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Activities of Bay Y 3118, Levofloxacin, and Ofloxacin Alone or in Combination with Ethambutol against *Mycobacterium avium* Complex In Vitro, in Human Macrophages, and in Beige Mice

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Levofloxacin, ofloxacin, and Bay Y 3118 are new fluoroquinolones with variable in vitro bacteriostatic and bactericidal activities against the *Mycobacterium avium* complex (MAC). The potential therapeutic activities of these agents both alone and combined with ethambutol were evaluated in a human macrophage test system and in the beige mouse animal test system with MAC strain 101. Bay Y 3118 at a human-equivalent dose of 30 mg/kg/day for 4 weeks caused a significant reduction in mortality compared with that in untreated controls ($P = 0.02$). Bay Y 3118 also caused significant reductions in the number of MAC organisms in the blood, liver tissue, and spleen tissue compared with those in untreated controls. Levofloxacin at a human-equivalent dose of 200 mg/kg/day was associated with a significant reduction in mortality (10 versus 39%); however, treatment with either levofloxacin or ofloxacin (200 mg/kg/day) did not result in significant reductions in the numbers of MAC organisms in blood, liver, and spleen compared with those in untreated controls. When Bay Y 3118 was combined with ethambutol, there was no enhancement in therapeutic activity except in the spleen in terms of CFU per gram (reductions of 89% compared with the untreated control, 63% compared with Bay Y 3118 alone, and 72.5% compared with ethambutol alone). Levofloxacin in combination with ethambutol was more active than either drug alone in the reduction of organisms in blood, liver, and spleen. Bay Y 3118 was the most active fluoroquinolone for monotherapy of MAC infection in beige mice, and the combination of ethambutol plus either levofloxacin or ofloxacin was at least additive. In summary, this study demonstrates that quinolones, although active, are inhibitory against MAC in vivo and that there is little correlation between the activity of quinolones in vitro and the activity in mice.

Antimicrobial agents with clinically useful activity against the *Mycobacterium avium* complex (MAC) include the new macrolides (azithromycin, clarithromycin, and roxithromycin), amikacin, rifabutin, and ethambutol (5–7, 13, 14, 19, 21, 30), while the activity of clofazimine in vivo is still controversial (11, 19). Nevertheless, additional agents, especially bactericidal agents, are necessary to improve efficacy (most importantly, eradication) and to prevent resistance. In these regards, the potential of fluoroquinolones for the treatment of MAC infections is of considerable interest because of the proven clinical activity of these agents against other mycobacterial species, including *Mycobacterium tuberculosis* and rapidly growing mycobacteria (26, 28, 29), as well as the general rapidly bactericidal activity and low toxicity of quinolones. However, the activities of several quinolones against MAC in vitro and in animal models of infection have been disappointingly poor; e.g., only 30% of MAC isolates have ciprofloxacin MICs within a therapeutic range, and ciprofloxacin has not proven to be clearly active either in animal studies or in limited studies with humans (16, 18, 22). The nature of this apparent paradox has not been explained, but it may reflect host-microbe interactions at the cellular level that cannot be easily mimicked in in vitro or macrophage studies.

Bay Y 3118 is a novel chlorofluoroquinolone with a broad spectrum of antibacterial activity (2, 4). It is as active as ciprofloxacin against gram-negative bacteria; however, its excellent activity against gram-positive bacteria distinguishes Bay Y 3118 from many other quinolones. Recent studies have shown that Bay Y 3118 was efficacious in treating *Listeria monocytogenes* infection in immunocompromised and immunocompetent murine models (23). Levofloxacin is the L enantiomer of ofloxacin and is considered to be the active form of ofloxacin (8). Both ofloxacin and levofloxacin have broad spectra of activity, including activity against both gram-positive and gram-negative bacteremia (4). In addition, anti-*M. tuberculosis* activity both in vitro and in mice (12, 17, 22) has been reported for both levofloxacin and ofloxacin. Here we report on the anti-MAC activity of these quinolones alone and in combination with ethambutol in vitro, in a human macrophage cell line, and in the beige mouse model of disseminated MAC disease.

MATERIALS AND METHODS

Antimicrobial agents. Levofloxacin and ofloxacin (racemic mixture of D and L forms) were provided by R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.; Bay Y 3118 was provided by Bayer (Miles) Pharmaceutical Inc., West Haven, Conn.; and ethambutol was purchased from Sigma Chemical Co., St. Louis, Mo. When such information was available, the potencies of drug stocks were confirmed by using the standard American Type Culture Collection quality control strains *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213. Antimicrobial agents were prepared fresh or stored frozen according to the recommendations of the manufacturers.

Mycobacteria. The strains of MAC used in this study were isolated from the blood of patients with disseminated MAC disease, and each isolate was identified by using commercially available DNA probes (GenProbe Inc., San Diego, Calif.). MAC strain 101 (serovar 1) was used for all macrophage and animal studies. MAC 101 is the most virulent strain we have tested in the beige mouse test

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system, and this strain causes reproducible levels of infection and mortality in beige mice (3). MAC organisms were cultured in Middlebrook agar 7H10 medium (Difco Laboratories, Detroit, Mich.), supplemented with oleic acid, albumin, glucose, and catalase (OADC) (Difco), for 10 days at 37°C. Only transparent colony types were used in the in vitro studies. Also, for macrophage and animal studies transparent colonies were harvested and suspended in Hanks balanced salt solution to a concentration of 3×10^8 CFU/ml by comparison with a McFarland no. 1 turbidity standard; samples were plated onto Middlebrook 7H10 agar to confirm the concentration (inoculum size). Beige mice were infected with 100 μl of the original suspension; however, before the mice were infected, the suspension was vortex agitated for 2 min in order to disperse clumps.

In vitro susceptibility testing. (i) MICs were determined by using a radiometric broth macrodilution method and the T100 method of data analysis (15). The inoculum for susceptibility testing was prepared by picking 5 to 10 colonies (only transparent colony variants) from a 7H11 agar plate onto 7H9 broth and was tested directly or frozen at −70°C. The inoculum was adjusted to approximately 5×10^4 CFU/ml by comparison with a McFarland no. 1 turbidity standard. Isolates that clumped and could not be easily dispersed were shaken with glass beads. The controls included the inoculum undiluted without drug added (no-drug control), the inoculum diluted 1:100 (99% control), and the inoculum diluted 1:1,000 (99.9% control). In addition, one vial was inoculated with a suspension of mycobacteria which were boiled for 5 min prior to inoculation in order to monitor the non-growth-related release of carbon dioxide in the Bactec system. The period of observation and end points were determined by daily monitoring of the control and test cultures, but a period of 7 days was sufficient for most isolates. MAC 101 was tested against amikacin to control for overall test performance. Each antimicrobial agent was tested at five concentrations in increments of log 2 (2). Time-kill curves were used to measure the bactericidal activity of each quinolone either alone or in combination with ethambutol. Bactericidal activity was measured at drug concentrations 1-, 5-, and 10-fold above the respective MIC for each strain tested (MAC 101 and MAC 108). The final concentration of mycobacteria was approximately 10^6 CFU/ml, and this was confirmed by quantitative plate counts. Samples were serially diluted in sterile distilled water and plated onto 7H11 agar plates; the minimum dilution was 1:250 in order to avoid any significant carryover of drug or combination of drugs. Significant bactericidal activity was defined as a reduction in the CFU per milliliter of 2 orders of magnitude over the period of the test (usually 72 h). The time-kill curve assay was done according to the recommendations of Amsterdan (1).

Human macrophage studies. The source of macrophages was the human monocyte cell line U937 cultured in RPMI 1640 medium (pH 7.2) (Gibco, Chicago, Ill.) supplemented with 5% fetal bovine serum (Sigma Chemical Co.) and 2 mM L-glutamine. Cells were grown to a density of 5×10^8 cells per ml and then centrifuged, washed, and resuspended in supplemented RPMI 1640 medium. The concentration of cells was adjusted to 10^6 cells per ml, and 1 ml of the cell suspension was added to each well of a 24-well tissue culture plate (Costar, Cambridge, Mass.). Monolayers were treated with 0.5 mg of phorbol myristate acetate per ml for 24 h to stimulate maturation of the monocytes. The monolayers were monitored for the number of cells, and no difference was observed in the extent of cell detachment among the several treatment and control groups.

MAC 101 was cultured for 10 days in Middlebrook agar 7H10 medium (Difco Laboratories). On the day of the experiment, bacteria were harvested, washed twice in Hanks balanced salt solution, suspended in Hanks balanced salt solution, and sonicated for 5 s to disperse clumps of bacilli. The turbidity of the suspension was adjusted to be equivalent to that of a McFarland no. 1 turbidity standard, and the suspension was diluted to a final concentration of approximately 5×10^7 CFU/ml. Each monolayer was infected with 100 ml of the final suspension, and the actual number of CFU per milliliter of the final suspension was determined by quantitative plate counts. At 4 h after infection, the CFU of mycobacteria per well of macrophage monolayer was determined by lysing the macrophages and performing quantitative plate counts. Ice-cold sterile water (0.5 ml) was added to each monolayer well, and the mixture was allowed to stand for 10 min at room temperature. Then, 0.5 ml of a second lysing solution (1.1 ml of Middlebrook 7H9 broth plus 0.4 ml of 0.25% sodium dodecyl sulfate [SDS] in phosphate buffer) was added to each well, and the mixture was allowed to stand for an additional 10 min. The wells were vigorously scraped with a rubber policeman, and the macrophage lysates were resuspended in 0.5 ml of 20% bovine serum in sterile water to neutralize the SDS. The suspension was then vortex agitated for 2 min to ensure complete lysis of the macrophages. Finally, the lysate was sonicated for 5 s to disrupt clumps of bacilli. To control for the osmotic stability of the mycobacteria, a suspension of mycobacteria alone was plated for quantitation before and after being subjected to the lysis procedure as described above, and in each instance there was no change in the CFU per milliliter before or after the lysis treatment procedure.

The final macrophage lysate suspension was serially diluted, and 0.1 ml was plated on 7H10 agar. The plates were allowed to dry at room temperature for 15 min and incubated at 37°C in 5% CO_2 for 2 weeks. Duplicate plates were prepared for each well, and the results were reported as the mean CFU per milliliter of macrophage lysate. Each experiment was performed in triplicate, and each experiment was repeated six times.

TABLE 1. Treatment regimens, dosages, and number of mice per experimental group

Treatment regimen	Dosage (mg/kg/day)	No. of mice
Bay Y 3118	30	21
Ethambutol	100	24
Bay Y 3118 + ethambutol	30 + 100	23
Ofloxacin	200	20
Levofloxacin	200	17
Ofloxacin + ethambutol	200 + 100	19
Levofloxacin + ethambutol	200 + 100	20
Untreated control	0	44

Animal test system. The potential therapeutic efficacies of antimicrobial agents were determined by using the beige mouse test system as previously described (9, 14); this system employs 6- to 7-week old female C57 BL6 bg^+/bg^+ mice (Jackson Laboratories, Bar Harbor, Maine). Briefly, mice were infected through the caudal vein with 3×10^7 CFU/ml, and after 7 days, treatment was initiated with Bay Y 3118 (30 mg/kg/day), levofloxacin (200 mg/kg/day), or ofloxacin (200 mg/kg/day) or with each of the quinolones (at the aforementioned dosages) plus ethambutol (100 mg/kg/day). Drugs were administered by daily gavage for 28 days. A control group of mice was infected but received water in place of an antibiotic. An additional group of mice was examined 7 days after infection in order to establish the level of infection before the initiation of therapy. The treatment regimens and the number of mice used in each experimental group are shown in Table 1. At the termination of treatment, the livers and spleens of control and treated mice were aseptically removed, and the organs were weighed and then homogenized in 5 ml of Middlebrook 7H9 broth (Difco) with a tissue homogenizer. The tissue suspensions were serially diluted in 7H9 broth and plated on 7H11 agar (Difco) plates supplemented with OADC (Difco) for quantitation of viable bacteria. The level of mycobacteremia was determined by collecting 0.05 ml of blood at day 7 and at day 28. The CFU per milliliter of blood was determined by inoculating a measured volume of blood into 4 ml of Bactec 12B medium (Johnston Laboratories, Sparks, Md.) and using the T100 method of data analysis as previously described (15).

Statistical analysis. The differences between results for untreated control and experimental groups in macrophage experiments at identical time points were determined by the Student *t* test. The statistical significance of the differences between the numbers of viable organisms recovered from the spleen, liver, and blood were evaluated by one- or two-variable analyses of variance. Differences between results for experimental groups and between results for experimental groups and control groups were considered statistically significant if P values were <0.05.

RESULTS

MIC studies. The MIC range, MIC at which 50% of the isolates were inhibited (MIC_{50}), and MIC_{90} (in micrograms per milliliter) of levofloxacin were <1 to >8, 8, and >8, respectively, and those of Bay Y 3118 were 0.06 to 2, 0.25, and 1, respectively. Bay Y 3118 was significantly more active than levofloxacin on a weight basis, while lexofloxacin was approximately twice as active as ofloxacin (a mixture of D- and L enantiomers) on a weight basis (data not shown). When MAC strain 101 was tested against a combination of ethambutol and each of the quinolones, there was a 1-dilution decrease in the combined MIC (based on a fractional inhibitory concentration calculation) with ethambutol at 1 μg/ml. The MICs of ethambutol, Bay Y 3118, ofloxacin, and levofloxacin against MAC 101 were 4, 0.5, 8, and 16 μg/ml, respectively. The fractional inhibitory concentration was 0.75, suggesting that the interaction between drugs was additive or slightly synergistic (data not shown). The MIC ranges indicate that the MAC organisms are quite heterogeneous in their susceptibilities to these quinolones, with a nearly continuous increase in the percentage of strains susceptible at increasing concentrations of drug.

Time-kill curve studies. Bay Y 3118 was potentially bactericidal against MAC 101, with a 3-order-of-magnitude (99.9%) reduction in survival of mycobacteria exposed to one or five times the MIC for 60 to 70 h (Fig. 1). When combined with

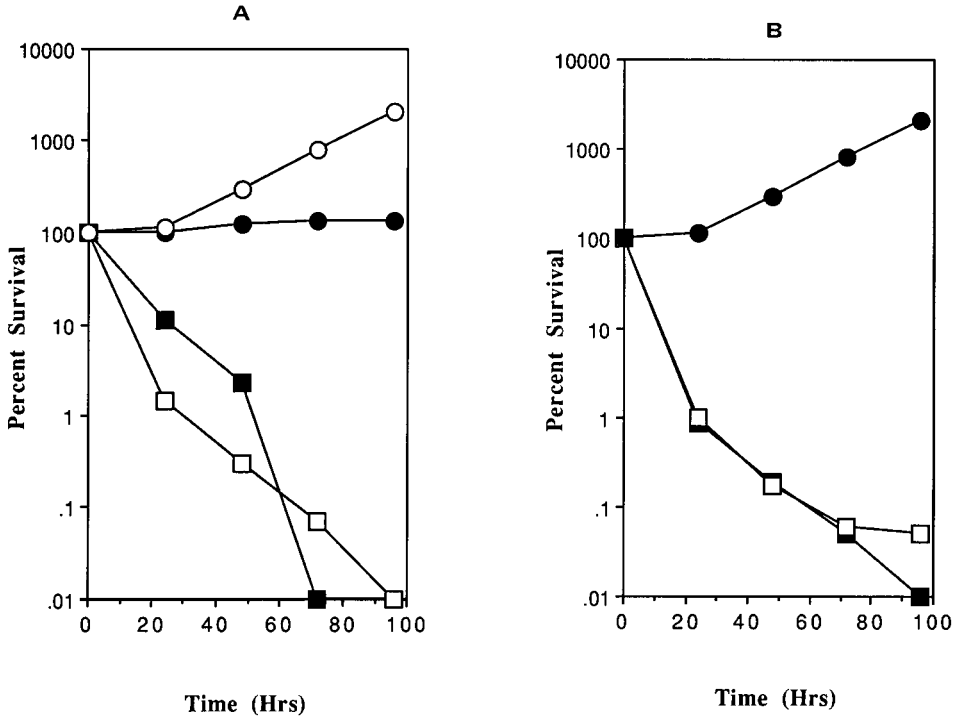

FIG. 1. Time-kill curves of Bay Y 3118 bactericidal activity in Middlebrook 7H9 broth with OADC over 96 h. (A) MAC strain 101 was incubated with no drug (○), ethambutol alone (●), one times the MIC of Bay Y 3118 (■), or five times the MIC of Bay Y 3118 (□). (B) MAC strain 101 was incubated with no drug (●), one times the MIC of Bay Y 3118 plus one times the MIC of ethambutol (■), or five times the MIC of Bay Y 3118 plus one times the MIC of ethambutol (□). The MICs of Bay Y 3118 and ethambutol were 0.5 and 4 μg/ml, respectively.

ethambutol, Bay Y 3118 was slightly more bactericidal at concentrations equal to or fivefold above the MIC for the test strain (Fig. 1). Levofloxacin and ofloxacin were also bactericidal for MAC 101 (but these activities were defined as a 2-order-of-magnitude decrease in CFU per milliliter within 72 h after exposure to the drug) at concentrations 5- to 10-fold above the MIC (Fig. 2). For a more susceptible strain (MAC 108) with a levofloxacin MIC of 4 μg/μl or an ofloxacin MIC of 8 μg/ml, the drugs were bactericidal at or near the MIC for both levofloxacin and ofloxacin (Fig. 2), while ethambutol had no effect on the killing activity of either quinolone against MAC 108 (data not shown). When combined with ethambutol, MAC 101 was killed at or near the MIC for both levofloxacin and ofloxacin (Fig. 2), while ethambutol had no effect on the killing activity of either quinolone against MAC 108 (data not shown).

Human macrophage studies. Bay Y 3118 significantly inhibited MAC growth within macrophage cell line U937 at 4 μg/ml, whereas the combination of Bay Y 3118, even at 0.25 μg/ml, and ethambutol (4 μg/ml) resulted in bactericidal activity (Table 2). Levofloxacin and ofloxacin were active against MAC in the human macrophage cell line at extracellular concentrations of 4 and 8 μg/ml, respectively. The combination of either of these quinolones with ethambutol resulted in significantly greater bacteriostatic activity; however, intracellular bacteria were not killed (Tables 3 and 4).

Animal studies of Bay Y 3118. Bay Y 3118 was administered at a dosage of 30 mg/kg/day for 4 weeks to MAC-infected beige mice in order to evaluate the therapeutic potential of this drug for disseminated MAC infection. The dosage of 30 mg/kg/day was based on pharmacokinetic studies [performed in collaboration with Bayer (Miles) Pharamaceutical Inc.] that showed that levels in serum and tissue achieved with 30 mg/kg/day given orally in beige mice, taking into consideration the rate of Bay Y 3118 metabolism in mice, are similar to the levels obtained in humans at an appropriate therapeutic dose (maximum concentration = 0.98 μg/ml; time to maximum concentration = 1 h; half-life = 4.03 h). The mortality in Bay Y 3118-treated animals was 14% compared with 39% in the untreated control group ($P = 0.002$). Furthermore, treatment with Bay Y 3118 at 30 mg/kg/day resulted in a significant reduction in the number of bacteria in the blood compared with that for the untreated control group ($P = 0.01$) and also was associated with significant reductions in the CFU per gram of liver ($P = 0.015$) and spleen ($P = 0.01$) compared with the levels of infection in the same sites in the untreated control

TABLE 2. Activity of Bay Y 3118 alone and in combination with ethambutol against MAC 101 within macrophages

Agent(s)[a]	Dose (μg/ml)	CFU/macrophage lysate[b] (mean ± SD)	P[c]
None	0	$(9.2 \pm 0.5) \times 10^5$	
BAY	0.25	$(3.8 \pm 0.2) \times 10^5$	
	1.0	$(1.0 \pm 0.4) \times 10^5$	1
	4.0	$(5.5 \pm 0.4) \times 10^3$	1
EMB	4.0	$(9.5 \pm 0.4) \times 10^4$	1
BAY + EMB	0.25 + 4.0	$(4.0 \pm 0.1) \times 10^3$	1, 2, 3
	1.0 + 4.0	$(6.1 \pm 0.2) \times 10^3$	1, 2, 3
	4.0 + 4.0	$(3.0 \pm 0.5) \times 10^3$	1, 3

[a] BAY, Bay Y 3118; EMB, ethambutol. Infected monolayers were treated with the antibiotic(s) daily for 4 days.

[b] Number of viable mycobacteria at day 4. Starting inoculum (day 0), $(2.4 \pm 0.6) \times 10^4$.

[c] 1, $P < 0.05$ compared with untreated control; 2, $P < 0.05$ compared with Bay Y 3118 alone; 3, $P < 0.05$ compared with ethambutol alone.

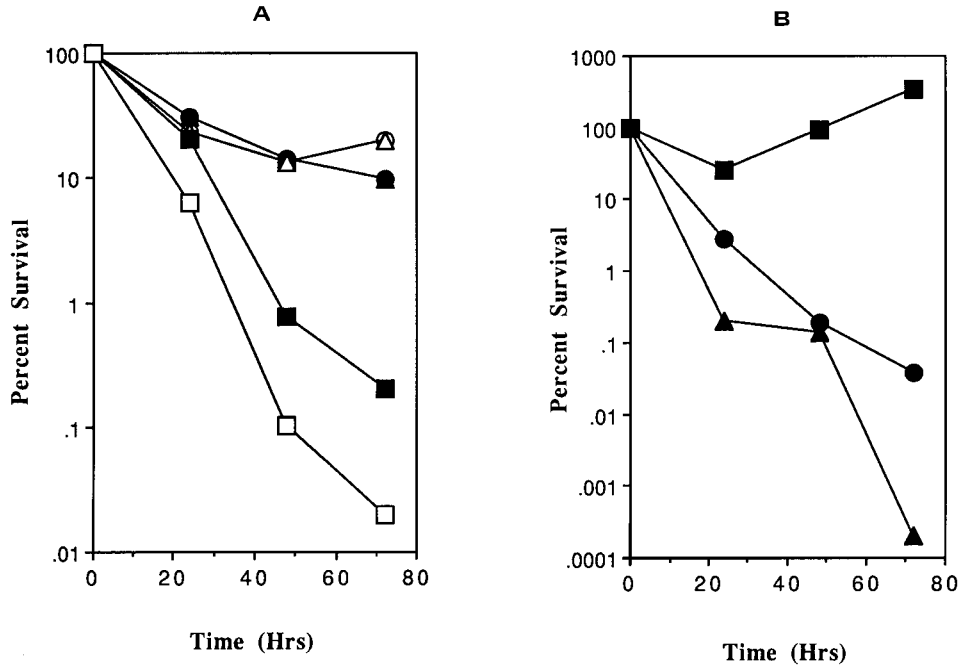

FIG. 2. Time-kill curves of ofloxacin and levofloxacin bactericidal activities in Middlebrook 7H9 broth with OADC over 96 h. (A) MAC strain 101 was incubated with 1 times the MIC (circles), 5 times the MIC (triangles), or 10 times the MIC (squares) of ofloxacin (open symbols) or levofloxacin (closed symbols). (B) MAC strain 101 was incubated with ethambutol alone (squares) or with ofloxacin plus ethambutol (circles) or levofloxacin plus ethambutol (triangles) (one times the MIC of each drug in each combination). The MICs of ofloxacin and levofloxacin for MAC strain 101 were 16 and 8 μg/ml, respectively. The curve for a no-drug control is shown in Fig. 1A.

group (Table 5). Nonetheless, treatment with Bay Y 3118 was only bacteriostatic and not bactericidal.

Animal studies of levofloxacin and ofloxacin. Preliminary dose range experiments with beige mice established that a levofloxacin or ofloxacin dose of 200 mg/kg was well tolerated and most likely to be effective, on the basis of the in vitro results. Treatment with levofloxacin resulted in a significant reduction of mortality compared with that in the untreated control group (10 versus 38%, respectively), but there was no difference in mortality between the ofloxacin-treated group and the untreated control group (37 and 38%, respectively). Neither levofloxacin nor ofloxacin treatment resulted in a significant reduction in bacteremia or CFU per organ (spleen or liver) compared with that in the untreated control group (Table 6).

Combinations of quinolones and ethambutol. When MAC-infected mice were treated with Bay Y 3118 (30 mg/kg/day) in combination with ethambutol (100 mg/kg/day), the mortality in this group (17%) was the same as that of mice treated with Bay Y 3118 alone (14%) and significantly better than those of mice treated with ethambutol alone (33%) or the untreated control mice (39%) ($P < 0.05$). The effect of Bay Y 3118 alone and in combination with ethambutol on the mycobacteremia is shown in Table 5. The combination reduced the bacteremia compared with the result with either Bay Y 3118 and ethambutol alone; the difference was not statistically significant when the combination was compared with Bay Y 3118, but the combination of Bay Y 3118 and ethambutol significantly ($P = 0.04$) reduced the bacteremia compared with the result with ethambutol alone. In addition, treatment with the combination of Bay Y 3118 and ethambutol led to a significant reduction in CFU per gram in both the spleen and liver compared with ethambutol alone ($P = 0.02$) and in the spleen compared with Bay Y 3118

TABLE 3. Activity of levofloxacin alone and in combination with ethambutol against MAC 101 within macrophages

Agent(s)[a]	Dose (μg/ml)	CFU/macrophage lysate[b] (mean ± SD)	P[c]
None	0	$(6 \pm 0.4) \times 10^5$	
EMB	4.0	$(3.6 \pm 0.4) \times 10^5$	1
LFX	8.0	$(1.6 \pm 0.5) \times 10^5$	1
LFX + EMB	4.0 + 4.0	$(7.3 \pm 0.4) \times 10^4$	1, 2
	8.0 + 4.0	$(3.1 \pm 0.4) \times 10^4$	1, 2, 3
EMB	4.0	$(9.6 \pm 0.3) \times 10^4$	1

[a] LFX, levofloxacin; EMB, ethambutol. Infected monolayers treated with the antibiotic(s) for 4 days.
[b] Number of viable mycobacteria at day 4. Starting inoculum (day 0), $(1.4 \pm 0.5) \times 10^4$.
[c] 1, $P < 0.05$ compared with untreated control; 2, $P < 0.05$ compared with levofloxacin alone; 3, $P < 0.05$ compared with ethambutol alone.

TABLE 4. Activity of ofloxacin alone and in combination with ethambutol against MAC 101 within macrophages

Agent(s)[a]	Dose (μg/ml)	CFU/macrophage lysate[b] (mean ± SD)	P[c]
None	0	$(2 \pm 0.6) \times 10^5$	
OFX	4.0	$(2.4 \pm 0.3) \times 10^5$	
	8.0	$(9.5 \pm 0.4) \times 10^4$	1
OFX + EMB	4.0 + 4.0	$(8.6 \pm 0.4) \times 10^4$	1
	8.0 + 4.0	$(4 \pm 0.3) \times 10^4$	1, 2, 3
EMB	4.0	$(7.8 \pm 0.4) \times 10^4$	1

[a] OFX, ofloxacin; EMB, ethambutol. Infected monolayers were treated with the antibiotic(s) for 4 days.
[b] Number of viable mycobacteria at day 4. Starting inoculum (day 0), $(4.1 \pm 0.4) \times 10^4$.
[c] 1, $P < 0.05$ compared with untreated control; 2, $P < 0.05$ compared with ofloxacin alone; 3, $P < 0.05$ compared with ethambutol alone.

TABLE 5. Effect of Bay Y 3118 alone and in combination with ethambutol on bacteremia and load of bacteria in livers and spleens of beige mice infected with MAC 101

Regimen	Dosage (mg/kg/day)	Mean bacteremia (SEM)[a,b]	Mean CFU/g (SEM)[b,c] in:	
			Liver	Spleen
Bay Y 3118	30	−0.81 (0.15)*	2.8×10^8 (3.3×10^7)	6.5×10^{8}* (1×10^8)
Ethambutol	100	−0.45 (0.22)*	3.1×10^{8}* (6.7×10^7)	8.0×10^{8}* (1.0×10^8)
Bay Y 3118 + ethambutol	30 + 100	−0.97 (0.23)*	1.3×10^{8}* (3.1×10^7)	2.4×10^{8}† (4.6×10^7)
Untreated control		0.43 (0.32)	8.6×10^8 (1.3×10^8)	2.2×10^9 (1.3×10^8)
1-wk control			2.2×10^8 (3.1×10^7)	4.8×10^8 (8.0×10^7)

[a] Obtained for each experimental animal as the difference in log CFU per milliliter between week 1 and week 4.
[b] *, $P < 0.05$ compared with untreated control.
[c] †, $P = 0.06$.

alone ($P = 0.04$) and with the untreated control group ($P < 0.001$) 28 days after infection (Table 5).

Levofloxacin (200 mg/kg/day) administered in combination with ethambutol (100 mg/kg/day) resulted in a significant reduction in the mycobacteremia (−1.52 log CFU compared with −0.36 log CFU for levofloxacin alone, −0.80 log CFU for ethambutol alone, and 1.02 log CFU for the untreated control). This combination also resulted in a significant reduction of tissue infection, with a reduction of 95% in the liver (versus 29% with levofloxacin alone and 88% with ethambutol alone; $P < 0.05$ for each comparison) and a reduction of 90% in the spleen (versus 35% for levofloxacin alone and 82% for ethambutol alone; $P < 0.05$ for each comparison). Treatment with ofloxacin (200 mg/kg/day) in combination with ethambutol yielded similar results. The combination of ofloxacin and ethambutol was significantly more active than ethambutol alone in the blood (−1.53 log CFU versus −0.80 log CFU; $P < 0.05$), liver (−93% versus −88%; $P < 0.04$), and spleen (−91% versus −82%; $P = 0.01$) (Table 6).

DISCUSSION

In vitro, Bay Y 3118 is one of the most active (on a weight basis) quinolones that we or others have tested against MAC. Bay Y 3118 was bactericidal for two MAC strains at concentrations 5 to 10 times above the MIC; i.e., at concentrations that are believed to be achieved in phagocytic cells at usual dosages. The in vitro activity of Bay Y 3118 was reflected in the studies carried out with the U937 macrophage cell line, which showed that there was a dose-dependent effect on intracellular mycobacteria. The difference in CFU between Bay Y 3118-treated and untreated MAC-infected U937 macrophages was statistically significant, with an approximately 200-fold reduction in CFU per macrophage compared with that for untreated controls. The addition of ethambutol to Bay Y 3118 resulted in a statistically significant increase in bactericidal activity, but whether the increase was biologically significant was questionable.

Bay Y 3118 was marginally effective as a monotherapeutic agent in the beige mouse model of disseminated MAC infection. When Bay Y 3118 was combined with ethambutol, there was an additive effect in spleen tissue only. Ethambutol is a cell wall synthesis inhibitor, and there is evidence that ethambutol potentiates the activities of other agents, including quinolones, by increasing the permeability of the MAC cell wall (24); however, the lack of a uniform additive effect detracts from the likelihood of this conclusion. The effect of Bay Y 3118 was primarily bacteriostatic against MAC in vivo; for example, the CFU per gram of liver and spleen of mice treated with Bay Y 3118 alone was greater than that 7 days after the initiation of therapy but lower than that in the untreated control group of animals after 4 weeks of therapy. The bactericidal effect of Bay Y 3118 in combination with ethambutol in spleen tissue is consistent with the in vitro results; however, again this was not a uniform observation.

Levofloxacin and ofloxacin are fluoroquinolones which are active against *M. tuberculosis* in vitro, in macrophages, and in animal models (12, 17, 22), and ofloxacin has proven clinical utility in the treatment of tuberculosis in humans (27). Against MAC, levofloxacin and ofloxacin were marginally active, with MIC_{50}s and MIC_{90}s that exceeded or equaled maximum concentrations in serum. In vivo, levofloxacin and ofloxacin (at doses of 200 mg/kg/day) were inactive against MAC. However, the combination of either quinolone with ethambutol resulted in a statistically significant increase in activity. This observation may be clinically relevant, since ethambutol monotherapy may have a significant effect in reducing MAC bacteremia in AIDS patients (20).

The results of the studies reported here largely add to the paradox of findings that quinolones with good to excellent in

TABLE 6. Effect of treatment with levofloxacin and ofloxacin alone or in combination with ethambutol against MAC 101 in blood, spleens, and livers of beige mice

Regimen	Dosage (mg/kg/day)	Mean bacteremia (SEM)[a,b]	CFU/g (SEM)[b] in:	
			Liver	Spleen
Levofloxacin	200	−0.36*	6.2×10^8 (7.2×10^7)	1.1×10^9 (2.0×10^8)
Ofloxacin	200	0.07	6.0×10^8 (1.2×10^8)	1.2×10^9 (2.2×10^8)
Ethambutol	100	−0.80*	9.4×10^7 (1.1×10^7)	2.9×10^8 (4.7×10^7)*
Levofloxacin + ethambutol	200 + 100	−1.52†	4.3×10^7 (4.8×10^6)	1.1×10^8 (1.4×10^7)*†
Ofloxacin + ethambutol	200 + 100	−1.52†	5.3×10^{7}*† (7.6×10^6)	1.5×10^8 (3.2×10^7)
Untreated control		1.02	8.7×10^{8}* (1.3×10^8)	1.7×10^9 (2.7×10^8)
1-wk control			1.3×10^8 (2.5×10^7)	1.8×10^8 (3.1×10^7)

[a] Obtained for each experimental animal as the difference in log CFU per milliliter between week 1 and week 4.
[b] *, $P < 0.05$ compared with control; †, $P < 0.05$ compared with either quinolone alone or with ethambutol alone.

vitro activity against MAC have marginal activity in the beige mouse test system for disseminated MAC disease and humans (20, 31). It is possible that quinolones, although concentrated within uninfected macrophages, cannot achieve bactericidal concentrations in infected phagocytes. Garcia et al. (10) recently showed that Bay Y 3118 uptake was significantly decreased in human leukocytes infected with *S. aureus*, and we recently determined (1a) that infection of macrophages with MAC (for 3 days in vitro) triggers the efflux of the rifamycin KRM 1648. Intriguing, however, is the recent observation that levofloxacin is active against *M. tuberculosis* in vivo (17), suggesting that such a quinolone efflux system may not be present in *M. tuberculosis*. Quinolone efflux from *S. aureus* has been described and is regulated by the *norA* gene (18). The possibility that the intramacrophage environment triggers a similar mechanism in MAC seems unlikely on the basis of the macrophage studies reported here, but this possibility may warrant further study.

The treatment of disseminated MAC infection in AIDS patients remains a challenge (20, 25, 26), and quinolones remain an attractive class of antimicrobial agents for treating this disease; however, the use of fluoroquinolones in experimental animals as well as in human clinical trials for MAC disease has been disappointing (20, 31). Clearly, if there is a real role for quinolones in the treatment of MAC disease, the basis for the paradox described in this paper is likely to provide valuable insight into the rational design of quinolones with potent therapeutic effectiveness.

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