Selection of a Moxifloxacin Dose That Suppresses Drug Resistance in Mycobacterium tuberculosis, by Use of an In Vitro Pharmacodynamic Infection Model and Mathematical Modeling

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Background. Moxifloxacin is a quinolone antimicrobial that has potent activity against Mycobacterium tuberculosis. To optimize moxifloxacin dose and dose regimen, pharmacodynamic antibiotic-exposure targets associated with maximal microbial kill and complete suppression of drug resistance in M. tuberculosis must be identified.

Methods. We used a novel in vitro pharmacodynamic infection model of tuberculosis in which we exposed M. tuberculosis to moxifloxacin with a pharmacokinetic half-life of decline similar to that encountered in humans. Data obtained from this model were mathematically modeled, and the drug-exposure breakpoint associated with the suppression of drug resistance was determined. Monte-Carlo simulations were performed to determine the probability that 10,000 clinical patients taking different doses of moxifloxacin would achieve or exceed the drug-exposure breakpoint needed to suppress resistance to moxifloxacin in M. tuberculosis.

Results. The ratio of the moxifloxacin-free (non-protein-bound) area under the concentration-time curve from 0 to 24 h to the minimum inhibitory concentration associated with complete suppression of the drug-resistant mutant population was 53. For patients taking moxifloxacin doses of 400, 600, or 800 mg/day, the calculated target-attainment rates to suppress drug resistance were 59%, 86%, and 93%, respectively.

Conclusion. A moxifloxacin dose of 800 mg/day is likely to achieve excellent M. tuberculosis microbial kill and to suppress drug resistance. However, tolerability of this higher dose is still unknown.

Mycobacterium tuberculosis currently infects 2 billion people worldwide and is the most common infectious cause of death [1]. Clinical disease most often occurs when there is reactivation of latent infection [2], although individuals with severe immunodeficiency may develop rapidly progressive primary tuberculosis (TB) [3]. In reactivation of TB, the largest population of M. tuberculosis may reach a density of $10^9$ cfu/mL in a pulmonary cavity [4] and consists largely of organisms in exponential-phase growth in an oxygen-rich environment [5]. This large bacterial load—together with such clinical factors as poor adherence to the treatment regimen, HIV-associated malabsorption, pharmacokinetic mismatch, and inadequate dosing practices that lead to subtherapeutic drug levels—increases the probability of emergence of drug resistance [6–9].

In developing countries, the prevalence of multidrug-resistant (MDR) TB is high. For example, in Mumbai, India, 80% of previously untreated patients were infected with M. tuberculosis resistant to at least 1 drug, and 51% were infected with M. tuberculosis resistant to both isoniazid and rifampin [10]. Failure of treatment of MDR-TB occurs in 20%–50% of patients [11–14]. MDR-TB increases treatment costs by a factor of 10–100-fold, and TB control programs may spend up to 30% of their budgets on the <3% of patients with TB who have with MDR-TB [15]. The directly observed therapy–plus strategy [15, 16] used for treatment of MDR-TB results in patients being exposed to toxic and less-effective second-line drugs for up to 24 months, even though the benefit of this strategy is controversial.
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[16]. Clearly, there is an urgent need to develop treatment strategies that can suppress emergence of drug resistance. Although the most common strategy used to suppress the emergence of drug resistance in M. tuberculosis has been the use of combination therapy, the strategy clearly needs to be improved by use of drug doses that optimize the activity of each drug and by development of new classes of anti-TB drugs.

One of the strategies we have used to suppress the emergence of drug resistance and to maximize microbial kill of other microorganisms is pharmacodynamically driven dosing. Previously, we have applied pharmacodynamic principles in conjunction with mathematical models, to determine the impact of incremental drug pressure on amplification and suppression of the drug-resistant subpopulations in Pseudomonas aeruginosa infection in a mouse-thigh model [17]. The methods developed in such studies enabled us to determine the drug-exposure breakpoint needed to suppress emergence of drug resistance in P. aeruginosa while optimizing kill of drug-sensitive subpopulations.

A second strategy used to deal with the problem of drug resistance in M. tuberculosis is the development of new anti-TB drugs. Newer fluoroquinolones, especially those with a C-8 methoxy substitution, have a potential role as first-line agents in the treatment of TB and could play a central role in the treatment of MDR-TB. One drug in this class, moxifloxacin, was recently demonstrated to dramatically improve the bactericidal activity of the standard multidrug regimen when it was substituted for isoniazid in the treatment of murine TB [18]. In human studies of early bactericidal activity, moxifloxacin demonstrated the same magnitude of microbial kill as did rifampin [19]. Unfortunately, resistance to moxifloxacin in M. tuberculosis has already been reported [20], making it imperative to develop dosing strategies that would optimally suppress the emergence of drug resistance.

To perform pharmacodynamic investigations of the emergence of drug resistance, dose-escalation studies are required, which, in some instances, may be toxic to the animals used in disease models of TB. For example, humans achieve a total serum area under the concentration-time curve from 0 to 24 h (AUC0–24) of 36 mg × h/L when the standard dose of moxifloxacin (400 mg/day) is administered, but mice develop toxicity when exposed to a total AUC0–24 >9 mg × h/L [21]. This makes it difficult to study drug resistance in mice at drug exposures relevant to human patients. We have developed an in vitro hollow-fiber pharmacodynamic infection model of TB to overcome such difficulties. Our in vitro pharmacodynamic model of TB allows us to expose M. tuberculosis to anti-TB drugs while mimicking the half-life and dose schedules of those encountered in human patients [22]. In the present study, we used the in vitro pharmacodynamic infection model of M. tuberculosis logarithmic-phase growth to study emergence of resistance to a range of moxifloxacin exposures. The data obtained were mathematically modeled to identify the drug-exposure breakpoint associated with maximal suppression of drug resistance and maximal microbial kill of the drug-sensitive subpopulation. After the drug-exposure breakpoint had been identified, Monte-Carlo simulations were performed to evaluate 3 different doses of moxifloxacin for their ability to achieve the drug-exposure target predicted to suppress drug resistance and maximize kill of M. tuberculosis in clinical patients.

MATERIALS AND METHODS

Organism. M. tuberculosis H37Ra (ATCC 25177) was used throughout the present study. Stock cultures of M. tuberculosis were stored at ~80°C in Middlebrook 7H9 broth (Becton Dickinson). For each study, the bacterial stock was thawed and incubated with shaking conditions in 7H9 broth, for 4 days at 35°C, to achieve M. tuberculosis exponential-phase growth.

Drug. Moxifloxacin hydrochloride powder (batch number 661093E; potency, 92%) was donated by Bayer Pharmaceuticals (West Haven, CT). The drug was dissolved in sterile water and then serially diluted, in 7H9 broth, to the drug concentrations required for study.

Determination of MIC. MIC was defined as the lowest concentration that allowed growth of M. tuberculosis of <1%, compared with untreated controls. The MIC testing was performed as described in the literature and by NCCLS [23, 24].

In vitro pharmacodynamic model of TB. The hollow-fiber bioreactor system (HFS) has been used for harvesting recombinant proteins, as well as for cultivation of bacteria and viruses [25, 26]. Its use as a pharmacokinetic system that mimics human plasma concentration-time profiles of drugs was first described by Blaser et al. [25] and has recently been described for HIV pharmacodynamic studies by Billello et al. [26]. The HFS allows M. tuberculosis to grow in the peripheral compartment (extra-capillary compartment in figure 1) of a hollow-fiber cartridge. The peripheral compartment is separated from the central compartment by semipermeable hollow fibers, with pore sizes that allow nutrients, drugs, and bacterial metabolites to freely transverse in and out of the peripheral compartment but that are too small for bacteria to leave the peripheral compartment.

M. tuberculosis was grown to exponential-phase growth in 7H9 broth, as described above. On the fourth day, the mutation frequency in the cultures was determined by plating 1 mL of M. tuberculosis at a density of 10⁸ cfu/mL on the surface of 100 Middlebrook 7H10 agar plates (New York Department of Health) that had been supplemented with 1.5 mg/L moxifloxacin. Fifteen milliliters of M. tuberculosis at a density of 10⁸ cfu/mL (total population, 1.5 × 10⁹ cfu) was then inoculated into the peripheral compartment of 7 HFSs (Fibercell Systems) that had been preconditioned with 7H9 broth and maintained in incubators for 72 h at 35°C. Twenty-four hours after the HFSs
Figure 1. In vitro hollow-fiber pharmacodynamic infection model of Mycobacterium tuberculosis that simulates human pharmacokinetics. The central compartment of the hollow-fiber system is composed of the central reservoir, the inner lumen of the hollow-fiber capillaries, and the oxygen-permeable flow path connecting the central reservoir to and from the hollow fibers. The peripheral compartment is the space outside the hollow-fiber capillaries that is enclosed by an impermeable plastic encasement.

were inoculated with M. tuberculosis, the mycobacteria in each HFS were treated with different doses of moxifloxacin at 24-h intervals for 10 days. Moxifloxacin was administered by computer-controlled pumps through a dosing port in the central compartment. Nominal values for the moxifloxacin-free (non-protein-bound [assuming 50% protein binding]) AUC0–24 were 0, 4.5, 9, 18, 36, 54, and 72 mg × h/L. By use of computer-controlled peristaltic pumps, fresh 7H9 broth was pumped into the afferent port of the central compartment of the HFS while drug-containing media was isovolumetrically removed from the efferent port of the system at rates programmed to simulate the 13-h half-life encountered in humans. Pharmacokinetic profiles of moxifloxacin attained in the HFSs were validated by sampling the central compartment of each HFS at 1.5, 4, 7, 11, 14, 22, 23.8, 25.5, 29, 36, 45, and 47.8 h after the first infusion. The drug concentrations were measured as described below.

Quantitative cultures were performed on bacterial samples collected from each HFS at 0, 3, 7, and 10 days after treatment was started. The samples were obtained just before administration of the next scheduled dose of antibiotics. To prevent drug carryover, each 1-mL sample was washed twice with sterile water before the bacterial suspension was serially diluted in saline for quantitative cultures. Each dilution was then plated onto antibiotic-free 7H10 agar plates. To assess the effect of each treatment regimen on the moxifloxacin-resistant M. tuberculosis subpopulation, each sample was also cultured on 7H10 agar plates that had been supplemented with 1.5 mg/L moxifloxacin. This drug concentration was chosen to satisfy 2 conditions. First, the clinical M. tuberculosis susceptibility breakpoint for moxifloxacin is 1 mg/L [24]. Second, a single gyrA point mutation increases the MIC >4-fold [17, 20, 27]. The MIC for our organism was 0.25 mg/L.

Drug assay. Moxifloxacin concentrations in samples collected from the central compartment of the HFSs were measured by liquid chromatography/mass spectrometry (LC/MS) by use of a method that we developed. Gatifloxacin (Bristol-Myers Squibb) was used as the internal standard. Analysis was performed by LC/MS by use of the Agilent model 1100 (Agilent Technologies). Separation was accomplished on a Zorbax SB-C18 (2.1 × 30 mm internal diameter) column (Agilent Technologies). The mobile phase consisted of 0.1% heptfluorobutyric acid and acetonitrile (75:25 [vol/vol]). For detection, samples were ionized by electrospray interface and examined in positive-ion mode. Moxifloxacin-ion monitoring was performed for a mass:charge ratio of 402, and gatifloxacin-ion monitoring was performed for a mass:charge ratio of 376. The assay was linear over a range of 0.02–10 mg/L.

Pharmacokinetic/pharmacodynamic analysis. All the mea-
sured outputs of the HFS (drug concentrations, total organism population, and drug-resistant organism subpopulation) for all regimens examined were simultaneously analyzed by use of a nonparametric adaptive grid program (Big NPAG) [28] (see Appendix). Next, we calculated parameter estimates for each of the regimens by maximal a posteriori probability (MAP) Bayesian techniques, by use of the “population of one” utility within Big NPAG. These MAP-Bayesian parameters were then used to make predictions of M. tuberculosis subpopulation responses. In addition, an inhibitory sigmoid E-max effect model was developed for the day-10 values for the total organism population. The inhibitory sigmoid E-max relationship is described by the Hill equation:

\[ E = E_{\text{con}} - E_{\max} \times \frac{(\text{AUC}_{0-24}:\text{MIC})^H}{[(\text{AUC}_{0-24}:\text{MIC})^H + (\text{EC}_{50})^H]}, \]

where \( E \) is microbial effect, \( E_{\text{con}} \) is the estimated microbial density (colony-forming units per milliliter) in the control arm, \( E_{\max} \) is the estimated microbial density (colony-forming units per milliliter) with maximal change in microbial density, \( EC_{50} \) is the moxifloxacin-free \( AUC_{0-24}:\text{MIC} \) ratio for which there is 50% of maximal kill, and \( H \) is Hill’s constant. The inhibitory sigmoid E-max model was fit to the data by use of the ADAPT II software package of D’Argenio and Schumitzky [29]. Next, published population pharmacokinetics described for human subjects [30, 31] were used in Monte-Carlo simulations. The population pharmacokinetic parameters were entered into subroutine PRIOR of ADAPT II and were used to produce 10,000-subject Monte-Carlo simulations. The point estimates of the model parameters were used to examine how suppression of drug resistance was met or exceeded in patients who would receive moxifloxacin doses of 400, 600, or 800 mg/day. An expectation was calculated over the entire MIC distribution of the 243 M. tuberculosis isolates described by Rodriguez et al. [32], to derive population target-attainment rates for prevention of drug resistance by human patients administered moxifloxacin at the stated doses.

RESULTS

The moxifloxacin MIC of this M. tuberculosis H37Ra strain was 0.25 mg/L. The mutation frequency of M. tuberculosis to 1.5 mg/L moxifloxacin was \( 1.53 \times 10^{-6} \). The effect of drug exposure on the total population after 10 days of therapy was described by an inhibitory sigmoid E-max relationship (figure 2), in which a moxifloxacin-free \( AUC_{0-24}:\text{MIC} \) ratio of 25.2 mediated 50% of maximal effect (\( EC_{50} \)). Of importance, this dose-response curve presents a composite picture in which only the total population is examined. Therefore, we also examined the changes in both the total population (composed of both drug-sensitive and drug-resistant bacteria) and the drug-resistant subpopulation in response to the fluctuating drug exposure over the 10-day study period. The results are illustrated in figure 3. There was early amplification of the drug-resistant subpopulation during therapy, in response to a moxifloxacin-free \( AUC_{0-24}:\text{MIC} \) ratio of 24.3 (figure 3B), and the drug-resistant population had completely replaced the drug-sensitive subpopulation by day 10 of treatment. Amplification of the drug-resistant subpopulation was slower to emerge in response to an \( AUC_{0-24}:\text{MIC} \) ratio of 40.4 but had increased by 1.7 log\(^10\) cfu/mL by day 10 of treatment (figure 3C). Of importance, these regimens resulted in a reduction in the total population despite amplification of drug resistance. At an \( AUC_{0-24}:\text{MIC} \) ratio of 40.4–101.6, the drug-resistant subpopulation was suppressed. The drug exposures with an \( AUC_{0-24}:\text{MIC} \) ratio \( \geq 101.6 \) (figure 3D) killed the drug-sensitive subpopulations and were not associated with emergence of drug resistance.

Next, we determined the drug-exposure breakpoint associated with suppression of drug resistance. The changes in moxifloxacin concentration with time and the growth profiles of the total and the drug-resistant populations over time were described by a system of simultaneous inhomogeneous differential equations (see Appendix). The point estimates of the model parameters and their SDs are displayed in table 1. We used these parameters as Bayesian prior estimates to obtain individual-regimen MAP-Bayesian parameter estimates, which were used to examine how well the model fit the data and to calculate the breakpoint moxifloxacin-free \( AUC_{0-24}:\text{MIC} \) ratio associated with suppression of drug resistance. The observed versus the predicted plots are shown in figure 4A–C. The models explain well the changes in drug concentration (\( r^2 = 0.975 \)) and the resultant changes in the total population composed of both the drug-
Figure 3. The responses of the total (diamonds) and the drug-resistant (circles) *Mycobacterium tuberculosis* populations over the course of 10 days of moxifloxacin exposure (free drug). Please note that untreated controls contained some drug-resistant mutants, but they were few. When the colony-forming units per milliliter values were transformed to log$_{10}$ values, they tended toward 0, and the graph in A (circles) appears to be flat. The panels show responses of the total *M. tuberculosis* population and the drug-resistant subpopulation to different moxifloxacin exposures: moxifloxacin-free area under the concentration-time curve from 0 to 24 h [AUC$_{0-24}$]:MIC ratios are 0 (control) (A), 24.3 (B), 40.4 (C), and 101.6 (D).

sensitive and the drug-resistant subpopulations ($r^2 = 0.954$), as well as in the drug-resistant subpopulation ($r^2 = 0.742$) ($P<0.001$ for all regressions). The breakpoint moxifloxacin-free AUC$_{0-24}$:MIC ratio associated with suppression of the drug-resistant mutant population was 53.

We performed a 10,000-subject Monte-Carlo simulation to determine the proportion of patients who would achieve or exceed the drug-exposure target of a moxifloxacin-free AUC$_{0-24}$:MIC ratio of 53, which is needed to suppress drug resistance (target-attainment rate) in patients administered moxifloxacin doses of 400, 600, and 800 mg/day. The rates of achieving the target as a function of the distribution of *M. tuberculosis* MICs are shown in figure 5A–5C. In patients taking 400 mg/day, the target-attainment probability for suppression of drug resistance (AUC$_{0-24}$:MIC ratio of 53) was 59.3%, and, in patients taking 600 and 800 mg/day, the target-attainment rates were 86.4% and 93.1%, respectively.

**DISCUSSION**

In clinical practice, anti-TB therapy is given in 2 phases [33]. In the initial phase, combination therapy is given for 2 months, with the aim to kill *M. tuberculosis* in exponential-phase growth and to prevent emergence of drug resistance. During the continuation phase, therapy is given intermittently for 4 more months, to kill nonreplicating, persistent *M. tuberculosis* during brief episodes of sporadic metabolism [5, 33]. Drugs that are most active during the initial phase of therapy are designated as having bactericidal activity, and those most active during the continuation phase are designated as having sterilizing activity [5, 33]. Moxifloxacin has been demonstrated to have excellent bactericidal activity, as judged by microbial kill of the total population [18, 19]. However, since resistance to fluoroquinolones develops rapidly in patients with TB, especially in those exposed to monotherapy [20], we performed a study of the
Table 1. Population-median parameter estimates of pharmacodynamic model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance rate, L/h</td>
<td>8.926</td>
<td>0.777</td>
</tr>
<tr>
<td>Volume of central compartment, L</td>
<td>179.349</td>
<td>27.041</td>
</tr>
<tr>
<td>$K_{\max-S}$, log$_{10}$ cfu/mL/h</td>
<td>0.415</td>
<td>0.411</td>
</tr>
<tr>
<td>$C_{50g}$, mg/L</td>
<td>5.103</td>
<td>3.909</td>
</tr>
<tr>
<td>$H_g$</td>
<td>4.759</td>
<td>5.276</td>
</tr>
<tr>
<td>$K_{\max-R}$, log$_{10}$ cfu/mL/h</td>
<td>0.023</td>
<td>0.014</td>
</tr>
<tr>
<td>$C_{50g}$, mg/L</td>
<td>1.090</td>
<td>1.178</td>
</tr>
<tr>
<td>$H_g$</td>
<td>8.857</td>
<td>8.179</td>
</tr>
<tr>
<td>$K_{\max-S}$, log$_{10}$ cfu/mL/h</td>
<td>8.699</td>
<td>4.047</td>
</tr>
<tr>
<td>$C_{50k}$, mg/L</td>
<td>14.009</td>
<td>12.710</td>
</tr>
<tr>
<td>$H_k$</td>
<td>5.122</td>
<td>3.693</td>
</tr>
<tr>
<td>$K_{\max-R}$, log$_{10}$ cfu/mL/h</td>
<td>10.459</td>
<td>12.187</td>
</tr>
<tr>
<td>$C_{50k}$, mg/L</td>
<td>16.867</td>
<td>11.864</td>
</tr>
<tr>
<td>$H_k$</td>
<td>3.316</td>
<td>1.126</td>
</tr>
<tr>
<td>$POP_{MAX}$, cfu/mL</td>
<td>$2.547 \times 10^6$</td>
<td>$3.078 \times 10^6$</td>
</tr>
<tr>
<td>Total population, cfu/mL</td>
<td>$1.962 \times 10^6$</td>
<td>$5.878 \times 10^5$</td>
</tr>
<tr>
<td>Drug-resistant population, cfu/mL</td>
<td>1.123</td>
<td>0.059</td>
</tr>
</tbody>
</table>

**NOTE.** $K_{\max}$ is the rate constant for maximal bacterial growth, $K_{\max-k}$ is the rate constant for maximal bacterial kill, $C_{50k}$ is the drug concentration needed to achieve 50% effect on maximal growth rate, $H_k$ is the sigmoidicity constant for microbial kill, and $H_g$ is the sigmoidicity constant for drug effect on microbial growth. These are shown for the drug-sensitive (S) and the drug-resistant (R) subpopulations. $POP_{MAX}$ is the estimated maximal size of the bacterial density in the control hollow-fiber bioreactor system after bacterial growth enters the stationary phase.

pharmacodynamics of moxifloxacin, to establish a drug-exposure target that would optimally kill $M. tuberculosis$ and suppress resistance during the initial phase of therapy. Others have approached the problem of drug resistance from a conceptual standpoint and have established antibiotic concentrations on agar plates that selectively enrich the population of drug-resistant mutants (mutant-selection window) and a concentration that must be exceeded to kill first-step drug-resistant mutants (mutant-prevention concentration) [23, 34]. However, these concepts have not yet been validated in the clinical arena. On the other hand, pharmacodynamic methods have been validated and are already in use in clinical practice [35]. The optimal microbial effect of each drug on a particular microbial species is best explained by one of the pharmacodynamic parameters, such as AUC$_{2-24}$:MIC ratio [35, 36], and by a breakpoint value of this pharmacodynamic parameter—that is, a drug-exposure value that enables us to select the correct size of a drug dose [17, 35, 36].

Moxifloxacin is already in use for treatment of TB [37] and is recommended by the American Thoracic Society, Centers for Diseases Control and Prevention, and the Infectious Diseases Society of America [33] for treatment of patients intolerant of first-line anti-TB agents. In addition, quinolones, including moxifloxacin, are the central drug in treatment regimens for MDR-TB [33]. Given that most drugs used for treatment of MDR-TB have limited effectiveness [14], moxifloxacin may be the only drug in the regimen with efficacy that matches that of first-line drugs. It is only when each drug and drug dose is optimized for substantial microbial kill and for suppression of drug resistance in $M. tuberculosis$ that we can expect combi-
Figure 5. The proportion of 10,000 simulated patients who attained the moxifloxacin exposure (area under the concentration-time curve from 0 to 24 h [AUC0–24]:MIC ratio = 53) that would suppress drug resistance after administration of 400, 600, or 800 mg of moxifloxacin, for the distribution of moxifloxacin MIC ratio for 243 M. tuberculosis clinical isolates [32]. The panels show target-attainment rates for moxifloxacin at 400 (A), 600 (B), and 800 (C) mg/day.

nation therapy to work better and to prevent emergence of drug resistance. Our study has demonstrated that, even when drug exposures are associated with excellent efficacy (as measured by the drug’s effect on the total population), those same drug exposures may inadvertently amplify the emergence of a drug-resistant subpopulation. For example, an AUC0–24:MIC ratio of 25.2 (EC50) mediates kill of the total M. tuberculosis population that is >3 log10 cfu/mL beyond simple stasis, yet it is associated with near-maximal amplification of the drug-resistant subpopulation. This phenomenon is similar to that observed by us in studies of resistance of other bacteria exposed to fluoroquinolones [38–40]. Because the drug exposure associated with maximal microbial kill of a drug-sensitive subpopulation may amplify the drug-resistant subpopulation, to optimize outcome of treatment of TB, it is the drug exposure associated with suppression of emergence of drug resistance that should be targeted. For moxifloxacin, this drug-exposure target for suppression of the drug-resistant subpopulation is a moxifloxacin-free AUC0–24:MIC ratio of 53. This AUC0–24:MIC ratio should be viewed as particular to M. tuberculosis, since, even with the same antibiotic, different microbial species have different drug-exposure targets for suppression of drug resistance [38–41].

To put the in vitro M. tuberculosis data into clinical perspective, we performed Monte-Carlo simulations. The Monte-Carlo simulations allowed us to determine moxifloxacin doses that are likely to achieve the drug exposure effective for killing drug-sensitive M. tuberculosis and preventing drug-resistant mutants from arising during therapy in clinical patients with TB. They take into account the variability of distribution and clearance of the drug in human subjects, as well as the variability of the MICs of clinical isolates of M. tuberculosis. The currently recommended dose of 400 mg/day is likely to achieve the important goal of suppressing drug resistance in ∼60% of patients, whereas 86% would achieve this goal with 600 mg/day, and 93% would achieve it with 800 mg/day. In places such as Makati City, Philippines, where resistance to ciprofloxacin and ofloxacin ranges between 26% and 35% for non-MDR-TB and is >50% for MDR-TB strains [42], the dose of 400 mg/day is even less satisfactory, given the well-known cross-resistance between quinolones [27, 32, 43]. Therefore, on the basis of these data, we recommend a dose of moxifloxacin of 600–800 mg/day for treatment of TB. Unfortunately, there is a lack of published clinical data on the tolerability of 800 mg/day [44]. Therefore, even though moxifloxacin at 800 mg/day would be the dose most likely to achieve drug exposures that would suppress drug resistance in M. tuberculosis, use of this higher dose in patients should await further evaluation.

There are some limitations to the present study. We know from previous studies [45] that, as the length of treatment increases, the drug-exposure breakpoint associated with suppression of drug resistance may increase. This is important, given that treatment of TB lasts up to 6 months, whereas our study had a duration of only 10 days. Moreover, our study does not address moxifloxacin’s sterilizing activity against nonreplicating persistent M. tuberculosis nor does it address activity in acidic milieu [5, 33, 46], either of which may result in higher drug-exposure breakpoints for suppression of drug resistance. Since these limitations would lead to an underestimate of the drug-exposure breakpoint, they would make it even more unlikely...
that a moxifloxacin dose of 400 mg/day would be adequate in suppressing drug resistance in patients. Furthermore, we used an avirulent strain of *M. tuberculosis*. However, the exponential-phase growth characteristics and susceptibilities of the *M. tuberculosis* H37Ra and the virulent H37Rv strains are similar [47, 48], as are the in vitro pharmacodynamics of another fluoroquinolone, sparfloxacin [49], suggesting validity of our observations, even for virulent strains. Finally, although the moxifloxacin concentrations supplemented on 7H10 agar plates were chosen to select for *gyrA* mutations, in clinical practice, mechanisms involved in 25%–42% of fluoroquinolone-resistant clinical isolates of *M. tuberculosis* are unknown [43, 50] but are known to not be *gyrA* mutations. It is possible that these may be *gyrB* mutations or inducible efflux pumps, as has been demonstrated in laboratory-generated mutations of both *M. tuberculosis* H37Ra and *M. smegmatis* [27, 51]. Our experimental system may allow us to determine drug exposures that would suppress drug resistance, even when the mechanism of drug resistance is unclear.

In summary, we have determined an in vitro drug-exposure target in which moxifloxacin not only kills *M. tuberculosis* but also suppresses emergence of drug resistance in exponential-phase growth, as is encountered in patients during the initial phase of therapy. Using mathematical methods, we have identified that the dose of moxifloxacin most likely to achieve this target, with a high likelihood in human patients, is 600–800 mg/day.

### APPENDIX

**FULL-POPULATION MODELING OF TOTAL ORGANISM POPULATION, DRUG-RESISTANT ORGANISM POPULATION, AND DRUG EXPOSURE**

The pharmacokinetics and the resultant changes in the total (drug-resistant [R] and drug-sensitive [S]) *Mycobacterium tuberculosis* population and the drug-resistant subpopulation were described by use of the following equations:

\[
\frac{dX_r}{dt} = R(t) - \left(\frac{SCL}{V_c}\right) \times X_r ;
\]

Equation 1 describes drug pharmacokinetics in the central compartment of the hollow-fiber pharmacodynamic model (a standard 1-compartment open model with first-order elimination and zero-order, time-delimited input). \(X_r\) is the amount of drug in the central compartment; \(SCL\) (liters per hour) is the rate of clearance of drug from the central compartment; \(V_c\) is the volume of the central compartment.

\[
\frac{dN_s}{dt} = K_{g_{max}-s} \times (1 - L_s) \times N_s \times E - K_{k_{max}-s} \times M_s \times N_s ;
\]

Equations 2 and 3 also allow the antibacterial effect of the different drug doses administered to be modeled. For both the drug-sensitive and the drug-resistant subpopulations, there is an independent effect of the drug dose on the 2 populations: 1 mediated through equation 2 (drug-sensitive subpopulation) and 1 mediated through equation 3 (drug-resistant subpopulation). There is a maximal kill rate that the drug can induce for each subpopulation (\(K_{g_{max}-s}\) and \(K_{k_{max}-s}\)). The killing effect of the drug was modeled as a saturable Michaelis-Menten–type kinetic event (\(M\); equation 5). The effect of the drug on growth is constrained to approach zero. As the organisms approach maximal bacterial density, they approach stationary phase. This is accomplished by multiplying the first-order growth terms by a logistic growth term (\(E\); equation 4). The maximal bacterial density (POPMAX) is identified as part of the estimation process. Most of the information for identifying this parameter is derived from the bacterial growth in the control group.

\[
\frac{dN_r}{dt} = K_{g_{max}-r} \times (1 - L_r) \times N_r \times E - K_{k_{max}-r} \times M_r \times N_r ;
\]

\[
E = 1 - \frac{N_s + N_r}{\text{POPMAX}} ;
\]

\[
L = \frac{(X_r/V_c)^{1/4} + (X_r/V_c)^{1/4} + C_{50}^{1/4}}{H} ,
\]

where \(H = H_{g-s}\) or \(H_{g-r}\).

\[
M = \frac{(X_r/V_c)^{1/4} + (X_r/V_c)^{1/4} + C_{50}^{1/4}}{H} ,
\]

where \(H = H_{k-s}\) or \(H_{k-r}\).

\[
\frac{dN}{dt} = K_{g_{max}-s} \times (1 - L_s) \times N_s \times E - K_{k_{max}-s} \times M_s \times N_s ;
\]

\[
E = 1 - \frac{N_s + N_r}{\text{POPMAX}} ;
\]

\[
L = \frac{(X_r/V_c)^{1/4} + (X_r/V_c)^{1/4} + C_{50}^{1/4}}{H} ,
\]

where \(H = H_{g-s}\) or \(H_{g-r}\).

\[
M = \frac{(X_r/V_c)^{1/4} + (X_r/V_c)^{1/4} + C_{50}^{1/4}}{H} ,
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where \(H = H_{k-s}\) or \(H_{k-r}\).

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where \(H = H_{g-s}\) or \(H_{g-r}\).

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M = \frac{(X_r/V_c)^{1/4} + (X_r/V_c)^{1/4} + C_{50}^{1/4}}{H} ,
\]

where \(H = H_{k-s}\) or \(H_{k-r}\).
the drug-resistant populations, there are separate terms for H and C_{50}. The drug effect observed is a balance between growth and death induced by the drug concentrations achieved.

References

39. Tam VH, Louie A, Deziel MR, et al. Pharmacodynamics of BMS-284756 (B) and ciprofloxacin (C) against Pseudomonas aeruginosa (PA) and Klebsiella pneumoniae (KP) in a hollow fiber system (HFS) [abstract A-443]. In: Program and abstracts of the 41st Interscience Conference


