicate bacterial growth after 5 days of incubation at 37°C, and bars on the right side indicate the relative bactericidal effects of the drugs used. Results are means ± standard errors. Refer to the text for further details.

The initial bacterial inoculum was achieved for 5 of 10 MAC strains at concentrations of sparfloxacin below its C_{max} (Table 2).

By using a previously described radiometric X/Y quotient method (9, 10, 23, 27, 29), our data revealed that its activity is further enhanced in 2 of 10 strains by rifampin and in 7 of 10 strains by ethambutol (Table 2). A good correlation between the X/Y quotient data given above and bacterial viable counts was achieved (Table 3), and when individual drugs or the two-drug combinations were screened against four MAC strains intracellularly in murine and human macrophages, it was clear that ethambutol is an essential component in the enhancement of sparfloxacin activity both extracellularly and intracellularly. These results confirmed earlier proposition of Källenius et al. (11) that ethambutol may be a key component in MAC chemotherapy, because it may help to break the exclusion barrier of MAC organisms not only by inhibiting the mycolic acid transfer (33) but also by inhibiting synthesis of arabinogalactan (34) in the mycobacterial cell wall, resulting in enhanced drug susceptibility (23, 27, 29). Ultrastructural evidence showing alterations in the cell wall or outer layer of MAC bacteria treated with ethambutol has been obtained (27, 29), and recently, Sareen and Khuller (32) have shown biochemical changes associated with the cell wall and membrane composition of ethambutol-resistant M. tuberculosis.

Our data also confirm recently published observations of Perrone et al. (18) that show the bactericidal action of sparfloxacin against M. avium growing inside human macrophages. We thus agree on an earlier proposition of Heifets (7) that the MIC must be considered a quantitative measurement of the susceptibility of MAC organisms. Indeed, if tested by the routine critical concentration method, which works satisfactorily for tubercle bacilli (26), most of the MAC strains may be declared drug resistant. However, it appears to us that in the case of these difficult-to-treat mycobacterial infections, we must quantify the degree of susceptibility by MIC and MBC determinations. In our opinion, a drug must be considered promising in the case of
ANTIMYCOBACTERIAL ACTIVITY OF SPARAFLOXACIN

ACKNOWLEDGMENTS

REFERENCES


