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Moxifloxacin, Ofloxacin, Sparfloxacin, and Ciprofloxacin against *Mycobacterium tuberculosis*: Evaluation of In Vitro and Pharmacodynamic Indices That Best Predict In Vivo Efficacy[▽]

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Members of the fluoroquinolone class are being actively evaluated for inclusion in tuberculosis chemotherapy regimens, and we sought to determine the best in vitro and pharmacodynamic predictors of in vivo efficacy in mice. MICs for *Mycobacterium tuberculosis* H37Rv were 0.1 mg/liter (sparfloxacin [SPX]) and 0.5 mg/liter (moxifloxacin [MXF], ciprofloxacin [CIP], and ofloxacin [OFX]). The unbound fraction in the presence of murine serum was concentration dependent for MXF, OFX, SPX, and CIP. In vitro time-kill studies revealed a time-dependent effect, with the CFU reduction on day 7 similar for all four drugs. However, with a J774A.1 murine macrophage tuberculosis infection model, CIP was ineffective at up to 32× MIC. In addition, MXF, OFX, and SPX exhibited less activity than had been seen in the in vitro time-kill study. After demonstrating that the area under the concentration-time curve (AUC) and maximum concentration of drug in plasma were proportional to the dose in vivo, dose fractionation studies with total oral doses of 37.5 to 19,200 mg/kg of body weight (MXF), 225 to 115,200 mg/kg (OFX), 30 to 50,000 mg/kg (SPX), and 38 to 100,000 mg/kg (CIP) were performed with a murine aerosol infection model. MXF was the most efficacious agent (3.0 ± 0.2 $\log_{10}$ CFU/lung reduction), followed by SPX (1.4 ± 0.1) and OFX (1.5 ± 0.1). CIP showed no effect. The ratio of the AUC to the MIC was the pharmacodynamic parameter that best described the in vivo efficacy. In summary, a lack of intracellular killing predicted the lack of in vivo activity of CIP. The in vivo rank order for maximal efficacy of the three active fluoroquinolones was not clearly predicted by the in vitro assays, however.

Fluoroquinolones (FQs) exhibit potent in vitro and in vivo antimycobacterial activity (2, 10). There is a significant effort to include fluoroquinolones as new front-line agents (ofloxacin [OFX] and moxifloxacin [MXF] [17, 25, 29]) and second-line agents (ciprofloxacin [CIP] [27]). There is also a considerable effort in the industry to discover and develop newer fluoroquinolones, and some of them might have value in the treatment of tuberculosis (TB). However, the choice of fluoroquinolone for treatment of tuberculosis is dictated by its efficacy with murine models, and as a consequence, only a very few members of the class are tested due to the complexities of the animal models for tuberculosis. Recently we described an approach to determining the pharmacokinetic/pharmacodynamic (PK/PD) driver and its magnitude for efficacy with an aerosol infection model of murine tuberculosis for rifampin and isoniazid (13, 14). By applying such a process, it is possible to rationally screen fluoroquinolones that might otherwise be missed because of either the lack of or the inappropriate use of in vitro parameters.

Due to the broad spectrum of activity of fluoroquinolones, it may be possible to extrapolate from the findings from studies on gram-negative and gram-positive organisms in terms of the PK/PD driver and its magnitude required to treat tuberculosis. MFX, which yields the highest ratio of the area under the concentration-time curve (AUC) to the MIC (*f*AUC/MIC) in standard human doses, is also the most potent fluoroquinolone with a murine model of tuberculosis (16). However, the size of the *f*AUC/MIC value achievable in humans, at 70 to 90, remains well below the optimal value of 100 to 125 at which efficacy against gram-negative bacilli is demonstrated (6). If one were to extrapolate from the findings with gram-negative pathogens, none of the fluoroquinolones reaches the value of 100 to 125 as per current dosing regimens (16). For a gram-positive pathogen like *Streptococcus pneumoniae*, a *f*AUC/MIC value of 25 to 30 was required for efficacy (28). Thus, it is difficult to predict the pharmacodynamic parameter and its magnitude for efficacy of fluoroquinolones against tuberculosis. An in-depth study of the PK/PD of fluoroquinolones in a murine model of tuberculosis could not only provide a better choice among these agents but may also provide a rational basis for setting the appropriate dose for humans. In this investigation, we report the PK/PD basis of efficacy for fluoroquinolones against *Mycobacterium tuberculosis* with an aerosol infection model of tuberculosis in BALB/c mice.

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Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 2003 [Radha K. Shandil et al., abstr. A 310] and the Keystone Symposia on Tuberculosis: Integrating Host-Pathogen Biology, Whistler, British Columbia, Canada, 2005 [Shandil et al., abstr. 3060]).

MATERIALS AND METHODS

Reagents. MXF, OFX, sparfloxacin (SPX), and CIP were purchased from Sai Quest Laboratories, Hyderabad, India. The purity was reconfirmed in-house by high-performance liquid chromatography (HPLC), liquid chromatography-mass spectroscopy (LCMS), and nuclear magnetic resonance spectroscopy analysis. Fluoroquinolone stock solutions were made in alkaline distilled water (0.02 N NaOH in water) for use in in vitro and ex vivo experiments. Carboxymethyl cellulose (lot no. 77H1077) was purchased from Sigma. EDTA (lot no. 5-4514) was purchased from Hi-Media Labs, Mumbai, India. Acetonitrile (HPLC grade) was obtained from Spectrochem Pvt. Ltd., Mumbai, India.

Microbial cultures and cell lines. *Mycobacterium tuberculosis* H37Rv (ATCC 27294) and J774A.1 macrophages were prepared for in vitro, macrophage, and animal infection studies by previously described methods (14). The inocula used for all of the experiments are derived from a single seed lot maintained at −70°C, which was made from infected mouse lungs followed by a single round of broth amplification. Briefly, *M. tuberculosis* H37Rv (ATCC 27294), a strain sensitive to all of the standard antimycobacterial agents, was grown in roller bottles in Middlebrook 7H9 broth supplemented with 0.2% glycerol, 0.25% Tween 80 (Sigma, St. Louis, MO), and 10% albumin dextrose catalase (Difco Laboratories) at 37°C for 7 to 10 days. Cells were harvested by centrifugation, washed twice in 7H9 broth, and resuspended in fresh 7H9 broth. Aliquots (0.5 ml each) were dispensed, and the seed lot suspensions were stored at −70°C.

Animals. The Institutional Animal Ethics Committee, registered with the government of India (registration no. CPCSEA 99/5) approved all animal experimental protocols and usage. Six- to eight-week-old BALB/c mice purchased from the National Institute of Nutrition, Hyderabad, India, were randomly assigned at seven per cage with the restriction that all cage members were within a 1- to 2-g weight of each other. They were allowed 2 weeks' acclimation before intake into experiments. Feed and water were given ad libitum.

MIC in broth. MICs of fluoroquinolones in broth were determined against *M. tuberculosis* by the standard microdilution method using drug concentrations ranging from 256 mg/liter to 0.01 mg/liter as per procedures described previously (14). The MIC was defined as the lowest concentration at which there was no visible turbidity.

Protein binding using equilibrium dialysis. Mouse plasma protein binding of fluoroquinolones was determined by using a Hoefer Scientific Equilibrium micro volume dialyzer with cellulose acetate membranes (12,000 to 14,000 nominal molecular weight limit; Hoefer Scientific, Germany) as described previously (14), followed by quantification of the concentration by HPLC-UV or LCMS. The concentrations studied for CIP, OFX, and SPX were 0.01, 0.1, 0.5, 1, 10, and 50 mg/liter; the concentrations for MXF were 0.01, 0.05, 0.1, 0.5, 1, and 10 mg/liter. Each concentration was analyzed in duplicate and the mean values reported. The relationship between concentration and fraction unbound (fu) was described by the Boltzman equation (GraphPad Prism, San Diego, CA).

Killing kinetics in vitro. Killing kinetics of fluoroquinolones was analyzed over a wide range (256 mg/liter to 0.01 mg/liter) with Bactec 7H12B media. Before addition of the drug, day-0 plating was carried out to estimate the initial bacterial count. Numbers of viable CFU following incubation with various concentrations of drugs after 1, 7, and 14 days were determined by plating on Middlebrook 7H11 agar plates (Difco Laboratories) as described previously (14).

Intracellular MIC and killing kinetics. Intracellular killing kinetics of fluoroquinolones was determined with *M. tuberculosis*-infected J774A.1 macrophages (MOI, 1:8; macrophage, H37Rv) at concentrations ranging from 32 mg/liter to 0.5 mg/liter as described previously (14) for 3 days. The intracellular MIC was defined as the minimal concentration that produces a static effect on the bacilli after 3 days of drug exposure. The intracellular efficacy was measured as the $\log_{10}$ CFU/ml reduction over the assay period.

Pharmacokinetic measurements. The concentration of fluoroquinolones in mouse plasma was determined by HPLC-UV or LCMS. The assays were linear over a wide concentration range for all the FQs (0.625 to 256 μg/ml for OFX, 0.2 to 100 μg/ml for SPX, 0.019 to 80 μg/ml for CIP, 0.0625 to 32 μg/ml for MXF) with a correlation of 0.99. The limits of quantitation were 0.0625 μg/ml for MXF and OFX and 0.2 and 0.019 μg/ml for SPX and CIP, respectively. The recoveries ranged between 90 and 100%.

TABLE 1. MICs of drugs for *M. tuberculosis* H37Rv either in the presence of 7H9 broth or within J774A.1 murine macrophages

Category	MIC (mg/liter) of drug			
	MXF	OFX	SPX	CIP
Broth	0.5	0.5	0.1	0.5
Intracellular	1.0	2.0	2.0	4.0

Pharmacokinetics of OFX, MXF, CIP, and SPX in uninfected mice. For safety reasons, dose-ranging studies were conducted with uninfected mice to determine the linearity of pharmacokinetics for the four fluoroquinolones with oral administration by gavage as single ascending doses for each drug at a 10-ml/kg of body weight dose volume. Doses used were 37.5, 150, 600, 1,200 and 2,400 mg of OFX/kg, 6, 12, 50, 200, and 400 mg of MXF/kg, 37.5, 150, 600, 1,200, and 2,400 mg of CIP/kg, 5, 100, 250, 500, and 1,000 mg of SPX/kg and were administered as suspensions in 0.25% (wt/vol) carboxymethylcellulose. Blood was collected at various time points, ranging from 0.08 h to 50 h postdosing, by retroorbital sinus puncture and plasma harvested as described previously (14). Three animals were used per time point. The concentrations of fluoroquinolones in plasma were determined by HPLC/LCMS. PK analyses of the plasma concentration-time relationships for the four fluoroquinolones were performed with WinNonLin software (version 1.5; Scientific Consulting, Inc.). A noncompartmental library model (model 200) was used to calculate the PK parameters, such as the maximum concentration of drug in plasma (C_{max}), time to C_{max}, elimination rate constant, elimination half-life, and AUC from time zero to infinity ($AUC_{0-\infty}$). The fC_{max}, $fAUC_{0-\infty}$, and $fT_{>MIC}$ (percent) values were obtained by converting the total concentrations into unbound concentrations using the Boltzman equation obtained for each FQ, followed by noncompartmental analysis. Calculation of PK/PD parameters was as follows. The broth MICs of fluoroquinolones for *M. tuberculosis* (OFX, MXF, and CIP, 0.5 mg/liter; SPX, 0.1 mg/liter) were used to calculate PK/PD parameters. The fC_{max}/MIC value was defined as the ratio of fC_{max} to the MIC, the fAUC/MIC value was defined as the ratio of $AUC_{0-\infty}$ to the MIC for the period of 576 h divided by 24 h (OFX, MXF, and CIP) or 432 h divided by 24 h (SPX), and the percent $fT_{>MIC}$ value was defined as the percentage of time that each fluoroquinolone exceeded the MIC in 576 h (OFX, MXF, and CIP) or 432 h (SPX). The $fT_{>MIC}$ value was estimated by the first-order kinetics equation ($C = C_0e^{-kt}$). The relationship between dose and fC_{max}/MIC, fAUC/MIC, and $fT_{>MIC}$ (percent) was used to estimate PK/PD values for the doses used in the dose fractionation studies.

Aerosol infection in mice. We have used an aerosol infection model wherein drugs are evaluated following a respiratory infection with low numbers of tubercle bacilli (1, 24). Mice were infected via the inhalation route as described previously (14), in an aerosol infection chamber designed and constructed in the Mechanical Engineering Shop, University of Wisconsin—Madison.

Dose fractionation studies. Doses for fractionation were selected based on PK linearity up to the maximum doses tested. Four weeks after initiation of infection, mice were dosed orally with each fluoroquinolone over a period of 4 weeks (MXF, OFX, and CIP) or only 3 weeks (SPX) as per the regimens shown in Table 3. All single doses that exceeded the respective 50% lethal dose value for each fluoroquinolone were eliminated from the design. Three mice were used for each regimen, with the control mice receiving saline. At the onset and 24 h after completion of treatment, groups of mice were killed by exposure to CO_2 and the lungs aseptically removed for homogenization in a final volume of 2.0 ml, using Wheaton Teflon-glass tissue grinders (catalog no. W012576). Each suspension was serially diluted in 10-fold steps, and appropriate dilutions were plated on Middlebrook 7H11 agar supplemented with 10% albumin dextrose catalase (Difco Laboratories) and incubated at 37°C with 5% CO_2 for 3 weeks.

Statistical analysis. Colony counts obtained from plating were transformed to $\log_{10}(X + 1)$, where X equals the total number of viable tubercle bacilli calculated to be present in a given sample. Nonlinear regression (curve fit) analysis using an inhibitory sigmoid E_{max} response model with or without constants was done for the in vitro, macrophage, and in vivo kill data. GraphPad Prism (version 3; GraphPad Software, Inc., San Diego, CA) was used for all the above calculations. The 50% effective concentration was defined as the concentration of the drug required to achieve 50% of the E_{max} effect. A 1-$\log_{10}$ CFU kill effect was calculated from the dose-response curves according to the following equation (13):

$$E = E_0 - \{E_{max}(x)^N/[(EC_{50})^N + (x)^N]\}$$

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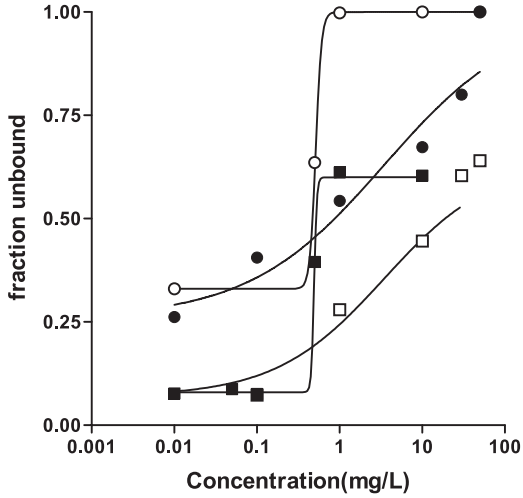

FIG. 1. Percentage of free drug as a function of the total concentration. ■, MXF; □, OFX; ●, SPX; ○, CIP.

where $E = \log_{10}$ CFU at any given concentration; $E_0 = \log_{10}$ CFU with no drug; $E_{max} =$ lowest $\log_{10}$ CFU achieved following treatment; $N =$ Hill slope; and $x = fC_{max}$/MIC ratio or fAUC/MIC ratio.

RESULTS

Broth MIC and protein binding. The results for broth MICs, plasma protein binding, and intracellular MICs for MXF, OFX, CIP, and SPX are shown in Table 1 and Fig. 1. The broth MICs of MFX, OFX, and CIP were 0.5 mg/liter, and that for SPX was 0.1 mg/liter. The intracellular MICs of MXF, OFX, SPX, and CIP were 2-, 4-, 20-, and 8-fold higher than their broth MICs, respectively. The fraction unbound for each of the four FQs was concentration dependent. The fu ranged from 0.33 to 1, 0 to 0.64, and 0.26 to 1 in the concentration range of 0.01 to 50 mg/liter for CIP, OFX, and SPX, respectively; the fu range was 0.077 to 0.6 for MXF in the concentration range of 0.01 to 10 mg/liter.

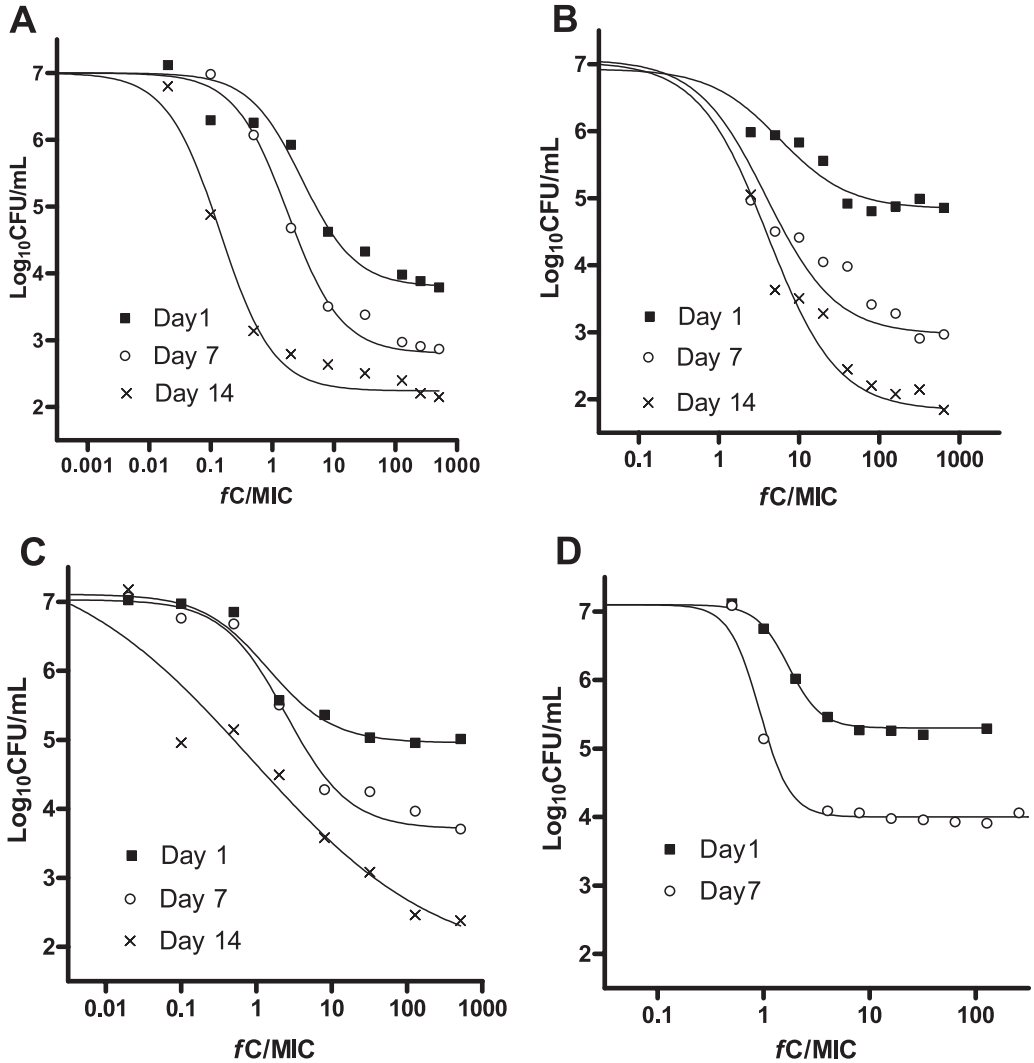

FIG. 2. Effects of increasing *fC*/MIC ratios on the bactericidal activities of MXF (panel A), OFX (B), SPX (C), and CIP (D) on days 1, 7, and 14 after the addition of drug. Each point represents the mean of triplicate values. The bactericidal effect is calculated on the basis of the initial inoculum prior to the addition of the drug.

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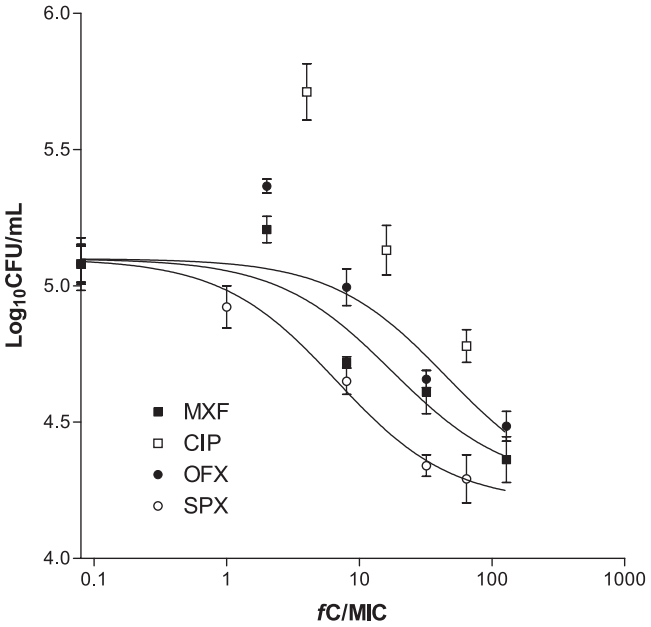

FIG. 3. Effects of increasing *f*C/MIC ratios on the intracellular bactericidal activities of the four fluoroquinolones, MXF, OFX, SPX, and CIP, against *M. tuberculosis* in the J774A.1 murine macrophage cell line after 3 days of exposure to the drug. Each point represents the mean ± standard deviation of triplicate values. The bactericidal effect is calculated on the basis of the initial inoculum prior to addition of the drug.

In vitro killing kinetics. Killing kinetics was analyzed with late-log-phase *M. tuberculosis* cultures over a concentration range of 0.01 to 256 mg/liter (Fig. 2). All four fluoroquinolones exhibited a clear time-dependent killing kinetics, ranging from a 3- to 4-$\log_{10}$ CFU/ml reduction for all of the four drugs on day 7. MXF exhibited the highest bactericidal activity on all days, i.e., 1-, 7-, and 14-day exposures, followed by OFX, SPX, and CIP. CIP was tested only up to a 7-day exposure and achieved a 3.2-$\log_{10}$ CFU/ml reduction.

Intracellular MIC and killing kinetics. In an intracellular infection model of J774A.1 macrophages, the MICs of all fluoroquinolones increased by 2- to 20-fold relative to the extracellular broth MICs (Table 1). MXF was least affected, with a 2-fold increase in the intracellular MIC, followed by OFX (4-fold), SPX (20-fold), and CIP (8-fold). The E_{max} values for all fluoroquinolones sharply decreased with the intracellular infection model compared to those with the broth cultures (Fig. 3). Fluoroquinolones at up to 32× MIC were either weakly bactericidal (maximum of 0.5- to 0.8-$\log_{10}$ CFU/ml reduction for OFX, MXF, and SPX) or ineffective (CIP). Intracellular bactericidal activities of the fluoroquinolones did increase with time from day 1 to day 3, similar to those observed in broth cultures.

TABLE 2. Pharmacokinetic parameters for four FQs, determined with uninfected BALB/c mice, following single ascending doses

Drug	Dose range (mg/kg)	C_{max} (μg/ml)	$AUC_{0-\infty}$ (μg · h/ml)	$t_{1/2}$ value (h)
OFX	37.5–2,400	1.7–101.2	29–661	4.93 ± 2.6
MXF	6–400	0.38–9.23	25–63	4.46 ± 0.66
CIP	37.5–2,400	1.2–31	29–125	4.74 ± 5.32
SPX	5–1,000	0.75–14	43–191	9.8 ± 5.32

Pharmacokinetics of fluoroquinolones in uninfected mice. In general, all the fluoroquinolones displayed linear PK in the dose range studied (Fig. 4). The PK parameters for the fluoroquinolones are summarized in Table 2. The elimination half-lives for MXF, OFX, and CIP were similar (4.5 to 4.9 h), with SPX having the longest (9.8 h). The fC_{max}, $AUC_{0-\infty}$, and $fT_{>MIC}$ (percent) values increased in proportion to the dose of fluoroquinolones administered (Fig. 4).

Dose fractionation studies. Dose fractionation studies with total oral doses of 225 to 115,200 mg/kg (OFX), 37.5 to 19,200 mg/kg (MXF), 31 to 50,000 mg/kg (SPX), and 38 to 100,000 mg/kg (CIP) were performed in an aerosol infection model of BALB/c mice, with fluoroquinolone tolerability limiting maximum doses (Table 3). MXF was the most efficacious (3.2 $\log_{10}$ ± 0.2 $\log_{10}$ CFU/lung reduction), followed by OFX (1.5 ± 0.1), SPX (1.4 ± 0.1), and CIP, which had no effect (Fig. 5). Of note, a plateau for the effect of MXF was not reached, probably due to toxicity preventing MXF being administered over a wider range of doses. The highest *f*AUC/MIC value achieved was lower than that achieved for OFX and SPX. *f*AUC/MIC was the primary pharmacodynamic parameter that best described the in vivo efficacies of MXF (r^2 = 0.94), OFX (r^2 = 0.82), and SPX (r^2 = 0.79). Although the $fT_{>MIC}$ (percent) showed higher correlations for MXF and SPX, the scatter was

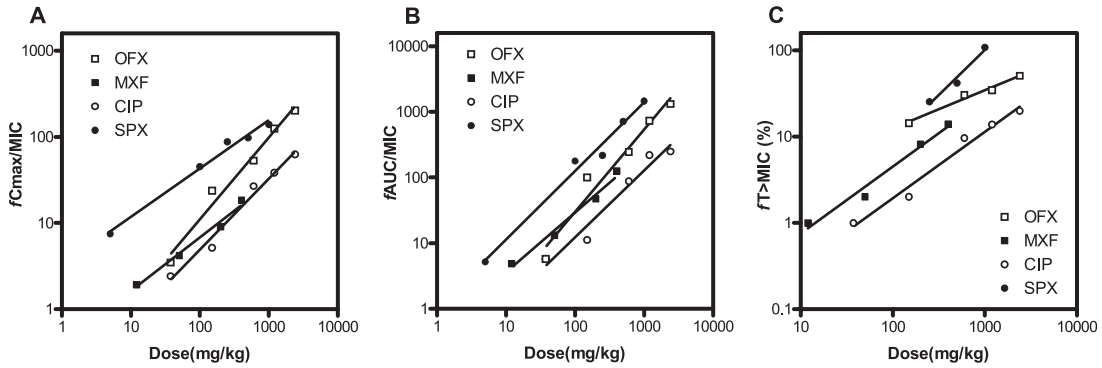

FIG. 4. Dose proportionality and limits of linearity for the four fluoroquinolones with respect to fC_{max}/MIC (A), *f*AUC/MIC (B), and $fT_{>MIC}$ (percent) (C).

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TABLE 3. Dose fractionation design for efficacy studies with an aerosol infection model of tuberculosis in BALB/c mice

Drug	Duration of therapy (wk)	Total dose (mg/kg)	Fractionation[a] (no. of doses)	*f*AUC/MIC	fC_{max}/MIC	$fT_{>MIC}$ (%)	LD_{50} (mg/kg) (reference)
MXF	4	38–19,000	1, 3, 6, 12, 18, 36, 48	0.11–177	1–11	0–100	600 (26)
OFX	4	225–115,000	1, 3, 6, 12, 24, 36, 48	2–320	1–117	0–58	3,000 (15)
SPX	3	31–50,000	1, 6, 12, 24, 36, 48	3–522	4–112	10–100	
CIP	4	38–100,000	1, 4, 12, 18, 36	4–201	5–70	10–100	2,000 (12)

[a] All single doses exceeding the LD_{50} were eliminated from the design.

greater for $fT_{>MIC}$ curves than for *f*AUC/MIC curves (Fig. 5). fC_{max}/MIC values showed a poor correlation for the three fluoroquinolones.

DISCUSSION

The WHO recommends the use of fluoroquinolones as second- and third-line drugs for the treatment of multidrug-resistant TB (27). In recent years, a few drugs of the fluoroquinolone class have been proposed as viable and ready-to-use alternatives to existing anti-TB drugs for the treatment of pulmonary TB (2, 10, 17, 18, 23). And in the coming decade, it is likely that more may be tried. However, a rational choice for the best option could be made if there were preclinical predictors of in vivo efficacy. The aim of this study was to identify the relevant in vitro parameter that best described the in vivo efficacy of MXF, along with OFX, CIP, and SPX, with an aerosol infection murine model for tuberculosis, using a dose fractionation design as described previously (14).

In general, the fluoroquinolones had potent MICs for *M. tuberculosis*, with SPX being fivefold more potent than the other three. All four fluoroquinolones displayed time-dependent killing kinetics for *M. tuberculosis*. This was evident from the analysis of the inhibitory dose-response curves for each of the four fluoroquinolones (Fig. 2). The E_{max} value for each fluoroquinolone increased from day 1 to day 14 for the same range of *fC*/MICs. An E_{max} value of 4.4- to 5.1-$\log_{10}$ CFU/ml reduction was seen approximately at an *fC*/MIC of 10 for each fluoroquinolone, indicating a narrow concentration range of activity. To our knowledge, this is the first report of fluoroquinolones displaying time-dependent killing kinetics in broth and is in contrast to the reports of concentration-dependent killing kinetics of fluoroquinolones against other gram-positive and gram-negative bacteria (7, 9, 30).

Even though the four fluoroquinolones were equipotent under broth conditions, their intracellular activities in the macrophage were substantially lower. CIP failed to show any activity, whereas MXF, OFX, and SPX showed maximal reductions of less than 1 $\log_{10}$ CFU/ml after 3 days of exposure. This may be partly explained by the phagosomal location of *M. tuberculosis* in the macrophage, whereas fluoroquinolones accumulate in the cytoplasm (3, 22). Further, the failure of CIP to kill intracellular bacteria could be attributed to mammalian cell efflux (21) as well as intrinsic CIP resistance mediated by the ABC efflux pump present in the mycobacterial cells (19). Recently an in vitro PD model attributed CIP failure to rapid emergence of resistance at clinically free drug levels, in contrast to MFX, to which no resistance emerged (11).

Protein binding was concentration dependent for all four FQs. The fu values obtained for CIP in this study were similar to that observed by Scaglione et al. (20). Saturation of fu was not achieved for OFX and SPX in the concentration range studied. With the murine model of tuberculosis, MXF showed the highest efficacy, followed by SPX and OFX, with CIP being ineffective. A complete inhibitory sigmoid curve was obtained for OFX, whereas it was incomplete for MXF and SPX. Although the fluoroquinolones showed time-dependent killing kinetics under in vitro conditions, the *f*AUC/MIC parameter best described their efficacy in vivo. This effect may be due to their postantibiotic effect on *M. tuberculosis* (4). This appears analogous to those for vancomycin, tetracycline, and azithromycin. These agents do not exhibit concentration-dependent killing in vitro, but *f*AUC/MIC best correlates with their in vivo efficacy, and this in turn has been linked to their lengthy in vivo postantibiotic effects (5). In the absence of a complete dose-response curve for MXF and SPX, the true potencies (*f*AUC/MIC 50% effective concentrations) of the FQs could not be compared. Since an efficacy of at least 1-$\log_{10}$ CFU/lung reduction was seen with MXF, OFX, and SPX, potencies were compared in terms of *f*AUC/MIC required for a 1-$\log_{10}$ CFU/lung reduction. An *f*AUC/MIC ratio of >100 to 150 was associated with a 1-$\log_{10}$ CFU/lung reduction with our model. This was in contrast to the observations that an *f*AUC/MIC ratio of approximately 30 was predictive of microbiological and clinical cure for fluoroquinolones against gram-positive pathogens (7). Clearly a PK/PD surrogate for significant antituberculosis activity in the lungs was not a constant number for different drugs in the fluoroquinolone class.

From these studies, it is apparent that neither the potency nor the efficacy in the broth was predictive of these indices with the murine model. On the contrary, the extent of killing observed with the macrophage model was indicative of the in vivo efficacy in the cases of CIP, OFX, and SPX. However, MXF's efficacy with the murine model was significantly higher than that with the macrophage model. Analysis of PK data showed that even though the plasma *f*AUC/MICs of MXF were lower than those of OFX and SPX, the highest efficacy was seen with MXF. We tested the hypothesis that the extent of distribution of fluoroquinolones into tissues determines their efficacy. Comparison of the volume of distribution at steady state for the four fluoroquinolones in mice showed that there was no correlation of volume of distribution at steady state with efficacy (our unpublished data). CIP has demonstrable early bactericidal activity in human tuberculosis (8, 23) and has been recommended as part of a treatment for multidrug-resistant tuberculosis. Presently, OFX and MXF are undergoing various clinical trials for inclusion into the primary regimen as part of

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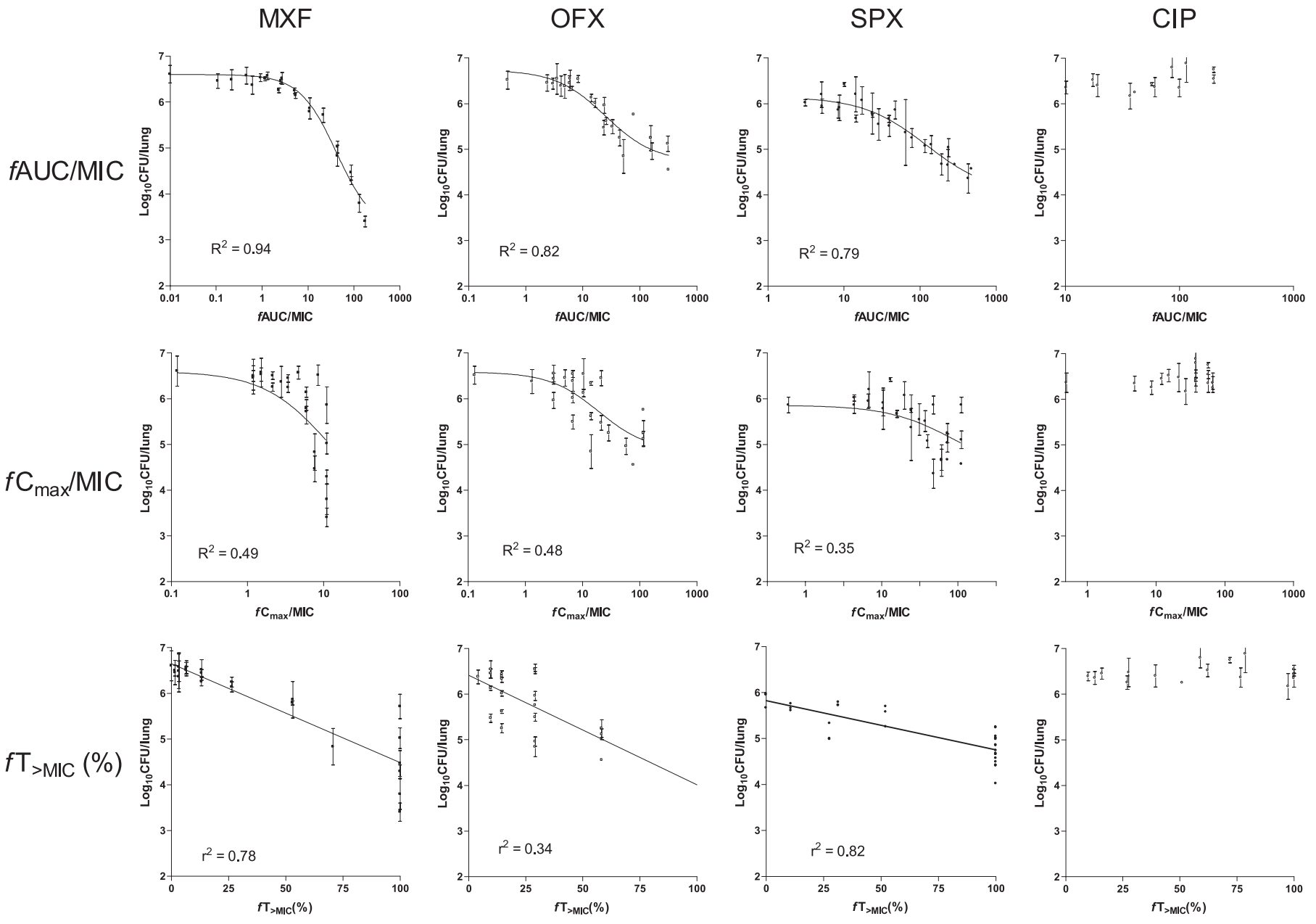

FIG. 5. Relationship between *f*AUC/MIC, fC_{max}/MIC, and $fT_{>MIC}$ (percent) of the four fluoroquinolones and $\log_{10}$ CFU/lung of *M. tuberculosis* when the total dose is fractionated as per the design shown in Table 3. Each point represents the mean ± standard deviation of triplicate values.

the induction phase of the directly observed therapy short course program (18). Our in vivo findings further strengthen the case for the clinical trial of moxifloxacin but do not support the inclusion of ciprofloxacin in the treatment of tuberculosis.

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