

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

Tawanda Gumbo,¹ Arnold Louie,^{1,2} Mark R. Deziel,^{1,2} Linda M. Parsons,² Max Salfinger,² and George L. Drusano^{1,2}

¹Emerging Infections and Host Defense Section, Ordway Research Institute, and ²The New York State Department of Health, Albany

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 10⁹ cfu/mL in a pulmonary cavity [4] and consists largely of organisms in exponential-phase growth in an oxygen-rich envi-

ronment [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

Received 12 February 2004; accepted 18 May 2004; electronically published 24 September 2004.

Financial support: Charitable Leadership Foundation. Moxifloxacin was donated by Bayer Pharmaceuticals.

Potential conflict of interest: G.L.D. receives research support from and is a consultant for Bayer Pharmaceuticals.

Reprints or correspondence: Dr. George L. Drusano, Emerging Infections and Host Defense Section, Ordway Research Institute, 150 New Scotland Ave., Albany, NY 12208 (gdrusano@ordwayresearch.org).

The Journal of Infectious Diseases 2004;190:1642–51

© 2004 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2004/19009-0016\$15.00

[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 (AUC_{0-24}) of $36 \text{ mg} \times \text{h/L}$ when the standard dose of moxifloxacin (400 mg/day) is administered, but mice develop toxicity when exposed to a total $AUC_{0-24} > 9 \text{ mg} \times \text{h/L}$ [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 Bilello et al. [26]. The HFS allows *M. tuberculosis* to grow in the peripheral compartment (extracapillary 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 10^6 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 10^6 cfu/mL (total population, 1.5×10^7 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

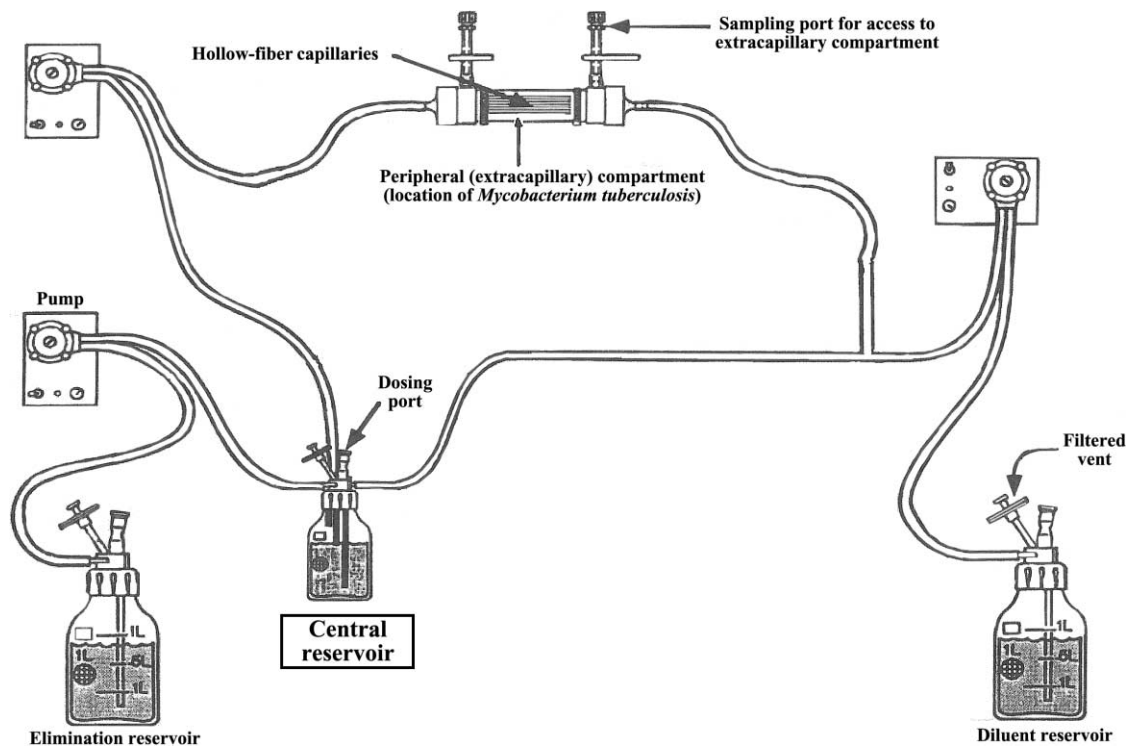


Figure 1. In vitro hollow-fiber pharmacodynamic infection model of *Mycobacterium tuberculosis* that simulates human pharmacokinetics. The central compartment of the hollow-fiber system is composed of the central reservoir, the inner lumina of the hollow-fiber capillaries, and the oxygen-permeable flow 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]) AUC_{0-24} were 0, 4.5, 9, 18, 36, 54, and 72 mg \times 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 \times 30 mm internal diameter) column (Agilent Technologies). The mobile phase consisted of 0.1% heptafluorobutyric 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 model 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:

$$E = E\text{-con} - E\text{-max} \times (AUC_{0-24}:\text{MIC})^H / [(AUC_{0-24}:\text{MIC})^H + (EC_{50})^H],$$

where E is microbial effect, E-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, to determine the probability that the $AUC_{0-24}:\text{MIC}$ ratio needed to suppress 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 1.53×10^{-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

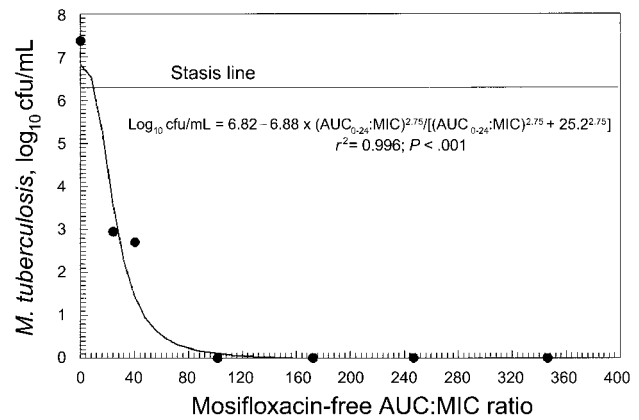


Figure 2. Response of the total *Mycobacterium tuberculosis* population to 10 days of exposure to moxifloxacin. The intersection of the stasis line with the inhibitory sigmoid E-max curve indicates the moxifloxacin-free area under the concentration-time curve from 0 to 24 h ($AUC_{0-24}:\text{MIC}$ ratio) at which there was no growth above the density of *M. tuberculosis* in the bacterial suspension inoculated into the hollow-fiber system at 0 h.

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 ≥ 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 the sum of both the drug-

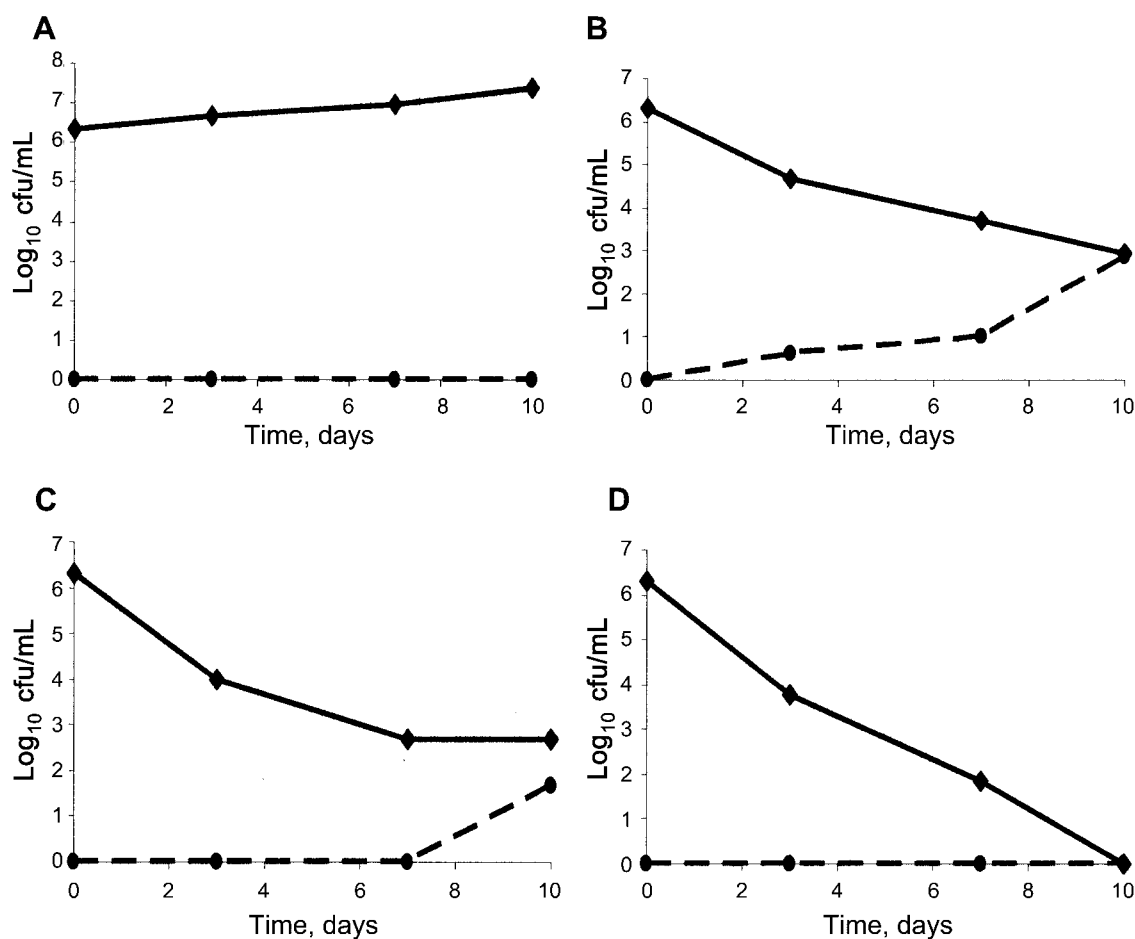


Figure 3. The responses of the total (*diamonds*) and the drug-resistant (*circles*) *Mycobacterium tuberculosis* populations over the course of 10 days of moxifloxacin exposure (free drug). Please note that untreated controls contained some drug-resistant mutants, but they were few. When the colony-forming units per milliliter values were transformed to log₁₀ 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 [AUC₀₋₂₄]: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 ($r^2 = 0.742$) ($P < .001$, for all regressions). The breakpoint moxifloxacin-free AUC₀₋₂₄: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 AUC₀₋₂₄: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 (AUC₀₋₂₄: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.

Parameter	Estimate	SD
Clearance rate, L/h	8.926	0.777
Volume of central compartment, L	179.349	27.041
K_{gmax-S} , \log_{10} cfu/mL/h	0.415	0.411
C_{50g-S} , mg/L	5.103	3.909
H_{g-S}	4.759	5.276
K_{gmax-R} , \log_{10} cfu/mL/h	0.023	0.014
C_{50g-R} , mg/L	1.090	1.178
H_{g-R}	8.857	8.179
K_{kmax-S} , \log_{10} cfu/mL/h	8.699	4.047
C_{50k-S} , mg/L	14.009	12.710
H_{k-S}	5.122	3.693
K_{kmax-R} , \log_{10} cfu/mL/h	10.459	12.187
C_{50k-R} , mg/L	16.867	11.864
H_{k-R}	3.316	1.126
POPMAX, cfu/mL	2.547×10^9	3.078×10^9
Total population, cfu/mL	1.962×10^6	5.878×10^5
Drug-resistant population, cfu/mL	1.123	0.059

NOTE. K_{gmax} is the rate constant for maximal bacterial growth, K_{kmax} is the rate constant for maximal bacterial kill, C_{50k} is the drug concentration needed to achieve 50% of maximal kill rate, C_{50g} is the drug concentration needed to achieve 50% effect on maximal growth rate, H_g is the sigmoidicity constant for microbial kill, and H_k is the sigmoidicity constant for drug effect on microbial growth. These are shown for the drug-sensitive (S) and the drug-resistant (R) subpopulations. POPMAX 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 $AUC_{0-24}:MIC$ 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-

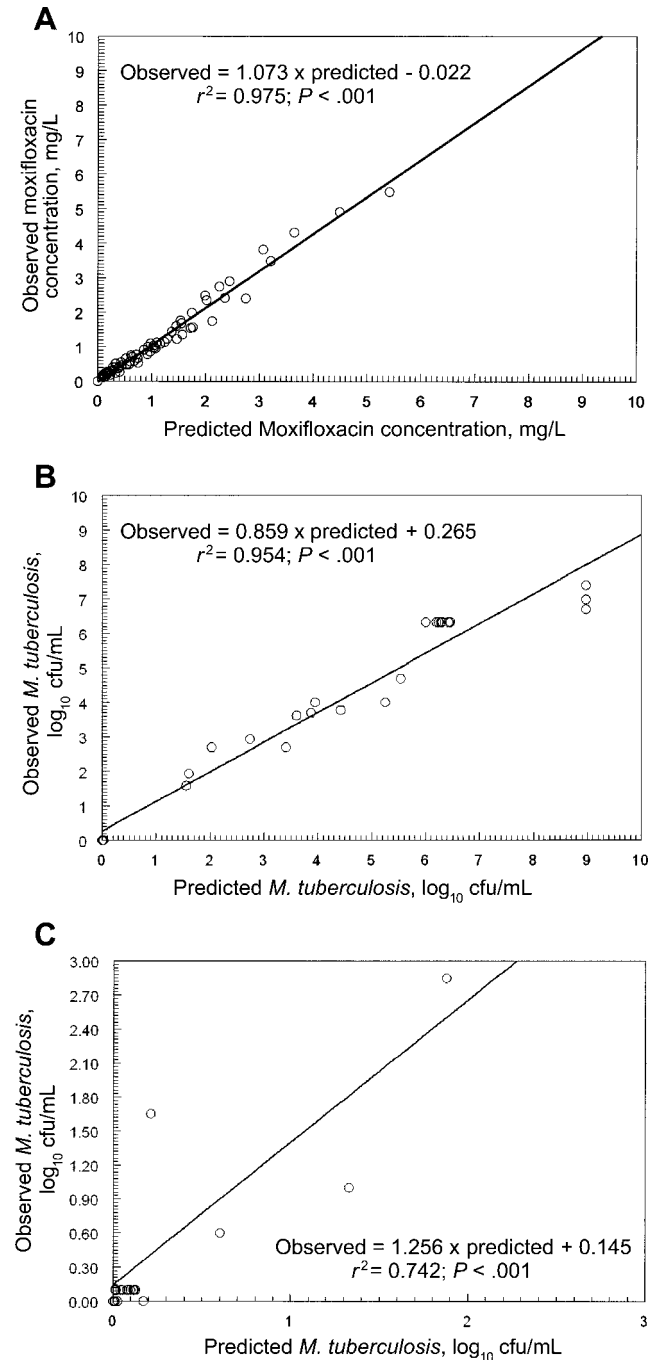


Figure 4. Plots of predicted versus observed values, after maximal a posteriori probability Bayesian approximation for moxifloxacin concentrations (A), the resultant change in the total population (B), and the changes in the drug-resistant subpopulation (C). *M. tuberculosis*, *Mycobacterium tuberculosis*.

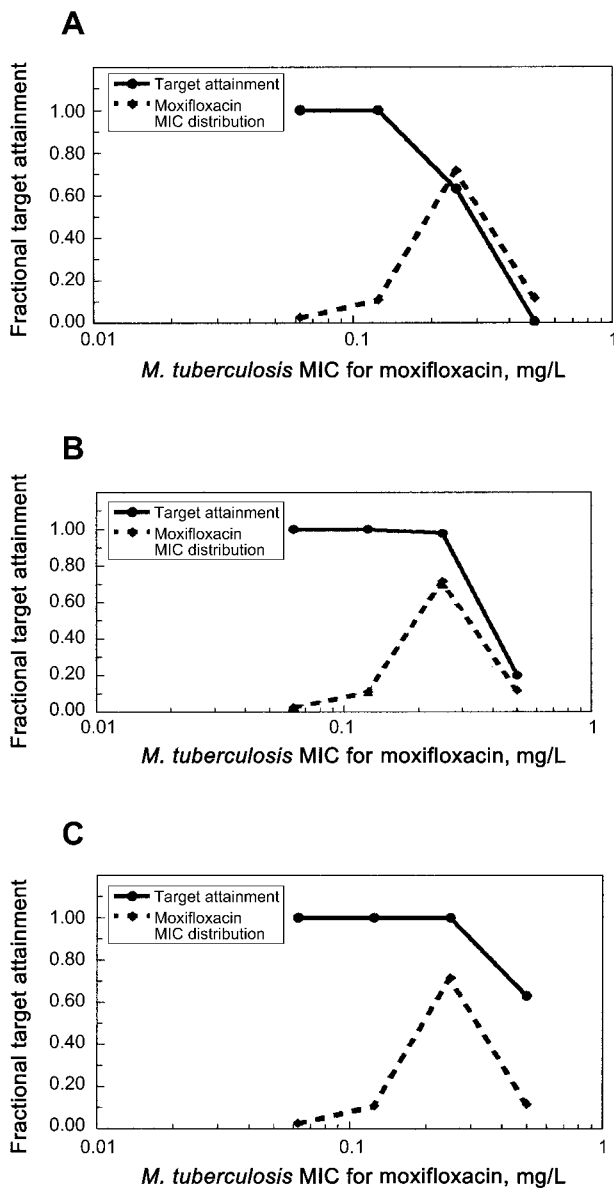


Figure 5. The proportion of 10,000 simulated patients who attained the moxifloxacin exposure (area under the concentration-time curve from 0 to 24 h [AUC_{0-24}]:MIC ratio = 53) that would suppress drug resistance after administration of 400, 600, or 800 mg of moxifloxacin, for the distribution of moxifloxacin MIC ratio for 243 *Mycobacterium 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 AUC_{0-24} :MIC ratio of 25.2 (EC_{50}) mediates kill of the total *M. tuberculosis* population that is $>3 \log_{10}$ cfu/mL beyond simple stasis, yet it is associated with near-maximal amplification of the drug-re-

sistant 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 AUC_{0-24} :MIC ratio of 53. This AUC_{0-24} :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:

$$dX_1/dt = R(1) - (SCL/V_c) \times X_1 ; \quad (1)$$

$$dN_s/dt = K_{g_{max-s}} \times (1 - L_s) \times N_s \\ \times E - K_{k_{max-s}} \times M_s \times N_s ; \quad (2)$$

$$dN_r/dt = K_{g_{max-r}} \times (1 - L_r) \times N_r \\ \times E - K_{k_{max-r}} \times M_r \times N_r ; \quad (3)$$

$$E = 1 - (N_r + N_s)/POP_{MAX} ; \quad (4)$$

$$L = (X_1/V_c)^H / [(X_1/V_c)^H + C_{50} g^H], \\ \text{where } H = H_{g-s} \text{ or } H_{g-r} , \quad (5)$$

$$M = (X_2/V_c)^H / [(X_2/V_c)^H + C_{50} k^H], \\ \text{where } H = H_{k-s} \text{ or } H_{k-r} . \quad (6)$$

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_1 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.

Equations 2 and 3 describe the rates of change of the drug-sensitive and the drug-resistant subpopulations, respectively, over time. The model equations for describing the rate of change of the numbers of organisms in the drug-sensitive and the drug-resistant subpopulations were developed on the basis of the in vivo observation that bacteria at the site of infection are in logarithmic-phase growth in the absence of drug and exhibit an exponential density-limited growth rate (equation 4). There is 1 equation to describe the drug-sensitive subpopulation (equation 2) and 1 to describe the drug-resistant subpopulation (equation 3). In each, first-order growth was assumed up to a density limit. Each subpopulation has an independent growth rate constant (drug sensitive, $K_{g_{max-s}}$; drug resistant, $K_{g_{max-r}}$). We allowed the drug to affect the growth rate independently of kill, through a saturable Michaelis-Menten-type kinetic event (L; 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 (POP_{MAX}) 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.

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 ($K_{k_{max-s}}$ and $K_{k_{max-r}}$). The killing effect of the drug was modeled as a saturable Michaelis-Menten-type kinetic event (M; equation 6) that relates the kill rate to serum drug concentration, where H is the slope constant and C_{50} (milligrams per liter) is the drug concentration at which the bacterial kill rate is half-maximal. For the drug-sensitive and

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

1. World Health Organization (WHO). Tuberculosis [fact sheet number 104]. Geneva: WHO, 2000.
2. Lillebaek T, Dirksen A, Vynnycky E, Baess I, Thomsen VO, Andersen AB. Stability of DNA patterns and evidence of *Mycobacterium tuberculosis* reactivation occurring decades after the initial infection. *J Infect Dis* 2003; 188:1032–9.
3. Lee MP, Chan JW, Ng KK, Li PC. Clinical manifestations of tuberculosis in HIV-infected patients. *Respirology* 2000; 5:423–6.
4. Canetti G. Present aspects of bacterial resistance in tuberculosis. *Am Rev Respir Dis* 1965; 92:687–703.
5. Mitchison DA. Basic mechanisms of chemotherapy. *Chest* 1979; 76: 771–81.
6. Pablos-Mendez A, Knirsch CA, Barr RG, Lerner BH, Frieden TR. Non-adherence in tuberculosis treatment: predictors and consequences in New York City. *Am J Med* 1997; 102:164–70.
7. Sahai J, Gallicano K, Swick L, et al. Reduced plasma concentrations of antituberculosis drugs in patients with HIV infection. *Ann Intern Med* 1997; 127:289–93.
8. Sandman L, Schluger NW, Davidow AL, Bonk S. Risk factors for rifampin-monoresistant tuberculosis: a case-control study. *Am J Respir Crit Care Med* 1999; 159:468–72.
9. Weiner M, Burman W, Vernon A, et al. Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine. *Am J Respir Crit Care Med* 2003; 167:1341–7.
10. Almeida D, Rodrigues C, Udawadia ZF, et al. Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. *Clin Infect Dis* 2003; 36:e152–4.
11. Goble M, Iseman MD, Madsen LA, Waite D, Ackerson L, Horsburgh CR. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. *N Engl J Med* 1993; 328:527–32.
12. Narita M, Alonso P, Lauzardo M, Hollender ES, Pitchenik AE, Ashkin D. Treatment experience of multidrug-resistant tuberculosis in Florida, 1994–1997. *Chest* 2001; 120:343–8.
13. Hadiarto M, Tjandra YA, Hudoyo A. Treatment of multidrug-resistant tuberculosis in Indonesia. *Chemotherapy* 1996; 42 (Suppl 3):24–9.
14. Park S K, Kim CT, Song SD. Outcome of chemotherapy in 107 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. *Int J Tuberc Lung Dis* 1998; 2:877–84.
15. Pablos-Mendez A, Gowda DK, Frieden TR. Controlling multidrug-resistant tuberculosis and access to expensive drugs: a rational framework. *Bull World Health Organ* 2002; 80:489–500.
16. Sterling TR, Lehmann HP, Frieden TR. Impact of DOTS compared with DOTS-plus on multidrug resistant tuberculosis and tuberculosis deaths: decision analysis. *BMJ* 2003; 326:574.
17. Jumbe N, Louie A, Leary R, et al. Application of a mathematical model to prevent in vivo amplification of antibiotic-resistant bacterial populations during therapy. *J Clin Invest* 2003; 112:275–85.
18. Nuermberger EL, Yoshimatsu T, Tyagi S, et al. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. *Am J Respir Crit Care Med* 2004; 169:421–6.
19. Gosling RD, Uiso LO, Sam NE, et al. The bactericidal activity of moxifloxacin in patients with pulmonary tuberculosis. *Am J Respir Crit Care Med* 2003; 168:1342–5.
20. Ginsburg AS, Woolwine SC, Hooper N, et al. The rapid development of fluoroquinolone resistance in *M. tuberculosis*. *N Engl J Med* 2003; 349:1977–8.
21. Yoshimatsu T, Nuermberger E, Tyagi S, Chaisson R, Bishai W, Grosset J. Bactericidal activity of increasing daily and weekly doses of moxifloxacin in murine tuberculosis. *Antimicrob Agents Chemother* 2002; 46:1875–9.
22. Gumbo T, Louie A, Deziel M, Drusano GL. Pharmacodynamic driven dosing of rifampin in a hollow fiber model of tuberculosis [abstract A-1157]. In: Program and abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology, 2003:16.
23. Dong Y, Xu C, Zhao X, Domagala J, Drlica K. Fluoroquinolone action against mycobacteria: effects of C-8 substituents on growth, survival, and resistance. *Antimicrob Agents Chemother* 1998; 42:2978–84.
24. NCCLS. Susceptibility testing of *Mycobacteria*, *Nocardiae*, and other aerobic *Actinomycetes*: approved standard [NCCLS document M24-A.2003]. Wayne, PA: NCCLS, 2003.
25. Blaser J, Stone BJ, Zinner SH. Two compartment kinetic model with multiple artificial capillary units. *J Antimicrob Chemother* 1985; 15(Suppl A):131–7.
26. Bilello JA, Bauer G, Dudley MN, Cole GA, Drusano GL. Effect of 2',3'-didehydro-3'-deoxythymidine in an in vitro hollow-fiber pharmacodynamic model system correlates with results of dose-ranging clinical studies. *Antimicrob Agents Chemother* 1994; 38:1386–91.
27. Kocagöz T, Hackbarth CJ, Unsal I, Rosenberg EY, Nikaido H, Chambers HF. Gyrase mutations in laboratory-selected, fluoroquinolone-resistant mutants of *Mycobacterium tuberculosis* H37Ra. *Antimicrob Agents Chemother* 1996; 40:1768–74.
28. Leary R, Jelliffe R, Schumitzky A, Van Guilder M. An adaptive grid non-parametric approach to pharmacokinetic and dynamic (PK/PD) models. In: Proceedings of the 14th IEEE Symposium on Computer-Based Medical Systems. Bethesda, MD: IEEE Computer Society, 2001: 389–94.
29. D'Argenio DZ, Schumitzky A. ADAPT II: a program for simulation, identification, and optimal experimental design. User manual. Los Angeles: Biomedical Simulations Resource, University of Southern California. 1997. Available at: <http://bmsr.usc.edu>. Accessed 17 September 2004.
30. Simon N, Sampol E, Albanese J, et al. Population pharmacokinetics of moxifloxacin in plasma and bronchial secretions in patients with severe bronchopneumonia. *Clin Pharmacol Ther* 2003; 74:353–63.
31. Stass H, Dalhoff A, Kubitz D, Schuhly U. Pharmacokinetics, safety, and tolerability of ascending single doses of moxifloxacin, a new 8-methoxy quinolone, administered to healthy subjects. *Antimicrob Agents Chemother* 1998; 42:2060–5.
32. Rodriguez JC, Ruiz M, Lopez M, Royo G. In vitro activity of moxifloxacin, levofloxacin, gatifloxacin and linezolid against *Mycobacterium tuberculosis*. *Int J Antimicrob Agents* 2002; 20:464–7.
33. Blumberg HM, Burman WJ, Chaisson RE, et al. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med* 2003; 167:603–62.
34. Drlica K. The mutant selection window and antimicrobial resistance. *J Antimicrob Chemother* 2003; 52:11–7.
35. Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. *Nat Rev Microbiol* 2004; 2:289–300.
36. Peloquin CA. Pharmacological issues in the treatment of tuberculosis. *Ann NY Acad Sci* 2001; 953:157–64.
37. Valerio G, Bracciale P, Manisco V, Quitadamo M, Legari G, Bellanova S. Long-term tolerance and effectiveness of moxifloxacin therapy for tuberculosis: preliminary results. *J Chemother* 2003; 15:66–70.
38. Tam VH, Louie A, Deziel MR, et al. Prevention of emergence of resistance of *Pseudomonas aeruginosa* (PA) through population co-modeling drug concentration, susceptible and resistant populations (P) [abstract A-2100]. In: Program and abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology, 2001.
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

- on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology, **2001**:11.
40. Tam VH, Louie A, Deziel MR, et al. Pharmacodynamics of BMS-284756 (B) in counter-selecting resistance in a hollow fiber system (H) [abstract A-442]. In: Program and abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology, **2001**:11.
 41. MacGowan AP, Rogers CA, Holt HA, Bowker KE. Activities of moxifloxacin against, and emergence of resistance in, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* in an in vitro pharmacokinetic model. *Antimicrob Agents Chemother* **2003**;47:1088–95.
 42. Grimaldo ER, Tupasi TE, Rivera AB, et al. Increased resistance to ciprofloxacin and ofloxacin in multidrug-resistant *Mycobacterium tuberculosis* isolates from patients seen at a tertiary hospital in the Philippines. *Int J Tuberc Lung Dis* **2001**;5:546–50.
 43. Cheng AE, Yew WW, Chan EW, Chin ML, Hui MM, Chan RC. Multiplex PCR amplimer conformation analysis for rapid detection of *gyrA* mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. *Antimicrob Agents Chemother* **2004**;48:596–601.
 44. Noel GJ, Natarajan J, Chien S, Hunt TL, Goodman DB, Abels R. Effects of three fluoroquinolones on QT interval in healthy adults after single doses. *Clin Pharmacol Ther* **2003**;73:292–303.
 45. Drusano G. Treating resistant organisms: how can pharmacodynamics help? [symposium 125]. In: Program and abstracts of the 41st Conference of the Infectious Diseases Society of America (San Diego). Alexandria, VA: The Infectious Diseases Society of America, **2003**. Available at: http://www.idsociety.org/Content/ContentGroups/Annual_Meeting1/20036/PowerPoints/Drusano_Treating.ppt#1. Accessed 17 September 2004.
 46. Michele TM, Ko C, Bishai WR. Exposure to antibiotics induces expression of the *Mycobacterium tuberculosis* sigF gene: implications for chemotherapy against mycobacterial persistors. *Antimicrob Agents Chemother* **1999**;43:218–25.
 47. Zhang M, Gong J, Lin Y, Barnes PF. Growth of virulent and avirulent *Mycobacterium tuberculosis* strains in human macrophages. *Infect Immun* **1998**;66:794–9.
 48. Collins L, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob Agents Chemother* **1997**;41:1004–9.
 49. Fietta A, Morosini M, Cascina A. Effects of continuous or pulsed exposure to rifabutin and sparflaxacin on the intracellular growth of *Staphylococcus aureus* and *Mycobacterium tuberculosis*. *J Chemother* **2001**;13:167–75.
 50. Sullivan EA, Kreiswirth BN, Palumbo L, et al. Emergence of fluoroquinolone-resistant tuberculosis in New York City. *Lancet* **1995**;345:1148–50.
 51. Liu J, Takiff HE, Nikaïdo H. Active efflux of fluoroquinolones in *Mycobacterium smegmatis* mediated by LfrA, a multidrug efflux pump. *J Bacteriol* **1996**;178:3791–5.