Title

Moxifloxacin, ofloxacin, sparfloxacin, and ciprofloxacin against *Mycobacterium tuberculosis*: Evaluation of in vitro and pharmacodynamic indices that best predict in vivo efficacy

Running Head: Activity of fluoroquinolones vs. *M. tuberculosis*

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Abstract

Members of the fluoroquinolone class are being actively evaluated for inclusion in TB chemotherapy regimens and we sought to determine the best in vitro and pharmacodynamic predictors of in vivo efficacy in mice. MICs for *M. tuberculosis* H37Rv were 0.1 mg/L (SPX), 0.5 mg/L (MXF, CIP and OFX). The unbound (fu) 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, in a J774A.1 murine macrophage tuberculosis infection model, CIP was ineffective at up to 32x MIC. In addition MXF, OFX, and SPX exhibited less activity than had been seen in the in vitro time kill study. After demonstrating that AUC and C<sub>max</sub> were proportional to dose in vivo, dose fractionation studies with total oral doses of 37.5 – 19200 mg/kg [MXF], 225 – 115200 mg/kg [OFX], 30 – 50000 mg/kg [SPX], and 38 – 100000 mg/kg [CIP] were performed in a murine aerosol infection model. MXF was the most efficacious agent (3.0 ± 0.2 Log<sub>10</sub>CFU/lung reduction), followed by SPX (1.4 ± 0.1) and OFX (1.5 ± 0.1). CIP showed no effect. fAUC/MIC was the pharmacodynamic parameter that best described the in vivo efficacy. In summary, lack of intracellular killing predicted the lack in vivo activity of CIP. The in vivo rank order for maximal efficacy of the three active fluoroquinolones was not, however, clearly predicted by the in vitro assays.

Key Words: Tuberculosis, Aerosol Infection, Fluoroquinolones, and Pharmacokinetics-Pharmacodynamics.
Introduction

Fluoroquinolones (FQs) exhibit potent in vitro and in vivo antimycobacterial activity (2,11). There is a significant effort to include fluoroquinolones as new front-line agents (OFX and MXF; 17, 27, 30) and second line agents (CIP; 28). There is also considerable effort in the industry to discover and develop newer fluoroquinolones and some of them might have value in the treatment of tuberculosis. However, the choice of the fluoroquinolone for treatment of tuberculosis is dictated by its efficacy in murine models and as a consequence only very few members of the class are tested due to the complexities in the animal models for tuberculosis. Recently, we described an approach to determine the PK/PD driver and its magnitude for efficacy in an aerosol infection model of murine tuberculosis for rifampicin and isoniazid (20, 21). By applying such a process, it is possible to rationally screen fluoroquinolones that might be otherwise 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 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 fAUC/MIC in standard human doses, is also the most potent fluoroquinolones in a murine model of tuberculosis (16). However, the size of the fAUC/MIC value achievable in humans, at 70–90, remains well below the optimal value of 100–125, at which the efficacy against gram-negative bacilli is demonstrated (6). If one were to extrapolate the findings from the Gram-negative pathogens, none of the fluoroquinolones reaches the value of 100–125 as per current dosing regimens (16). For a Gram-positive pathogen like Streptococcus pneumoniae a fAUC/ MIC of 25–30 was required for efficacy (29). 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 better choice among these agents but may also provide a rational basis for setting the appropriate dose in humans. In this investigation, we report the PK/PD basis of efficacy for fluoroquinolones against M. tuberculosis in an aerosol infection of tuberculosis in BALB/c mice.


Materials and Methods

Reagents: MXF, OFX, SPX and CIP were purchased from Sai Quest laboratories, Hyderabad, India. The purity was reconfirmed in house by HPLC, LCMS and NMR spectroscopy analysis. Fluoroquinolone stock solutions were made in alkaline distilled water (0.02N NaOH in water) for use in vitro and ex vivo experiments. Carboxymethyl cellulose (Lot No. 77H1077) was purchased from Sigma, USA. Ethylenediaminetetraacetic acid (ETDA-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 ATCC27294 and J774A.1 macrophages were prepared for in vitro, macrophage and animal infection studies by previously described methods (21). The inocula used for all the experiments are derived from a single seed-lot maintained at –700C, 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 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, USA) and 10% albumin dextrose catalase (ADC; Difco Laboratories, USA) at 37°C for 7-10 days. Cells were harvested by centrifugation, washed twice in 7H9 broth and resuspended in fresh 7H9 broth. 0.5 mL aliquots were dispensed and the seed-lot suspensions were stored at -70°C.

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

MIC in Broth: Broth MIC of fluoroquinolones was determined against *M. tuberculosis* by standard microdilution method using drug concentrations from 256 mg/L to 0.01 mg/L as per procedures described previously (21). The lowest concentration at which there was no visible turbidity was defined as the MIC.

Protein binding using Equilibrium Dialysis: Mouse plasma protein binding of fluoroquinolones was determined in a Hoefer Scientific Equilibrium micro volume dialyser with cellulose acetate membranes (12-14K NMWL, Hoefer Scientific, Germany) as described previously (21) followed by quantification of 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/L; the concentrations for MXF were 0.01, 0.05, 0.1, 0.5, 1 and 10 mg/L. Each concentration was analyzed in duplicates and the mean values reported. The relationship between concentration and fraction unbound (fu) was described by the Boltzman equation (GraphPad Prism, San Diego, California).
Killing kinetics in vitro: Killing kinetics of fluoroquinolones was performed over a wide range (256 mg/L to 0.01 mg/L) in Bactec 7H12B media. Before addition of the drug, day-0 plating was carried out to estimate the initial bacterial count. Number 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, USA) as described previously (21).

Intracellular MIC and killing kinetics: Intracellular killing kinetics of fluoroquinolones was determined in M. tuberculosis infected J774A.1 macrophages (MOI 1:8, macrophage: H37Rv) at concentrations ranging from 32 mg/L to 0.5 mg/L as described previously (21) for 3 days. Intracellular MIC was defined as the minimal concentration that produces a static effect on the bacilli after 3 days of drug exposure. Intracellular efficacy was measured as \( \log_{10} \text{CFU/mL} \) 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 ug/mL for OFX; 0.2 to 100 ug/mL for SFX; 0.019 to 80 ug/mL for CIP; 0.0625 to 32 ug/mL for MFX) with a correlation of 0.99. The LOQ was 0.0625 ug/mL for MFX and OFX, 0.2 and 0.019 ug/mL for SFX and CIP, respectively. The recoveries ranged between 90 and 100%.

Pharmacokinetics of OFX, MXF, CIP & SFX in uninfected mice: For safety reasons, dose-ranging studies were conducted in uninfected mice to determine the linearity of pharmacokinetics for the four fluoroquinolones when administered orally by gavage, as single ascending doses for each drug at 10mL/Kg dose volume. Doses used were 37.5, 150, 600, 1200 and 2400 mg of OFX/kg, 6, 12, 50, 200 and 400 mg of MXF/kg, 37.5, 150, 600, 1200 and 2400 mg of CIP/kg, 5, 100, 250, 500 and 1000 mg of SFX/kg and were administered as suspensions in 0.25% (wt/vol) carboxymethylcellulose (CMC). Blood was
collected at various time points ranging from 0.08h to 50h post dosing by retro-orbital sinus puncture and plasma harvested as described previously (21). Three animals were used per time point. The concentration of fluoroquinolones in plasma was determined by HPLC/LCMS. PK Analysis: PK analyses of the plasma concentration-time relationships for the four fluoroquinolones were performed with WinNonLin software (version 1.5; Scientific Consulting, Inc.). A non-compartmental library model (model 200) was used to calculate the PK parameters, such as the maximum concentration of drug in plasma ($C_{\text{max}}$), time to $C_{\text{max}}$ ($t_{\text{max}}$), elimination rate constant, elimination half-life, and AUC from time zero to infinity (AUC 0-$\infty$). The $fC_{\text{max}}$, $f\text{AUC}0-\infty$ and $fT_{>\text{MIC}}$ (%) was obtained by converting the total concentrations into unbound concentrations using the Boltzman equation obtained for each FQ and then followed by noncompartmental analysis.

Calculation of PK/PD parameters: The broth MIC of fluoroquinolones on $M. \ tuberculosi$s OFX, MXF and CIP (0.5 mg/L), SPX (0.2 mg/L) was used to calculate PK/PD parameters. The $fC_{\text{max}}/\text{MIC}$ was defined as the ratio of $fC_{\text{max}}$ to MIC, the $f\text{AUC}/\text{MIC}$, was defined as the ratio of AUC0-432 to MIC for the period of 576 h divided by 24 (OFX, MXF, CIP) or 432 h divided by 24 h (SPX) and the %$fT_{>\text{MIC}}$ was defined as the percentage of the time that each fluoroquinolone exceeded MIC in 576 h (OFX, MXF, CIP) or 432 h (SPX). The $fT_{>\text{MIC}}$ was estimated by the first order kinetics equation ($C= C_0e^{-kt}$). The relationship between dose and $fC_{\text{max}}/\text{MIC}$, $f\text{AUC}/\text{MIC}$, and $fT_{>\text{MIC}}$ (%) was used to estimate PKPD 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 (1,26). Mice were infected via the inhalation route as described previously (21), in an aerosol infection chamber designed and constructed in the Mechanical Engineering Shop, University of Wisconsin-Madison, USA.
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 PO with each fluoroquinolone over a period of 4 weeks (MXF, OFX and CIP) and only 3 weeks (SPX) as per the regimens shown in Table 3. All single doses that exceeded respective LD50 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 hrs after completion of treatment, groups of mice were killed by exposure to CO2 and the lungs aseptically removed for homogenisation in a final volume of 2.0 mL, using Wheaton Teflon-Glass tissue grinders [catalogue No: W012576]. Each suspension was serially diluted in 10-fold steps, and appropriate dilutions were plated on Middlebrook 7H11 agar supplemented with 10% ADC (Difco Laboratories, USA) and incubated at 37°C with 5% CO2 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. Non-linear regression (curve fit) analysis using an Inhibitory Sigmoid Emax 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, USA) was used for all the above calculations. EC50 was defined as the concentration of the drug to achieve 50% of the Emax effect. 1 Log$_{10}$CFU kill effect was calculated from the dose response curves according to the following equation (20):

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

Where,

\[
E = \text{Log}_{10}\text{CFU at any given concentration}
\]

\[
E_0 = \text{Log}_{10}\text{CFU at no drug}
\]
\[ E_{\text{max}} = \text{Lowest } \log_{10}\text{CFU achieved following treatment} \]

\[ N = \text{Hill slope} \]

\[ X = f_{C_{\text{max}}}/\text{MIC ratio or } f_{\text{AUC}}/\text{MIC ratio} \]

**Results**

**Broth MIC and protein binding:** The results for broth MIC, plasma protein binding and intracellular MIC for MXF, OFX, CIP and SPX are shown in table 1 and figure 1. The broth MICs of MXF, OFX and CIP was 0.5 mg/L and for SPX it was 0.1 mg/L. 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 all the 4 FQs were concentration dependent. The \( f_u \) ranged between 0.33 to 1, 0 to 0.64, and 0.26 to 1 in the concentration range 0.01 to 50 mg/L for CIP, OFX, and SPX, respectively; the \( f_u \) range was 0.077 to 0.6 for MXF in the concentration range 0.01 to 10 mg/L.

**In vitro killing kinetics:** Killing kinetics was performed on late-log phase *M. tuberculosis* cultures over a concentration range between 0.01 – 256 mg/L. All four fluoroquinolones exhibited a clear time dependent killing kinetics ranged from 3 to 4 \( \log_{10}\text{CFU/mL} \) reduction for all the 4 drugs on day 7. MXF exhibited 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 7-day exposure and achieved a 3.2 \( \log_{10}\text{CFU/mL} \) reduction.

**Intracellular MIC and killing kinetics:** In an intracellular infection model of J774A.1 macrophages, the MIC of all fluoroquinolones increased by 2-20 fold than the extra cellular broth MIC (table 1). MXF was least affected with 2-fold increase in intracellular MIC followed by OFX (4 fold), SPX (20-fold) and CIP (8-fold). The \( E_{\text{max}} \) of all the fluoroquinolones sharply decreased in the intracellular infection model as compared to the broth cultures (figure 3). Fluoroquinolones at up to 32x MIC were either weakly...
bactericidal (maximum of 0.5–0.8 Log$_{10}$CFU/mL reduction, OFX, MXF, SPX) or ineffective (CIP). Intracellular bactericidal activity of the fluoroquinolones did increase with time from day 1 to day 3 similar to that observed in broth cultures.

Pharmacokinetics of fluoroquinolones in uninfected mice: In general, all the fluoroquinolones displayed linear PK in the dose range studied (figure 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 (9.8h). The fC$_{max}$, AUC$_{0-\infty}$ and fT$_{>MIC}$ (%) increased in proportion to the dose of fluoroquinolones administered (figure 4).

Dose-fractionation studies: Dose fractionation studies with total oral doses of 225 - 115200 mg/kg [OFX], 37.5 - 19200 mg/kg [MXF], 31 - 50000 mg/kg [SPX], and 38 - 100000 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 ± 0.2 Log$_{10}$CFU/lung reduction), followed by OFX (1.5 ± 0.1), SPX (1.4 ± 0.1) and CIP had no effect (figure 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 fAUC/MIC achieved was lower than that achieved for OFX and SPX. fAUC/MIC was the primary pharmacodynamic parameter that best described the in vivo efficacy of MXF (r$^2 = 0.94$), OFX (r$^2 = 0.82$), and SPX (r$^2 = 0.79$). Although the fT$_{>MIC}$ (%) showed higher correlations for MXF and SPX the scatter was more in fT$_{>MIC}$ curves than for fAUC/MIC curves (figure 5). fC$_{max}$/MIC 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 MDR–TB (28). In the recent years a few drugs of the fluoroquinolone class have been proposed as a viable and ready to use alternative to existing anti-TB drugs for the treatment of pulmonary TB (2, 11, 17, 18, 25). And in the coming decade, it is likely that some more maybe tried. However a rational choice for the best option could be made if there were pre-clinical 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 in an aerosol infection murine model for tuberculosis, using dose fractionation design as described previously (21).

In general, the fluoroquinolones had potent MICs on \textit{M. tuberculosis}, with SPX being 5 fold more potent than the other three. All the four fluoroquinolones displayed time dependent killing kinetics of \textit{M. tuberculosis}. This was evident from the analysis of the inhibitory dose response curves for each of the four fluoroquinolones (see fig 2). The Emax for each fluoroquinolone increased from day 1 to day 14 for the same range of fC/MICs. An Emax of 4.4-5.1 Log_{10} mL CFU/mL reduction was seen approximately at a 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, 31).

Even though all the four fluoroquinolones were equipotent under broth conditions, their intracellular activity in the macrophage was 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 maybe partly explained by the phagosomal
location of *M. tuberculosis* in the macrophage, whereas fluoroquinolones accumulate in the cytoplasm (3, 23). Further, the failure of CIP to kill intracellular bacteria could be attributed to mammalian cell efflux (22) as well as intrinsic CIP resistance mediated by ABC efflux pump present in the mycobacterial cells (19). Recently, an in vitro PD model attributed CIP failure due to rapid emergence of resistance at clinically free drug levels in contrast to MFX that has no resistance (12).

The protein binding was concentration dependent for all the four FQs. The fu values obtained for CIP in this study was similar to that observed by Scaglione et al (24). Saturation of fu was not achieved for OFX and SPX in the concentration range studied. In 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, fAUC/MIC best described their efficacy in vivo. This effect maybe due to their post-antibiotic effect (PAE) on *M. tuberculosis* (4). This appears analogous to that for vancomycin, tetracycline and azithromycin. These agents do not exhibit concentration dependent killing in vitro but fAUC/MIC best correlates with their in vivo efficacy and this, in turn, has been linked to their lengthy in vivo PAEs (5). In the absence of a complete dose response curve for MXF and SPX, the true potencies (fAUC/MIC EC50) 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 fAUC/MIC required for a 1-Log$_{10}$CFU/lung reduction. An fAUC/MIC ratio > of 100-150 was associated with a 1 Log$_{10}$CFU/lung reduction in our model. This was in contrast to the observations that an fAUC/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 anti-tubercular 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 in the murine model. On the contrary, the extent of killing observed in the macrophage model was indicative of the in vivo efficacy in the case of CIP, OFX and SPX. However, MXF’s efficacy in the murine model was significantly higher than in the macrophage model. Analysis of PK data showed that even though the plasma fAUC/MICs of MXF was lower than 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 (Vss) for the 4 fluoroquinolones in mice showed that there was no correlation of Vss with efficacy (our unpublished data). CIP has demonstrable early bactericidal activity in human tuberculosis (8, 25) and has been recommended as part of treatment for multi-drug resistant tuberculosis. Presently, OFX and MXF are undergoing various clinical trials for the inclusion into the primary regimen as part of the induction phase of the DOTS 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.
References


TABLE 1: MIC (mg/L) on *M. tuberculosis* H37Rv either in the presence of 7H9 broth or within J774A.1 murine macrophages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MXF</th>
<th>OFX</th>
<th>SPX</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth MIC</td>
<td>0.5</td>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Intracellular MIC</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>
TABLE 2: Pharmacokinetic parameters for four FQs determined in uninfected Balb/c mice, following single ascending doses.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose range (mg/kg)</th>
<th>$C_{\text{max}}$ (ug/mL)</th>
<th>$\text{AUC}_{0-\infty}$ (ug.h/mL)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFX</td>
<td>37.5 to 2400</td>
<td>1.7 to 101.2</td>
<td>29 to 661</td>
<td>4.93 +/- 2.6</td>
</tr>
<tr>
<td>MXF</td>
<td>6 to 400</td>
<td>0.38 to 9.23</td>
<td>25 to 63</td>
<td>4.46 +/- 0.66</td>
</tr>
<tr>
<td>CIP</td>
<td>37.5 to 2400</td>
<td>1.2 to 31</td>
<td>29 to 125</td>
<td>4.74 +/- 5.32</td>
</tr>
<tr>
<td>SPX</td>
<td>5 to 1000</td>
<td>0.75 to 14</td>
<td>43 to 191</td>
<td>9.8 +/- 5.32</td>
</tr>
</tbody>
</table>
TABLE 3: Dose fractionation design for efficacy studies in an aerosol infection model of tuberculosis in Balb/c mice

<table>
<thead>
<tr>
<th></th>
<th>MXF</th>
<th>OFX</th>
<th>SPX</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of Rx [weeks]</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total dose (mg/kg)</td>
<td>38 – 19000</td>
<td>225 – 115,000</td>
<td>31 – 50,000</td>
<td>38 – 100,000</td>
</tr>
<tr>
<td>Fractionation*(No. Doses)</td>
<td>1, 3, 6, 12,</td>
<td>1, 3, 6, 12,</td>
<td>1, 6, 12,</td>
<td>1, 4, 12,</td>
</tr>
<tr>
<td></td>
<td>18, 36, 48</td>
<td>24, 36, 48</td>
<td>24, 36, 48</td>
<td>18, 36</td>
</tr>
<tr>
<td>fAUC/MIC</td>
<td>0.11-177</td>
<td>2-320</td>
<td>3-522</td>
<td>4-201</td>
</tr>
<tr>
<td>fC&lt;sub&gt;max&lt;/sub&gt;/MIC</td>
<td>1 -11</td>
<td>1-117</td>
<td>4-112</td>
<td>5 – 70</td>
</tr>
<tr>
<td>fT&lt;sub&gt;&gt;&lt;/sub&gt;MIC (%)</td>
<td>0 – 100</td>
<td>0-58</td>
<td>10-100</td>
<td>10 – 100</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt; mg/kg</td>
<td>600&lt;sup&gt;14&lt;/sup&gt;</td>
<td>3000&lt;sup&gt;15&lt;/sup&gt;</td>
<td>2000&lt;sup&gt;10&lt;/sup&gt;</td>
<td>2000&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*All single doses that exceed LD<sub>50</sub> were eliminated from the design
FIGURE 1: Percentage of free drug as a function of the total concentration. ■, MXF; □, OFX; ●, SPX; ○, CIP.
FIGURE 2: Effect of increasing fC/MIC ratios on the bactericidal activity 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.
FIGURE 3: Effects of increasing fC/MIC ratios on the intracellular bactericidal activity 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 ± SD of triplicate values. The bactericidal effect is calculated on the basis of the initial inoculum prior to addition of the drug.
FIGURE 4: Dose proportionality and limits of linearity for the four fluoroquinolones with respect to $fC_{\text{max}}/\text{MIC}$ (panel A), $f\text{AUC}/\text{MIC}$ (panel B) and $fT > \text{MIC}$ (%) (panel C).
FIGURE 5: Relationship between $f_{AUC/MIC}$, $f_{C_{max}/MIC}$ and $f_{T>MIC}$ (%) 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 ± SD of triplicate values.