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In Vitro Activities of Levofloxacin Used Alone and in Combination with First- and Second-Line Antituberculous Drugs against *Mycobacterium tuberculosis*

NALIN RASTOGI,[1]* KHYE SENG GOH,[1] ANDRE BRYSKIER,[2,3] AND ANNE DEVALLOIS[1]

Unité de la Tuberculose et des Mycobactéries, Institut Pasteur, 97165-Pointe à Pitre Cedex, Guadeloupe, French West Indies,[1] and Domaine Antibiothérapie, Hoechst-Marion-Roussel, 93230 Romainville,[2] and Laboratoire de Microbiologie, Centre Hospitalier Victor Dupouy, 95107 Argenteuil Cedex,[3] France

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By using the radiometric BACTEC 460-TB methodology, the inhibitory and bactericidal activity of the optically active L-isomer of ofloxacin (levofloxacin) was compared with those of the D-isomer and the commercially available mixture containing equal amounts of DL-isomers (ofloxacin) against the *Mycobacterium tuberculosis* complex (type strain H37Rv, a panel of drug-susceptible and -resistant clinical isolates including multidrug-resistant isolates of *M. tuberculosis*, as well as *M. africanum*, *M. bovis*, and *M. bovis* BCG). Levofloxacin MICs (range, 0.50 to 0.75 μg/ml) were about 1 dilution lower than those of ofloxacin (MIC range, 0.75 to 1.00 μg/ml) and 5 to 6 dilutions lower than those of the D-isomer (MIC range, 32 to 60 μg/ml). The MICs of levofloxacin, ofloxacin, and D-ofloxacin at which 90% of the strains are inhibited were 0.50, 1.00, and 64 μg/ml, respectively. The multidrug-resistant *M. tuberculosis* strains resistant to first-line drugs were as susceptible to quinolones as the wild-type drug-susceptible isolates. Levofloxacin at 0.5 μg/ml showed bactericidal activity comparable to the activities of 1.0 μg of ofloxacin per ml and 64 μg of D-ofloxacin per ml, with MBCs within the range of 0.5 to 2.0 μg/ml, compared with MBCs of 0.75 to 4.0 μg of ofloxacin per ml for *M. tuberculosis*, *M. africanum*, *M. bovis*, and *M. bovis* BCG. Combination testing of sub-MICs of levofloxacin with other first-line (isoniazid, rifampin, and ethambutol) and second-line (amikacin and clofazimine) antituberculous drugs was evaluated with various two-, three-, and four-drug combinations; enhanced drug activity was observed in 8 of 25, 12 of 20, and 8 of 15 tests, respectively, indicating that levofloxacin acts in synergy with other antituberculous drugs.

The treatment of multidrug-resistant (MDR) tuberculosis (MDR-TB) remains extremely difficult (12) and requires meticulous laboratory studies to characterize the susceptibilities of the *Mycobacterium tuberculosis* isolates to drugs that have high levels of bactericidal activity and that act by mechanisms other than those involved with first-line antituberculous drugs. However, established critical concentrations are unavailable for most of the second-line and newer antituberculous drugs, and consequently, a variety of second-line antituberculous drugs, new analogs of existing drugs, and newer drug combinations against *M. tuberculosis* should be investigated as a priority research issue (28). Furthermore, considering that at least two or more bactericidal drugs are required to avoid the emergence of resistance in *M. tuberculosis* (as a result of spontaneous mutations) and reports of increasing resistance to isoniazid and rifampin (3, 33), rapid isolation, presumptive identification, and drug susceptibility results are a must in the fight against tubercle bacilli, which can be accomplished today by routinely using the radiometric BACTEC 460-TB methodology (23).

One of the first reports describing the use of quinolones in the chemotherapy of tuberculosis provided the results of a study of ofloxacin monotherapy of 19 patients with advanced cavitary disease who did not respond positively to chemotherapy with conventional antituberculous drugs; 5 patients converted to *M. tuberculosis* negativity, whereas the remaining 14 patients had substantial decreases in the numbers of bacilli in their sputum, leading to the conclusion that ofloxacin was indeed an active antituberculous drugs (39).

Ofloxacin consists of equal amounts of two optically active isomers, the D- and the L-isomers (9). Levofloxacin is the optically active L-isomer of ofloxacin, and the antimicrobial activity of levofloxacin is 8 to 128 times greater than that of the corresponding D-isomer (9). Levofloxacin is about twice as potent as ofloxacin against various microorganisms including *M. tuberculosis*, a variety of atypical mycobacteria, and *M. leprae* (5, 7, 9, 15, 18, 20, 34, 38, 40). At the pharmacokinetic level, both levofloxacin and ofloxacin are characterized by similar absorption rates (7, 19, 38, 40), with a higher intracellular concentration/extracellular concentration ratio for levofloxacin compared with that for ofloxacin in cultured human macrophages (38.44 ± 1.1 versus 27.1 ± 0.8 μg/ml, respectively) (18). As for the other quinolones (2), the higher in vitro activity of levofloxacin compared with that of ofloxacin against bacterial DNA gyrase (11) is an important parameter relating to its potency. Information concerning the in vitro activity of levofloxacin against *M. tuberculosis* is limited (13, 18, 34), and the results of studies concerning its use in combination with other first-line and second-line antituberculous drugs are lacking.

Consequently, the present investigation was planned to evaluate the antimicrobial activity of the commercially available DL-mixture of ofloxacin compared with those of its D- and L-isomers against the *M. tuberculosis* complex (comprising *M. tuberculosis*, *M. africanum*, *M. bovis*, and *M. bovis* BCG) and to assess the activity of levofloxacin in combination with other antituberculous drugs which included isoniazid, rifampin, ethambutol, amikacin, and clofazimine.

* Corresponding author. Mailing address: Unité de la Tuberculose et des Mycobactéries, Institut Pasteur, B.P. 484, 97165-Pointe à Pitre Cedex, Guadeloupe, French West Indies. Phone: (590) 893-881. Fax: (590) 893-880.

TABLE 1. Spectra of activity of D-ofloxacin, ofloxacin, and levofloxacin against members of the *M. tuberculosis* complex

Species	D-Ofloxacin MIC (μg/ml)[a]	Ofloxacin			Levofloxacin		
		MIC (μg/ml)	MBC (μg/ml)[b]	MBC/MIC ratio	MIC (μg/ml)	MBC (μg/ml)[b]	MBC/MIC ratio
M. tuberculosis (drug-susceptible isolates)							
Type strain H37Rv	64.0	1.00	4.0	4	0.50	2.0	4
Clinical isolate 001-005	64.0	1.00	4.0	4	0.50	2.0	4
Clinical isolate 001-006	64.0	1.00	2.0	2	0.50	1.0	2
Clinical isolate 001-007	64.0	1.00	2.0	2	0.50	1.0	2
Clinical isolate 001-008	64.0	0.75	1.5	2	0.50	1.0	2
Clinical isolate 001-009	64.0	1.00	4.0	4	0.50	1.0	2
M. tuberculosis (drug-resistant isolates)							
Clinical isolate 001-010[c]	32.0	0.75	1.5	2	0.50	1.0	2
Clinical isolate 001-011[d]	32.0	0.75	1.5	2	0.50	1.0	2
Clinical isolate 001-012[e]	64.0	1.00	2.0	2	0.75	0.75	1
Clinical isolate 001-013[e]	64.0	1.00	4.0	4	0.75	1.5	2
M. africanum							
Type strain ATCC25420	64.0	1.00	4.0	4	0.50	2.0	4
Clinical isolate 003-002	64.0	0.75	1.5	2	0.50	1.0	2
Clinical isolate 003-003	64.0	1.00	2.0	2	0.50	1.0	2
M. bovis							
Type strain ATCC 19210	64.0	0.75	3.0	4	0.50	2.0	4
Clinical isolate 002-002	64.0	0.75	3.0	4	0.50	1.0	2
Clinical isolate 002-003	64.0	1.00	4.0	4	0.50	2.0	4
M. bovis BCG							
BCG Pasteur	64.0	0.75	1.5	2	0.50	1.0	2
BCG Denmark	32.0	0.75	0.75	1	0.50	0.5	1
BCG Russia	64.0	1.00	2.0	2	0.50	1.0	2

[a] MICs were determined radiometrically with BACTEC 12B medium (pH 6.8 ± 0.2).
[b] MBCs were defined as the minimal drug concentration resulting in ≥2-log killing of the initial bacterial inoculum.
[c] MDR-TB clinical isolate resistant to isoniazid, rifampin, ethambutol, streptomycin, ethionamide, and D-cycloserine by the proportional method on 7H11 agar.
[d] Clinical isolate resistant to isoniazid and streptomycin by using 7H11 medium.
[e] MDR-TB clinical isolate resistant to isoniazid and rifampin by using 7H11 medium.

MATERIALS AND METHODS

Organisms. Nineteen strains from our own culture collection belonging to the *M. tuberculosis* complex (see Table 1) were selected for the study. The strains were kept frozen at −40°C as small aliquots and were cultured in Löwenstein-Jensen (LJ) medium prior to the experiments.

MIC and MBC determinations in 7H12 broth. In agreement with a recent study describing levofloxacin MICs and MBCs for *M. tuberculosis* (18), the MIC was defined as the lowest drug concentration that inhibited more than 99% of the bacterial population within 7 to 8 days of observation, and the MBC was defined as the lowest drug concentration that was able to kill the bacterial population by 2 or more $\log_{10}$ units within the same period of incubation. MICs were essentially determined as reported previously (17, 18, 25–27, 35, 36); the bacteria were scraped from fresh LJ slants, resuspended in 3 ml of diluting fluid, and homogenized with glass beads (2 mm in diameter). The suspension was left to stand for a few minutes to sediment the bacterial clumps, and 0.1 ml of homogeneous supernatant (turbidity adjusted with diluting fluid to be equivalent to that of a no. 1 McFarland standard) was injected into a BACTEC 12B vial (Becton-Dickinson Diagnostics Instruments Systems, Sparks, Md.). The contents of this vial were used as the primary inoculum after the growth index (GI) reached 500, as follows. A total of 0.1 ml of the bacterial suspension (10^4 to 10^5 CFU/ml) from the preculture vial was injected into drug-containing vials. Drug concentrations initially ranged in a twofold dilution from 0.25 to 8.0 μg/ml for ofloxacin and levofloxacin and from 4.0 to 64.0 μg/ml for D-ofloxacin. Strains for which the MIC range was ≥0.5 to ≤1.0 μg/ml were retested with intermediate twofold concentration ranges of 0.75 to 6.0 μg/ml for more precise MIC and MBC determinations.

The two controls included a first vial inoculated with the same number of organisms as the drug-containing vials and a second control vial (also called the 1:100 control vial) containing an initial bacterial inoculum diluted 100-fold (10^2 to 10^3 CFU/ml). Test vials as well as control vials were incubated at 37°C, and the GI was recorded daily. When the GI of the 1:100 control vial reached 30, the GI was read at least 1 additional day before the test was terminated. The results were interpreted as follows. If the difference in the GI values from the previous day (ΔGI) in the case of the drug-containing vials was less than the ΔGI of the 1:100 control vial, then the drug concentration tested was considered to have inhibited more than 99% of the bacterial population and was designated the MIC. This approach has been justified previously on the basis of CFU counts made in parallel from radiometric broth culture vials (17).

For the MBC determination, bacterial viability was determined by plating the bacterial suspensions from individual BACTEC vials at the beginning and at the end of the experiments (18, 25). For this purpose, 0.1 ml of the culture from BACTEC vials was taken and serially diluted 10-fold to provide successive dilutions ranging from 10^{-1} to 10^{-5}. Bactericidal activity was determined by plating a 0.1-ml aliquot from each of the 10-fold dilutions to 7H11 agar plates. CFU counts were assessed after 21 days of incubation at 37°C. The successive dilutions and the minimal plating volume used under our experimental conditions avoided any artifactual decrease in bacterial viable counts because of drug carryover. The results were expressed as mean viable count ± standard error.

Drug combination studies. Drug combination studies were performed as reported earlier for *M. avium* (29–31), except that the BACTEC vials were inoculated with 0.1 ml of an undiluted primary culture vial grown to a GI of 500 (because of the relatively slower growth rate of *M. tuberculosis* organisms compared with that of *M. avium* isolates). All of the drugs were used at sub-MICs, because at these concentrations, the drugs used alone were unable to significantly reduce the initial bacterial inoculum in the BACTEC vials (see Fig. 4A). Thus, any significant enhancement of drug activity obtained at these sub-MICs may indicate potential activity in *M. tuberculosis*-infected host cells, in which these drugs are available at much higher concentrations. For example, for a peak concentration of 15 μg/ml in serum and to achieve two to three times the extracellular concentration within the macrophages (23), rifampin was used in the range of only 0.02 to 0.2 μg/ml for drug-susceptible strains in radiometric combination studies. The action of the combined drugs was equal to *x/y*, where *x* was the BACTEC GI obtained with the combination of two or more drugs, and *y* was the lowest GI obtained at the same time with any of the drugs used alone. The sub-MICs used and the *x/y* quotient interpretations are provided in the footnotes to Table 2.

Drugs. Levofloxacin, ofloxacin, and D-ofloxacin (Roussel-Uclaf, Romainville, France) and clofazimine (Ciba-Geigy, Basel, Switzerland) were kindly provided by their manufacturers, whereas all other drugs used in the investigation were purchased from Sigma Chemical Co., St. Louis, Mo.

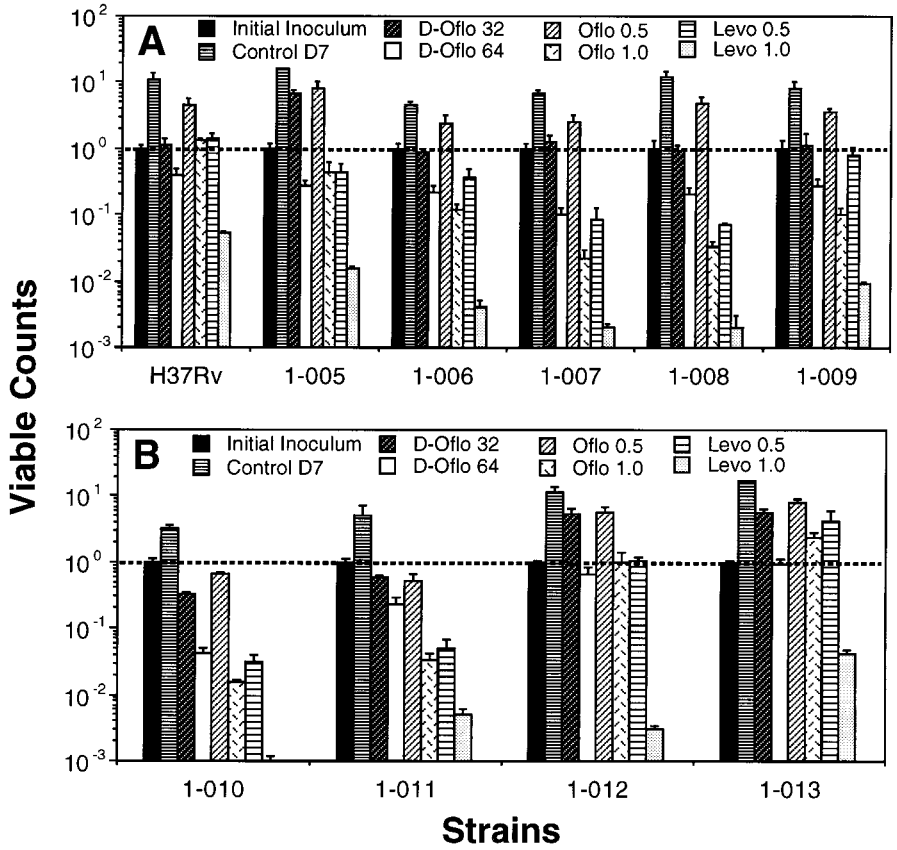

FIG. 1. Comparative bactericidal effects of levofloxacin, ofloxacin, and D-ofloxacin in BACTEC 7H12 vials against drug-susceptible (A) and drug-resistant (B) strains of *M. tuberculosis*. Results illustrate mean viable counts ± standard error in the presence of selected drug concentrations after 7 days of incubation at 37°C compared with those of the growth in untreated control vials. CFU were enumerated by plating the bacterial suspensions from individual BACTEC vials at the beginning and at the end of the experiments onto 7H11 agar medium. The initial inoculum (dashed line) varied from $(1.17 \pm 0.21) \times 10^4$ to $(7.5 \pm 1.5) \times 10^4$ CFU/ml, depending on the individual isolates, and was taken as 1 to facilitate comparison between various isolates. Control D7, control at day 7; D-Oflo, D-ofloxacin; Oflo, ofloxacin; Levo, levofloxacin; the numbers after the drug abbreviations indicate concentrations (in micrograms per milliliter).

RESULTS

Radiometric MICs. The comparative MICs of levofloxacin, ofloxacin, and D-ofloxacin for the type strain H37Rv, a panel of drug-susceptible and -resistant clinical isolates including MDR strains of *M. tuberculosis*, and a panel of strains belonging to the *M. tuberculosis* complex (*M. africanum*, *M. bovis*, and *M. bovis* BCG) are provided in Table 1. Both levofloxacin and ofloxacin were active against all 19 strains tested, including the MDR-TB isolates, whereas D-ofloxacin showed little inhibitory activity by itself (Table 1); levofloxacin MICs (range, 0.50 to 0.75 μg/ml) were about 1 dilution lower than those of ofloxacin (MIC range, 0.75 to 1.00 μg/ml) and 5 to 6 dilutions lower than those of the D-isomer (MIC range, 32 to 60 μg/ml). The MIC of levofloxacin, ofloxacin, and D-ofloxacin at which 90% of the strains are inhibited were 0.50, 1.00, and 64 μg/ml, respectively. The MDR-TB strains resistant to first-line drugs were as susceptible to the quinolones as the wild-type drug-susceptible isolates. Despite differences in the MICs observed among the three quinolones, the MICs of each drug were within an extremely narrow range for all 19 strains of the *M. tuberculosis* complex, indicating that the activities of quinolones remain unaltered in the case of MDR-TB isolates resistant to routinely used first-line drugs.

Bactericidal activity. The bactericidal effects of various quinolones in the BACTEC system against both drug-susceptible and drug-resistant clinical isolates of *M. tuberculosis* (Fig. 1) and other members of the *M. tuberculosis* complex (Fig. 2) were compared by plating the bacterial suspensions from individual BACTEC vials at the beginning and at the end of the experiments onto 7H11 agar medium for viable count enumeration. Levofloxacin at 0.5 μg/ml showed bactericidal activity comparable to those of 1.0 μg of ofloxacin per ml and 64 μg of D-ofloxacin per ml, and at 1.0 μg/ml, it resulted in more than 99% killing of the initial bacterial inoculum in 7 of 10 *M. tuberculosis* isolates (including 3 MDR-TB isolates) and 95 to 98% killing in the case of the remaining 3 isolates (including 1 MDR-TB isolate). For the 19 isolates, levofloxacin MBCs were within the range of 0.5 to 2.0 μg/ml, compared with ofloxacin MBCs of 0.75 to 4.0 μg/ml for *M. tuberculosis*, *M. africanum*, *M. bovis*, and *M. bovis* BCG (Table 1). Although viable counts were not performed for D-ofloxacin concentrations greater than 64 μg/ml (the highest concentration tested), this concentration did correspond to 2× the MIC for three strains for which MICs were 32 μg/ml: MDR-TB strains 001-010 and 001-011 and *M. bovis* BCG Denmark or BCG4 (Table 1, Fig. 1B, and Fig. 2). However, except for *M. bovis* BCG, against which D-ofloxacin resulted in more than 99% killing of the initial inoculum, 2× the MIC of D-ofloxacin resulted only in about 80 and 96% killing of the two MDR-TB strains, respectively, suggesting that the MBC/MIC ratios for D-ofloxacin were probably higher than those for levofloxacin and ofloxacin and varied from 2 to 4 and more.

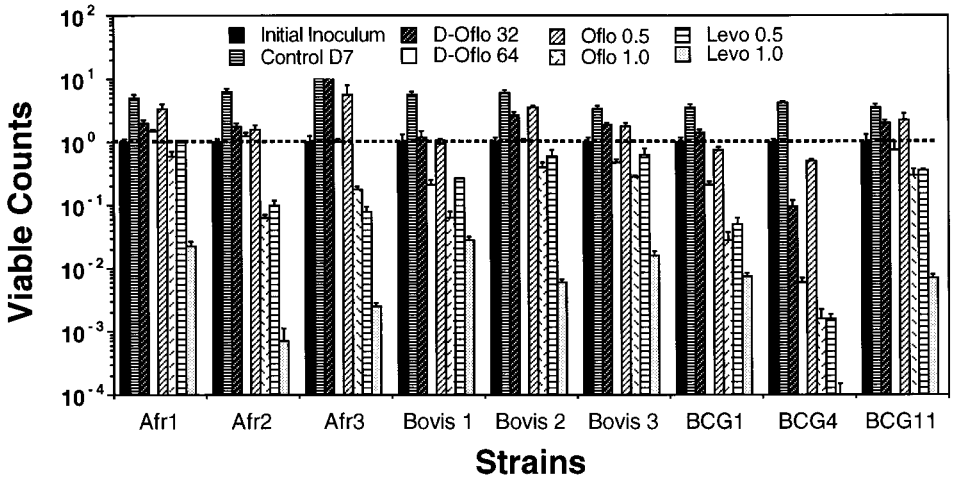

FIG. 2. Comparative bactericidal effects of levofloxacin, ofloxacin, and D-ofloxacin against three strains each of *M. africanum*, *M. bovis*, and *M. bovis* BCG. Afr1, Afr2, and Afr3 represent, the type strain ATCC 25420 and clinical isolates 003-002 and 003-003, respectively; Bovis 1, Bovis 2, and Bovis 3 represent the type strain ATCC 19210 and clinical isolates 002-002 and 002-003, respectively; BCG1, BCG4, and BCG11 represent BCG strains Pasteur, Denmark, and Russia respectively. The initial inoculum (dashed line) varied from $(1.45 \pm 0.15) \times 10^4$ to $(2.01 \pm 0.53) \times 10^4$ CFU/ml for *M. africanum*, $(2.9 \pm 0.54) \times 10^4$ to $(4.54 \pm 0.95) \times 10^4$ CFU/ml for *M. bovis*, and $(4.33 \pm 0.88) \times 10^4$ to $(5.67 \pm 1.76) \times 10^4$ CFU/ml for *M. bovis* BCG and was taken as 1 to facilitate comparison between various isolates. Refer to the legend to Fig. 1 for the definitions of abbreviations. The values after the drug abbreviations indicate concentrations (in micrograms per milliliter).

Drug combination studies. Comparative data obtained by using the *x/y* quotients for the assessment of the activities of various drug combinations against five strains of *M. tuberculosis* are summarized in Table 2, whereas typical radiorespirometry curves for the type strain H37Rv are illustrated in Fig. 3. When sub-MICs of levofloxacin and other first-line (isoniazid, rifampin, and ethambutol) and second-line (amikacin and clofazimine) antituberculous drugs were used in various two-, three-, and four-drug combinations, synergistic drug activity was observed in 8 of 25, 12 of 20, and 8 of 15 tests, respectively. Two-drug combinations of levofloxacin with ethambutol and levofloxacin with rifampin were synergistic against two of five strains each. When levofloxacin was used with isoniazid, synergistic activity was observed against four of five strains (including one isoniazid-resistant strain). In the case of three-drug combinations, the levofloxacin-ethambutol component was kept constant and the third drug was varied; the addition of isoniazid resulted in enhanced activity against all five strains tested, including the two clinical isolates resistant to isoniazid (Table 2); this was followed by the combination with rifampin, which was active against four of five strains (the exception was rifampin-resistant strain 001-013), and amikacin, which was

TABLE 2. In vitro enhancement of anti-*M. tuberculosis* activity of levofloxacin by selected drugs in two-, three-, and four-drug combinations

Drug[a]	Enhancement of drug activity (*x/y* quotient)[b]				
	Drug-susceptible strains			Drug-resistant strains	
	H37Rv	001-005	001-009	001-011 (Inh^r Sm^r)	001-013 (Inh^r Rif^r)
Levo + Emb	−	−	+ (0.32)	+ (0.49)	−
Levo + Rif	−	+ (0.33)	−	+ (0.45)	−
Levo + Inh	+ (0.49)	+ (0.33)	+ (0.26)	−	+ (0.32)
Levo + Amik	−	−	−	−	−
Levo + Clofa	−	−	−	−	−
Levo + Emb + Rif	+ (0.19)	+ (0.11)	+ (0.29)	+++ (0.028)	−
Levo + Emb + Inh	+ (0.30)	+ (0.11)	+++ (0.025)	+ (0.27)	+ (0.17)
Levo + Emb + Amik	−	+ (0.076)	+ (0.082)	+ (0.24)	−
Levo + Emb + Clofa	−	−	−	−	−
Levo + Emb + Rif + Inh	+++ (0.013)	+ (0.11)	+ (0.11)	+++ (0.005)	+ (0.15)
Levo + Emb + Rif + Amik	+ (0.14)	+ (0.076)	−	++ (0.027)	−
Levo + Emb + Rif + Clofa	−	−	−	−	−

[a] All drugs were used at sub-MICs. The concentrations chosen were as follows: levofloxacin (Levo), 0.25 μg/ml each; ethambutol (Emb), 0.25 μg/ml for H37Rv and 0.50 μg/ml for strains 001-005, 001-009, 001-011, and 001-013; rifampin (Rif), 0.05 μg/ml for strain 001-011, 0.1 μg/ml for H37Rv, 0.2 μg/ml for 001-005 and 001-009, and 1 μg/ml for the rifampin-resistant strain 001-013; isoniazid (Inh), 0.01 μg/ml for H37Rv, 0.02 μg/ml for strains 001-005 and 001-009, and 1 μg/ml for isoniazid-resistant strains 001-011 and 001-013; amikacin (Amik), 0.125 μg/ml for H37Rv and 0.25 μg/ml for strains 001-005, 001-009, 001-011, and 001-013; clofazimine (Clofa), 0.05 μg/ml for H37Rv, 0.1 μg/ml for strains 001-011 and 001-013, and 0.2 μg/ml for strains 001-005 and 001-009.

[b] A radiometric *x/y* quotient of <0.5 (two-drug combinations), <0.33 (three-drug combinations), or <0.25 (four-drug combinations) indicates enhanced drug action. The scores for *x/y* quotients are given as follows: +, <0.5; ++, <0.1; and +++, <0.05 (two-drug combinations); +, <0.33; ++, <0.066; and +++, <0.033 (three-drug combinations); +, <0.25; ++, <0.05; and +++, <0.025 (four-drug combinations).

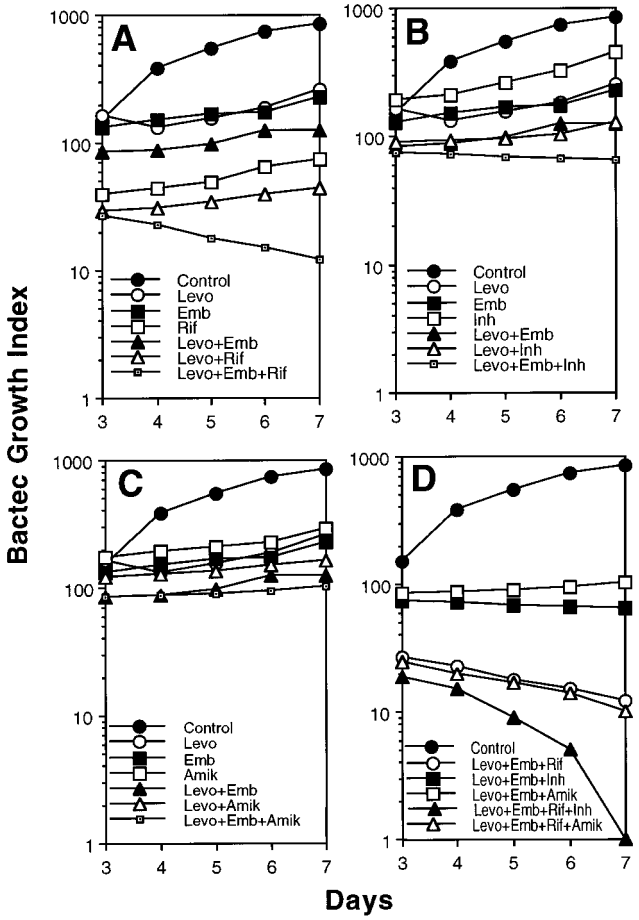

FIG. 3. Typical radiometric data showing the growth inhibition of *M. tuberculosis* H37Rv by levofloxacin used alone and in combination with other drugs (A, B, and C). (D) Comparison of various three- and four-drug combinations. All drugs were used at their sub-MICs (refer to footnote *a* of Table 2 for the concentrations used). Levo, levofloxacin; Emb, ethambutol; Rif, rifampin; Inh, isoniazid; Amik, amikacin.

active against three of five strains. On the other hand, the combination levofloxacin-ethambutol-clofazimine was not effective at all. The four-drug combination levofloxacin-ethambutol-rifampin-isoniazid was the most efficient four-drug combination tested (Table 2; Fig. 3).

The radiometric *x/y* quotients were compared in all cases by viable count determinations (Fig. 4). Considering the sub-MICs of the drugs used in combination testing, the killing by ≥ 2 $\log_{10}$ units of the initial inoculum by the combination levofloxacin-ethambutol-rifampin-isoniazid for three of five isolates (including one isoniazid-resistant strain) was exceptional. Neither amikacin nor clofazimine appeared to be a potential component of three- or four-drug combinations involving levofloxacin. The four-drug combinations containing amikacin gave discrepant results, despite their suggested additive activities against three of five strains (Table 2); viable count determinations showed an antagonistic effect of amikacin when it was included in the otherwise efficient three-drug combination of levofloxacin-ethambutol-rifampin (data not shown). In conclusion, a satisfactory correlation between the radiometric data (Fig. 3), *x/y* quotients (Table 2), and the viable count data (Fig. 4) was observed for all drugs except the four-drug combination that included amikacin.

DISCUSSION

Ofloxacin has been shown to be one of the most potent second-line drugs against both extracellular and intracellular *M. tuberculosis* bacilli (10, 24, 25); however, when it is used alone, its therapeutic efficacy was marginal against human pulmonary tuberculosis (39). In part, this discrepancy can be explained on the basis of its pharmacokinetic properties. For example, the dosages of 150 and 300 mg of ofloxacin per kg of body weight 6 times weekly in mice were shown to be equal to those of 400- and 800-mg doses in humans, respectively (with the latter dose being the maximal clinically tolerated dose in humans); however, even the maximal dose of 300 mg/kg in mice was less bactericidal than isoniazid or even as little as 50 mg of sparfloxacin per kg (13, 14, 16). In this context, the improved physicochemical and pharmacokinetic properties of levofloxacin compared with those of ofloxacin, e.g., lower MICs (7, 9, 15, 18, 20, 38, 40), higher levels of activity against bacterial DNA gyrase (11), similar absorption rates (7, 19, 38, 40), higher levels of intracellular accumulation (21, 22), and intracellular concentration/extracellular concentration ratios in human macrophages (18), led us to investigate in detail the activity of levofloxacin against the full spectrum of the mem-

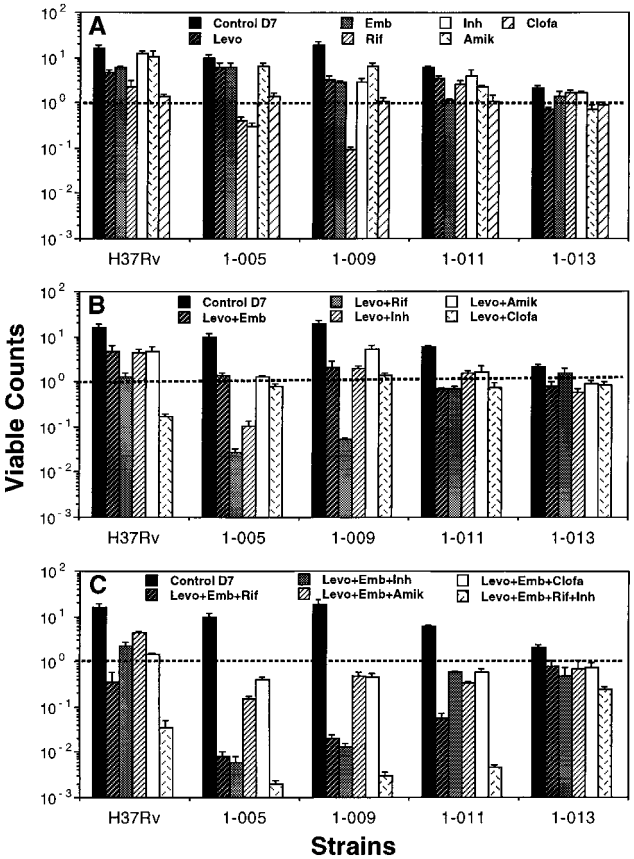

FIG. 4. Viable cell count data comparing the effects of various drugs used alone (A) and in combination (B and C) against three drug-susceptible and two drug-resistant strains of *M. tuberculosis*. The vials were initially inoculated to contain about 10^4 CFU/ml. The viable cell counts were performed by plating the cultures at the beginning and at the end of the experiment (Table 2 and Fig. 3). The initial inoculum (dashed line) was taken as 1 to facilitate comparison between various isolates. All drugs were used at their sub-MICs. Refer to footnote *a* of Table 2 and the legend to Fig. 3 for the concentrations and the abbreviations used, respectively; Clofa, clofazimine.

bers in the *M. tuberculosis* complex, as well as against drug-susceptible and clinical MDR-TB isolates.

For *M. tuberculosis*, which responds well to chemotherapy, it can be concluded that bactericidal drugs have in common lower MICs, lower critical concentrations, and lower intracellular bactericidal concentrations, whereas they have higher maximum concentrations in the plasma of humans (for a review, see reference 23). However, the progressive emergence of multiple drug resistance in an isolate of *M. tuberculosis* during unsuccessful chemotherapy was reported recently (32). This isolate was initially resistant to rifampin and isoniazid, but the standard four-drug therapy of isoniazid, rifampin, ethambutol, and pyrazinamide was administered to the patient for about 3 months, pending the isolation and identification of the organism and the results of drug susceptibility tests performed by routine methods. Although the regimen was changed once the results were available by incorporating one active drug each time, each successive isolate was found to be resistant to a wider range of antituberculous drugs than its predecessors, with the last isolate being resistant to 8 of 12 drugs tested. All successive isolates were established to be identical by using IS*6110* and pTBN restriction fragment length polymorphism patterns (32), and this finding raised two important aspects concerning MDR-TB isolates: first, that the rapid provision of drug susceptibility results to physicians is the best way to avoid the emergence of MDR-TB so that the physician can adapt the treatment, and second, in the case of resistance of the infecting organism to the components of the standard four-drug therapeutic regimens, at least two highly bactericidal second-line drugs acting on targets different from those against which the first-line drugs act are needed to avoid the underlying resistance to another drug that could have been selected during the initial therapy that was used while susceptibility test results were pending (6, 28).

The above discussion underlines the findings of the present investigation, because not only does levofloxacin appear to be a better candidate than ofloxacin for the treatment of tuberculosis but its activity against MDR-TB isolates is also highly remarkable. Moreover, the radiometric method described here guarantees rapid results of drug susceptibility and drug combination tests, compatible with the objectives discussed above and in numerous recent publications (1, 4, 12, 23, 28). One of the most remarkable findings of the present investigation was the ability of levofloxacin to act in synergy with ethambutol and isoniazid (and/or rifampin) to significantly kill even isolates resistant to isoniazid-rifampin or isoniazid-streptomycin. Our results corroborate recent findings of Skinner et al. (37), who showed that levofloxacin retained its exceptional bactericidal activity against an intracellularly growing isoniazid-resistant isolate of a tubercle bacillus, killing more than 2 $\log_{10}$ units of the initial bacterial inoculum at 2 μg/ml. It should be underlined, however, that contrary to combination testing of drugs in the case of multiresistant *M. avium* organisms which aimed to improve the efficacies of otherwise bacteriostatic drugs (29–31), the aim of combination testing with levofloxacin against tubercle bacilli in the present investigation was completely different. We used combination testing in the present investigation to determine whether levofloxacin retains its effectiveness when it is used in combination with other antituberculous drugs, with the conclusion not only that levofloxacin is highly bactericidal against tubercle bacilli but also that it may be used safely in association with other established antituberculous drugs.

Lastly, the pharmacokinetics and safety of levofloxacin were found to be unaltered in human immunodeficiency virus-infected patients in a phase I, double-blind, randomized (1:1), placebo-controlled trial (8); adsorption of levofloxacin was not rate limited by the gastrointestinal transit process. Adsorption was almost immediate following oral administration, with a slow elimination process. In addition, it appeared to be safe as a drug after the administration of multiple oral doses of 350 mg every 8 h to both healthy individuals and human immunodeficiency virus-infected patients (8), suggesting its potential use in *M. tuberculosis*-infected human immunodeficiency virus-positive patients, who are at increased risk of developing MDR-TB infections (28). In conclusion, levofloxacin appears to be a good candidate for treating tuberculosis, and its activity, alone and in combination with other antituberculous drugs, compared with that of ofloxacin should be assessed in controlled clinical trials.

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