ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1995, p. 1341–1344 Vol. 39, No. 6
0066-4804/95/$04.00+0

In Vitro and In Vivo Activities of Levofloxacin against *Mycobacterium tuberculosis*

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Received 30 January 1995/Returned for modification 21 March 1995/Accepted 10 April 1995

In tests with 18 drug-susceptible strains of *Mycobacterium tuberculosis*, the MIC at which 50% of the strains are inhibited by levofloxacin (LVFX) was one dilution less than that at which 50% of the strains are inhibited by ofloxacin (OFLO), but the MICs at which 90% of the strains are inhibited were similar. The in vivo activity of LVFX against *M. tuberculosis* was compared with the activities of isoniazid, OFLO, and sparfloxacin (SPFX). Mice were inoculated intravenously with 1.74×10^6 CFU of H37Rv, and treatments began the next day and were carried out six times weekly for 4 weeks. The severity of infection and effectiveness of treatment were assessed by survival rate, spleen weights, gross lung lesions, and enumeration of CFU in the spleen. In terms of CFU counts, the ranking of the anti-*M. tuberculosis* activities of the treatments used ran in the following order: LVFX (300 mg/kg of body weight) = SPFX (100 mg/kg) > isoniazid > SPFX (50 mg/kg) > OFLO (300 mg/kg) = LVFX (150 mg/kg) > OFLO (150 mg/kg) = LVFX (50 mg/kg). It seems, therefore, that the in vivo activity of LVFX is comparable to that produced by a twofold-greater dosage of OFLO. It is assumed that the maximal clinically tolerated dosage of LVFX is similar to that of OFLO, i.e., 800 mg daily, which is equivalent to 300 mg of LVFX per kg in mice. Because LVFX displayed powerful bactericidal activity, promising effects against human tuberculosis may be achieved if patients are treated with the maximal clinically tolerated dosage of LVFX.

Today, one-third of the world's population is infected with *Mycobacterium tuberculosis*, and pulmonary tuberculosis is one of the most serious public health problems in both developing and developed countries (17). Furthermore, *M. tuberculosis* isolates that are resistant to multiple drugs, especially to isoniazid (INH) and rifampin, are increasing (1, 22); for those multidrug-resistant patients, only a limited number of alternative chemotherapeutic regimens are available, none of them is very effective, and the mortality is high (22). Therefore, effective new antituberculosis drugs with bactericidal mechanisms different from those of the presently available agents, i.e., INH, rifampin, pyrazinamide, ethambutol, and streptomycin, are urgently needed.

Our previous experiments demonstrated that among the major commercially available fluoroquinolones, ofloxacin (OFLO) seemed to be the only compound active against *M. tuberculosis* both in vitro and in vivo (19). The MIC of OFLO at which 90% of the clinical isolates of *M. tuberculosis*, either susceptible or resistant to both INH and rifampin, are inhibited (MIC_{90}) was 2 μg/ml on Löwenstein-Jensen medium (19) or 7H11 agar (10), well below its achievable peak level in serum in mice or humans. The activity of OFLO in mice was dosage related: a dosage of 150 mg/kg of body weight six times weekly displayed only a modest degree of activity against *M. tuberculosis*, whereas the activity of a dosage of 300 mg/kg six times weekly was moderate (12, 19). The pharmacokinetic studies that will be discussed below estimated that doses of OFLO at 150 and 300 mg/kg in mice are equivalent, respectively, to 400- and 800-mg doses of OFLO in humans, with the latter dose being the maximal clinically tolerated dose (5, 9). Because OFLO at 150 or 300 mg/kg daily in mice showed only a modest or moderate degree of activity against *M. tuberculosis*, it is understandable that the maximal clinically tolerated dosage of OFLO displayed no more than a marginal degree of therapeutic effect against human tuberculosis (20).

Recently, we have demonstrated that the activity of sparfloxacin (SPFX) against *M. tuberculosis* was more powerful than that of OFLO (10, 12). Its MIC_{90} for *M. tuberculosis* was 0.5 μg/ml on 7H11 agar, two dilutions lower than that of OFLO (10); in the murine tuberculosis model, its minimal effective dosage, as assessed by the survival rate, spleen weights, and gross lung lesions, was 12.5 mg/kg daily, only one-sixth to one-eighth of that of OFLO (10). SPFX at a dosage of 50 mg/kg six times weekly in mice displayed stronger bactericidal activity than that found with OFLO at a dosage of 300 mg/kg six times weekly (12), and SPFX at a dosage of 100 mg/kg six times weekly showed very powerful bactericidal activity against *M. tuberculosis*, comparable to that of rifampin and significantly greater than those of INH, pyrazinamide, and SPFX at 50 mg/kg (12). Our pharmacokinetic studies indicated that, in terms of the area under the concentration-time curve, SPFX doses of 50 and 100 mg/kg in mice were equivalent, respectively, to 200- and 400-mg doses in men (12); thus, to obtain the optimal therapeutic effect for the treatment of pulmonary tuberculosis, SPFX should ideally be given at 400 mg daily. Unfortunately, at least for the time being, the manufacturer recommends that patients be treated with only 200 mg daily; on the basis of the results of mouse experiments, this is most likely a suboptimal dosage for the treatment of tuberculosis.

OFLO consists of equal amounts of two optically active isomers, i.e., DR-3355 [levofloxacin (LVFX), *l*-ofloxacin, or *S*-(−)-ofloxacin] and DR-3354 [*R*-(+)-ofloxacin] (6, 7). Preliminary studies demonstrated that LVFX displayed a broad spectrum of bactericidal activities and is approximately twice as active as OFLO and 8 to 128 times more potent than DR-3354 (6). These observations have been confirmed, both in

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vitro and in vivo, by other investigators who tested LVFX against a wide range of microorganisms, including *M. tuberculosis* (3, 11, 13, 15, 18, 21, 23), and the differences in the antimicrobial activities of the two drugs have been shown to be related to the differences in the anti-DNA gyrase activities of LVFX and OFLO (7). Because of the greater in vitro activity of LVFX compared with that of OFLO, because the absorption rates for the two are similar (3, 14, 18, 21), and because there is no evidence that the adverse reactions of fluoroquinolones are correlated with their anti-DNA gyrase activities, greater therapeutic effect may be achieved if LVFX is administered at the same dosage as OFLO. LVFX may be applied to the treatment of infections, such as tuberculosis, for which OFLO cannot be prescribed at the optimal therapeutic dosage. We, therefore, have compared the in vitro and in vivo activities of LVFX against *M. tuberculosis* with those of OFLO and SPFX.

MATERIALS AND METHODS

Antimicrobial agents. LVFX and OFLO were generously provided by Roussel Uclaf, Romainville, France; SPFX was from Rhone D.P.C. Europe, Antony, France; and INH was a gift from Roche, Neuilly, France. For in vitro experiments, the fluoroquinolones were initially dissolved in NaOH and subsequently diluted with distilled water. For mouse experiments, all the agents were suspended in 0.05% agar in distilled water at the desired concentrations; the suspensions were prepared weekly and stored at 4°C.

In vitro susceptibility. The MICs of LVFX, OFLO, and SPFX for 18 strains of drug-susceptible *M. tuberculosis*, including strain H37Rv, and 2 strains of fluoroquinolone-resistant *M. tuberculosis* (both isolated from OFLO-treated tuberculosis patients) were determined on 10% oleic acid-albumin-dextrose-catalase (OADC)-enriched 7H11 agar by an established method (19). The MIC was defined as the lowest drug concentration that inhibited more than 99% of the bacterial population after incubation at 37°C for 28 days.

Comparison of the in vivo activities of LVFX, OFLO, SPFX, and INH. Two hundred female outbred Swiss mice (age, 4 to 6 weeks) were inoculated intravenously with 0.5 ml of freshly prepared suspension containing 1.74×10^6 CFU of *M. tuberculosis* H37Rv. The next day (day 1), 10 inoculated mice were sacrificed to provide the baseline values for spleen weights, lung lesions, and the number of CFU in the spleen; the remaining mice were allocated randomly to an untreated control group with 30 mice and eight treated groups with 20 mice each. Treatments began on the same day. Drugs were administered through an esophageal cannula six times weekly, and each treated group received one of the following eight doses: INH, 25 mg/kg; OFLO, 150 or 300 mg/kg; SPFX, 50 or 100 mg/kg; and LVFX, 50, 150, or 300 mg/kg. The total duration of the treatment was 4 weeks, the last dose of the treatment was given on day 28, and all surviving mice were sacrificed on day 30. The severity of infection and the effectiveness of the treatment were assessed by the survival rate, the spleen weights, the gross lung lesions, and the amounts of CFU in the spleens. The severity of gross lung lesions was scored from 0 to 2+, with the latter referring to a lung extensively infiltrated with tubercles (10, 19).

Enumeration of CFU. During sacrifice, the spleens were removed aseptically and homogenized (4), and a suspension of up to 3.5 ml was made for each spleen. At least three serial 10-fold dilutions of the suspension were plated onto Löwenstein-Jensen medium, with three to five tubes per dilution. The results of the cultures were recorded after incubation at 37°C for 6 weeks. The bactericidal effect of the treatment was defined as a significant decrease in the mean number of CFU in the treated group compared with the pretreatment value.

Statistical analysis. The results were analyzed by the Student *t* test and Fisher's exact probability calculation. Differences were considered significant at the 95% level of confidence.

RESULTS

In vitro susceptibility. In tests with 18 drug-susceptible strains of *M. tuberculosis*, the MIC_{50} of LVFX was one dilution (log_2) less than that of OFLO, but its MIC_{90} was the same as that of OFLO; both the MIC_{50} and MIC_{90} of LVFX were one dilution more than those of SPFX (Table 1). For H37Rv, the MICs of OFLO, LVFX, and SPFX were 1.0, 0.5, and 0.12 μg/ml, respectively. The MICs of OFLO, LVFX, and SPFX against two quinolone-resistant strains were significantly higher than those for drug-susceptible strains and were 8.0, 8.0, and 4.0 and 8.0, 2.0, and 4.0 μg/ml, respectively.

TABLE 1. MICs of OFLO, LVFX, and SPFX for 18 drug-susceptible strains of *M. tuberculosis*

Drug	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
OFLO	0.5–2.0	1.0	1.0
LVFX	0.25–1.0	0.5	1.0
SPFX	0.12–0.5	0.25	0.5

In vivo activities. (i) Survival rates for 30 days after inoculation. As expected (10, 19), after the mice were inoculated with more than 10^6 CFU (0.1 mg wet weight) of virulent H37Rv per mouse, only 10% of the untreated controls survived after 30 days, and the first deaths occurred at 18 days after inoculation (Fig. 1); on the other hand, no mortality was observed for mice treated daily with INH, SPFX at 50 or 100 mg/kg, or OFLO at 300 mg/kg. The survival rate for mice treated daily with OFLO at 150 mg/kg was 70%, significantly lower than those for the four above-mentioned treated groups ($P < 0.01$). Various survival rates were observed for mice treated with LVFX, and the rates correlated with the dosages of the LVFX treatment. The survival rate for mice treated daily with LVFX at 50 mg/kg was only 20%, significantly lower than that for mice treated daily with OFLO at 150 mg/kg ($P < 0.01$), and it did not differ significantly from that for control mice. However, the rates for mice treated daily with LVFX at 150 or 300 mg/kg were high and did not differ significantly from those for mice treated daily with INH, SPFX, or OFLO at 300 mg/kg. One death in each group was observed on days 9 and 11 (Fig. 1), and the causes for the deaths were most likely unrelated to the tuberculosis infection.

(ii) Spleen weights. As Fig. 2 shows, the mean spleen weight of the control mice surviving 30 days after inoculation (Control D30) was significantly greater than that for the same group of mice sacrificed the day after inoculation (Control D1). The mean spleen weight of mice treated daily with INH remained at the pretreatment level, indicating that a highly effective treatment may prevent the development of splenomegaly caused by tuberculosis infection. The values for all quinolone-treated groups were significantly lower than those for the corresponding control mice but were dose related: the higher the dosages of the compound, the lower the spleen weights. However, except for those for mice treated daily with LVFX at 300 mg/kg, the spleen weights for all quinolone-treated mice were significantly greater than the pretreatment weights or those for mice treated with INH, suggesting that even the higher dose

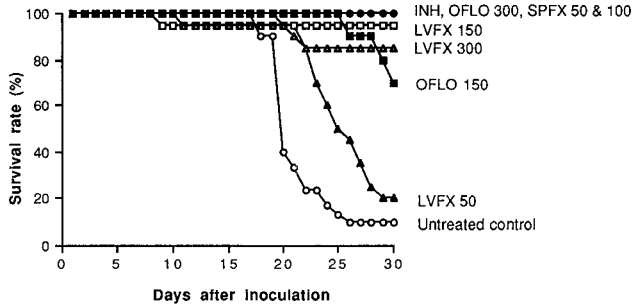

FIG. 1. Survival rates of mice for 30 days after intravenous infection with 1.74×10^6 CFU of *M. tuberculosis* H37Rv. At the time (day 1) the treatments were begun, there were 30 mice in the control group and 20 mice in each treated group. The numbers after the abbreviations for the various drugs indicate daily doses.

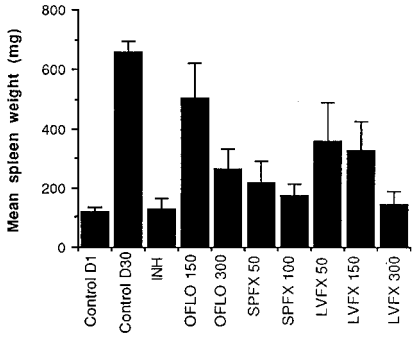

FIG. 2. Mean spleen weights for mice surviving at 30 days. The "control D1" bar represents the spleen weights for mice sacrificed the next day after intravenous infection with *M. tuberculosis* H37Rv. Error bars represent standard deviations. The numbers after the abbreviations for the various drugs indicate daily doses.

(100 mg/kg) of SPFX was unable to prevent completely the development of splenomegaly.

The mean spleen weight of mice treated with LVFX at 150 mg/kg was significantly lower than that for mice treated with OFLO at 150 mg/kg ($P < 0.01$) but higher than that for mice treated with OFLO at 300 mg/kg ($P < 0.05$) or with SPFX at 50 mg/kg ($P < 0.01$). The mean spleen weight for mice treated with LVFX at 300 mg/kg was lower than that for the latter two groups and did not differ significantly from that for mice treated with either INH or SPFX at 100 mg/kg.

(iii) Lung lesions. No lung lesions were observed in untreated mice sacrificed at day 1 or in mice that had been treated daily with INH or SPFX at 50 or 100 mg/kg or with LVFX at 300 mg/kg and that were sacrificed at day 30. Severe (++) lesions were encountered in all controls surviving on day 30 and in mice treated daily with OFLO at 150 mg/kg or with LVFX at 50 mg/kg daily. Moderate (+) lesions developed in 35% of the mice treated daily with OFLO at 300 mg/kg and in 65% of the mice treated daily with LVFX at 150 mg/kg. The difference between the two groups did not attain statistical significance ($n = 20$, $P > 0.05$).

(iv) Enumeration of CFU in the spleens. By the time the treatment was begun, i.e., 1 day after inoculation, all the spleens of 10 mice were culture positive for *M. tuberculosis* and the mean number of CFU ($\log_{10}$) per spleen was 5.20 ± 0.13. As Fig. 3 shows, the mean number of CFU for surviving control mice significantly increased at day 30 by comparison with the value for day 1 ($P < 0.01$). Because 90% of the control mice died from tuberculosis infection before day 30, it is very likely that the mean value of 6.31 ± 0.11 for the control group was underestimated. On day 30, the mean numbers of CFU for all treated groups were significantly smaller than those for the corresponding control mice ($P < 0.01$), indicating that all the treatments displayed various degrees of anti-*M. tuberculosis* activity. The mean numbers of CFU for mice treated daily with LVFX at 50 mg/kg or OFLO at 150 mg/kg were virtually the same, and both of them were significantly greater than the pretreatment value ($P < 0.01$), demonstrating that both treatments displayed only a partial bacteriostatic effect. The anti-*M. tuberculosis* activity of LVFX at 150 mg/kg daily was so close to that of OFLO at 300 mg/kg daily that the growth curves of *M. tuberculosis* for both groups overlapped (Fig. 3). For both groups, the mean number of CFU was reduced by about 0.5 $\log_{10}$ from the pretreatment value, suggesting that the treatments showed only modest bactericidal activities. The mean numbers of CFU were reduced by around 1.7, 2.3, 3.5, and 3.8 $\log_{10}$ for mice treated, respectively, with SPFX at 50 mg/kg, INH, SPFX at 100 mg/kg, and LVFX at 300 mg/kg daily, indicating that SPFX at 50 mg/kg displayed a stronger bactericidal activity than did OFLO at 300 mg/kg or LVFX at 150 mg/kg ($P < 0.01$). INH was more active than SPFX at 50 mg/kg ($P < 0.01$) but less potent than SPFX at 100 mg/kg and LVFX at 300 mg/kg ($P < 0.01$), and the bactericidal activities of the latter two treatments did not differ significantly.

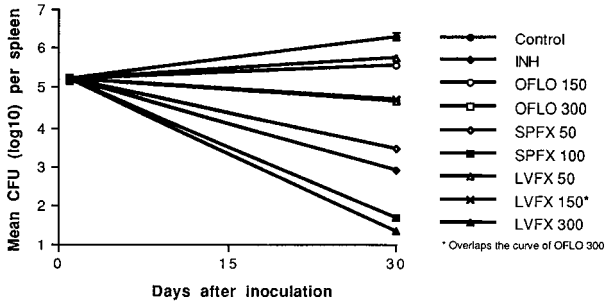

FIG. 3. Enumerations of *M. tuberculosis* CFU in the spleens of mice. Mice were inoculated intravenously with 1.74×10^6 CFU of *M. tuberculosis* H37Rv, and treatments were begun the day after inoculation. Drugs were administered by gavage six times weekly for 4 weeks. Each curve represents the mean numbers of CFU for 3 to 20 mice. The error bar represents the standard deviation for the control mice. The numbers after the abbreviations for the various drugs indicate daily doses.

DISCUSSION

In experimental infections of mice with various bacterial pathogens, the protective effect of orally administrated LVFX was superior to those of OFLO and ciprofloxacin (3, 11, 18). The superior in vivo activity of LVFX is probably due to its greater in vitro activity and also to its favorable physicochemical and pharmacokinetic properties. LVFX is 10 times more soluble in water than OFLO (21), is as well absorbed in mice and humans as OFLO (3, 14, 18, 21), and reached significantly higher intracellular concentrations than OFLO (16).

To date, information about the in vitro and/or in vivo activity of LVFX against *M. tuberculosis* is very limited (11, 13, 23). In terms of MIC and MBC, it was reported that LVFX exhibited twofold greater inhibitory and bactericidal activities than OFLO against either extracellular or intracellular tubercle bacilli (13, 23). The preliminary studies with a murine tuberculosis model indicated that the daily treatment with LVFX at 200 mg/kg had a more than twofold greater activity than OFLO at the same dosage but was less active than daily treatment with SPFX at 100 mg/kg (11). The results of the present experiments demonstrated that the MIC_{50} of LVFX for 18 drug-susceptible strains of *M. tuberculosis* was one dilution smaller than that of OFLO but that its MIC_{90} was the same as that of OFLO, indicating that the in vitro activity of LVFX was superior to but less than twofold greater than that of OFLO. In terms of CFU counts, the ranking of the anti-*M. tuberculosis* activities of the tested treatments ran in the following order: LVFX (300 mg/kg) = SPFX (100 mg/kg) > INH (25 mg/kg) > SPFX (50 mg/kg) > OFLO (300 mg/kg) = LVFX (150 mg/kg) > OFLO (150 mg/kg) = LVFX (50 mg/kg). A tendency for the same ranking was also observed for survival rate, spleen weights, and gross lung lesions, although the differences were less distinctive than those of the CFU counts. It is very encouraging to observe that daily treatment of mice with LVFX at 300 mg/kg resulted in a very powerful bactericidal activity against *M. tuberculosis*, an activity comparable to that of SPFX at 100

mg/kg and significantly greater than that of INH at 25 mg/kg. The latter observation was very different from that of other investigators, who claimed that LVFX at 300 mg/kg was far less active than INH (11). It appears that there are at least three potential explanations for the differences between the two experiments: the use of different strains of *M. tuberculosis* that probably have different degrees of virulence, the use of different in vivo protocols, and the possible variability inherent in a small study using only a single isolate. Because the results by all measures except the spleen weight were very similar for mice treated with LVFX at 150 mg/kg and mice treated with OFLO at 300 mg/kg and were significantly better than the results for mice treated with OFLO at 150 mg/kg, it seems reasonable to conclude that the in vivo activity of LVFX is comparable to that of a twofold greater dosage of OFLO.

Up to now, the pharmacokinetic data on LVFX in mice and humans have been very scant (3, 12, 16), and no chronic toxicological data are available. Therefore, it is very difficult to identify the optimal dosage of LVFX for the treatment of human tuberculosis by extrapolating the results of the mouse experiment. However, because preliminary studies indicated that the pharmacokinetic properties of LVFX in healthy volunteers and presumably also in mice were very similar to those of OFLO (14) and because there is no evidence that the adverse reactions to fluoroquinolones are correlated with their anti-DNA gyrase activities, it is likely that the maximal clinically tolerated dosage of LVFX is similar to that of OFLO, i.e., 800 mg per day (5, 9). Since the half-life of OFLO in humans (2, 8, 24) is significantly longer than that in mice (19), there is a very significant accumulation effect after multiple doses in healthy human volunteers (8) but probably not in mice; therefore, to extrapolate the results of the mouse experiments for the treatment of humans, it is appropriate to take into account the pharmacokinetic data for OFLO and LVFX in humans after multiple doses. Because the area under the concentration-time curve, 48.8 $\mu g \cdot h/ml$, for mice treated with a single dose of OFLO at 150 mg/kg (19) is very similar to the area under the concentration-time of 48.1 (8) or 41.20 $\pm$ 6.98 (2) $\mu g \cdot h/ml$ for humans treated with multiple doses of OFLO at 400 mg every 12 h, it is reasonable to estimate that in terms of the area under the concentration-time curve, OFLO at 300 mg/kg in mice is equivalent to OFLO at 800 mg, or the maximal clinically tolerated dosage, in humans. Daily treatment of mice with LVFX at 300 mg/kg revealed that LVFX has a very powerful bactericidal activity against *M. tuberculosis*. A similar promising effect could possibly be achieved for human tuberculosis if patients were treated daily with LVFX at 800 mg. Nevertheless, in order to confirm the powerful bactericidal activity of LVFX at 300 mg/kg in mice and the bioequivalence of LVFX at 300 mg/kg in mice and LVFX at 800 mg in humans, additional experiments should be carried out before a clinical trial is conducted.

ACKNOWLEDGMENT

This investigation was partially financed by Laboratories Roussel-Santé, Romainville, France.

REFERENCES

1. **Culliton, B. J.** 1992. Drug-resistant TB may bring epidemic. Nature (London) **356:**472.
2. **Flor, S. C., M. C. Rogge, and A. T. Chow.** 1993. Bioequivalence of oral and intravenous ofloxacin after multiple-dose administration to healthy male volunteers. Antimicrob. Agents Chemother. **37:**1468–1472.
3. **Fu, K. P., S. C. Lafredo, B. Foleno, D. M. Isaacson, J. F. Barrett, A. J. Tobia, and M. E. Rosenthale.** 1992. In vitro and in vivo antibacterial activities of levofloxacin (*l*-ofloxacin), an optically active ofloxacin. Antimicrob. Agents Chemother. **36:**860–866.
4. **Grosset, J., C. Truffot-Pernot, J. Fermanian, and H. Lecoeur.** 1982. Activité stérilisante des différents antibiotiques dans la tuberculose expérimentale de la souris. Pathol. Biol. **30:**444–448.
5. **Grosset, J. H., B. Ji, C. C. Guelpa-Lauras, E. G. Perani, and L. N. N'Deli.** 1990. Clinical trial of pefloxacin and ofloxacin in the treatment of lepromatous leprosy. Int. J. Lepr. **58:**281–295.
6. **Hayakawa, I., S. Atarashi, S. Yokohama, M. Imamura, K. Sakano, and M. Furukawa.** 1986. Synthesis and antibacterial activities of optically active ofloxacin. Antimicrob. Agents Chemother. **29:**163–164.
7. **Imamura, M., S. Shibamura, I. Hayakawa, and Y. Osada.** 1987. Inhibition of DNA gyrase by optically active ofloxacin. Antimicrob. Agents Chemother. **31:**325–327.
8. **Israel, D., J. G. Gillum, M. Turik, K. Harvey, J. Ford, H. Dalton, M. Towle, R. Echols, A. Heller, and R. Polk.** 1993. Pharmacokinetics and serum bactericidal titers of ciprofloxacin and ofloxacin following multiple oral doses in healthy volunteers. Antimicrob. Agents Chemother. **37:**2193–2199.
9. **Ji, B., E. G. Perani, C. Petinom, L. N'Deli, and J. H. Grosset.** 1994. Clinical trial of ofloxacin alone and in combination with dapsone plus clofazimine for treatment of lepromatous leprosy. Antimicrob. Agents Chemother. **38:**662–667.
10. **Ji, B., C. Truffot-Pernot, and J. Grosset.** 1991. In vitro and in vivo activities of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis*. Tubercle **72:** 181–186.
11. **Klemens, S. P., C. A. Sharpe, M. C. Rogge, and M. H. Cynamon.** 1994. Activity of levofloxacin in a murine model of tuberculosis. Antimicrob. Agents Chemother. **38:**1476–1479.
12. **Lalande, V., C. Truffot-Perenot, A. Paccaly-Moulin, J. Grosset, and B. Ji.** 1993. Powerful bactericidal activity of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* in mice. Antimicrob. Agents Chemother. **37:**407–413.
13. **Mor, N., J. Vanderkolk, and L. Heifets.** 1994. Inhibitory and bactericidal activities of levofloxacin against *Mycobacterium tuberculosis* in vitro and in human macrophages. Antimicrob. Agents Chemother. **38:**1161–1164.
14. **Nakashima, M., T. Uematsu, M. Kanamura, O. Okazaki, S. Hashimoto, and H. Tachizawa.** 1988. Pharmacokinetics of DR-3355, a new quinolone, in healthy volunteers, abstr. 951, p. 278. *In* Program and abstracts of the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
15. **Neu, H. C., and N.-X. Chin.** 1989. In vitro activity of *S*-ofloxacin. Antimicrob. Agents Chemother. **33:**1105–1107.
16. **Pascual, A., I. Garcia, and E. J. Perea.** 1990. Uptake and intracellular activity of an optically active ofloxacin isomer in human neutrophils and tissue culture cells. Antimicrob. Agents Chemother. **34:**277–280.
17. **Sudre, P., H. G. Ten Dam, and A. Kochi.** 1992. Tuberculosis: a global overview of the situation today. Bull. W. H. O. **70:**149–159.
18. **Tanaka, M., M. Otsuki, T. Une, and T. Nishino.** 1990. In-vitro and in-vivo activity of DR-3355, an optically active isomer of ofloxacin. J. Antimicrob. Chemother. **26:**659–666.
19. **Truffot-Pernot, C., B. Ji, and J. Grosset.** 1991. Activities of pefloxacin and ofloxacin against mycobacteria: in vitro and mouse experiments. Tubercle **72:**57–64.
20. **Tsukamura, M., E. Nakamura, S. Yoshii, and H. Amano.** 1985. Therapeutic effect of a new antibacterial substance ofloxacin (DL-8280) on pulmonary tuberculosis. Am. Rev. Respir. Dis. **131:**352–356.
21. **Une, T., T. Fujimoto, K. Sato, and Y. Osada.** 1988. In vitro activity of DR-3355, an optically active ofloxacin. Antimicrob. Agents Chemother. **32:** 1336–1340.
22. **World Health Organization Working Group.** 1991. Tuberculosis research and development. WHO/TB/91-162. World Health Organization, Geneva.
23. **Yew, W. W., L. J. Y. Piddock, M. S. K. Li, D. Lyon, C. Y. Chan, and A. F. B. Cheng.** 1994. In-vitro activity of quinolones and macrolides against mycobacteria. J. Antimicrob. Chemother. **34:**343–351.
24. **Yuk, J. H., C. H. Nightingale, R. Quintiliani, and K. R. Sweeney.** 1991. Bioavailability and pharmacokinetics of ofloxacin in healthy volunteers. Antimicrob. Agents Chemother. **35:**384–386.