Dose Fractionation of Moxifloxacin for Treatment of Tuberculosis: Impact of Dosing Interval and Elimination Half-Life on Microbial Kill and Resistance Suppression

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ABSTRACT The repurposed agent moxifloxacin has become an important addition to the physician's armamentarium for the therapy of *Mycobacterium tuberculosis*. When a drug is administered, we need to have metrics for success. As for most antimicrobial chemotherapy, we contend that for *Mycobacterium tuberculosis* therapy, these metrics should be a decline in the susceptible bacterial burden and the suppression of amplification of less-susceptible populations. To achieve optimal outcomes relative to these metrics, a dose and schedule of administration need to be chosen. For large populations of patients, there are true between-patient differences in important pharmacokinetic parameters. These distributions of parameter values may have an impact on these metrics, depending on what measure of drug exposure drives the metrics. To optimize dose and schedule choice of moxifloxacin, we performed a dose fractionation experiment in the hollow fiber infection model. We examined 12-, 24-, and 48-h dosing intervals with doses of 200, 400, and 800 mg for each interval, respectively. Within each interval, we had an arm where half-lives of 12, 8, and 4 h were simulated. We attempted to keep the average concentration (C_{avg}) or area under the concentration-time curve (AUC) constant across arms. We found that susceptible bacterial load decline was linked to C_{avg} , as we had indicated previously. Resistance suppression, a nonmonotonic function, had minimum concentration (C_{min}) as the linked index. The 48-h interval with the 4-h half-life had the largest less-susceptible population. Balancing bacterial kill, resistance suppression, toxicity (linked to peak concentration [C_{peak}]), and adherence, we conclude that the dose of 400 mg daily is optimal for moxifloxacin.

KEYWORDS *Mycobacterium tuberculosis*, dose fractionation, mathematical modeling, pharmacodynamics, resistance suppression

Moxifloxacin (MXF) is one of the repurposed agents that has expanded the therapeutic armamentarium for *Mycobacterium tuberculosis*. It is an important part of a regimen for multiple-drug-resistant *M. tuberculosis* strains (1). Recently, our laboratory demonstrated *in vitro* that MXF plus pretomanid and bedaquiline form a highly potent regimen that rapidly killed *M. tuberculosis* H37Rv and also suppressed amplification of less-susceptible subpopulations (2).

MXF has been shown previously (3) to have an area under the concentration-time curve (AUC)/MIC ratio as the pharmacodynamic driver most closely linked to bacterial load reduction for *M. tuberculosis*. Our group previously studied MXF in a 10-day hollow fiber infection model (HFIM) study (4) against log-phase organisms and in a 28-day

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study against acid-phase and nonreplicative persister (NRP)-phase organisms (5). In neither of these studies was any dose fractionation performed. MXF is a bit unusual in that we previously demonstrated different pharmacodynamic drivers are linked to resistance suppression (6, 7) for *Yersinia pestis* (peak/MIC ratio) versus *Bacillus anthracis* (minimum concentration [$C_{\min}$]/MIC ratio). Consequently, we felt that it was important to identify the pharmacodynamic driver for both bacterial load decline and resistance suppression simultaneously for *M. tuberculosis*. Further, because of between-patient variance in important pharmacokinetic parameters, large populations of patients will have substantial differences in these parameter values and will have different half-lives. We previously demonstrated (8) that this may have a substantial clinical impact. Consequently, in our experiment we kept the drug exposure (AUC or average concentration [C_{avg}]) relatively constant but varied the half-life in each of the fractionation schedules. This was accomplished by varying the peak concentration: if the clearance (CL) is constant, a shorter half-life gives a larger elimination rate constant (k_{el}), which forces the volume of distribution (V) to be smaller ($\text{CL} = V \times k_{\text{el}}$) and, therefore, the peak concentration to be higher. In this way, we could look for the phenomenon of driver switching (8, 9), in which the pharmacodynamic index (or driver) for either *M. tuberculosis* kill by MXF or resistance prevention changes from one index to another. This was seen for the antiviral drugs raltegravir, in which the pharmacodynamic driver for efficacy switched from AUC/90% effective concentration (EC_{90}) to time (T) $>$ EC_{90} in simulated and real people who rapidly eliminated these drugs from their bodies (8), and for zanamivir (9), where a longer half-life changed the pharmacodynamic driver from $T > \text{EC}_{90}$ to AUC/ EC_{90} .

RESULTS

MICs, resistance mutational frequency, and MIC values from colonies on moxifloxacin-containing plates. The resistance mutational frequency was 7.27×10^{-7} (1/10^{7.27} CFU) for H37Rv. The MXF MICs were 0.25 mg/liter on agar and 0.5 mg/liter on broth. The majority of isolates retested for MIC values from moxifloxacin-containing plates had values of 2 to 4 mg/liter.

Moxifloxacin activity against log-phase *M. tuberculosis* H37Rv. The bacterial effects (bacterial load reduction and resistance emergence) for 200 mg every 12 h, 400 mg daily, and 800 mg every other day with half-lives of 12, 8, and 4 h for each dose and schedule are displayed in Fig. 1. In all instances, there was emergence of resistance on all schedules and with all half-lives. The less-susceptible population ultimately took over the total population in all experimental arms (displayed in Fig. S1 in the supplemental material). At time zero, no less-susceptible population was identified in 7 of 9 arms. This is highly likely to be due to Poisson sampling. The total bacterial burden in each arm was always greater than the inverse of the mutational frequency to resistance. However, the independently measured baseline burdens were calculated to contain 1 to 6 less-susceptible colonies per ml. Given the sample size (200 μ l) for the MXF-containing resistance selection plates (containing $3 \times$ the baseline MIC of MXF), it was to be expected that most of the arms would not have MXF less-susceptible populations identified at baseline even though they were present at low numbers.

The actual total burden decline in the 9 active arms ranged from 4.05 to 5.76 log₁₀ CFU/ml. It should be recognized that these numbers are confounded by ongoing less-susceptible subpopulation amplification. We have described this finding previously (10).

In order to identify the independent effect of MXF on the susceptible population and the amplification of the less-susceptible population, it was necessary to fit a model to all the data (drug concentrations, total bacterial burden, and less-susceptible bacterial burden) simultaneously.

Mathematical modeling of all the data. The results of the mathematical modeling analysis are displayed in Table 1 and Fig. 2. The Bayesian parameter estimates for each treatment regimen are displayed in Table S1 in the supplemental material. In Table 1, we present the mean and median parameter vectors and the standard deviations (SD).

FIG 1 Bacterial load changes after exposure to moxifloxacin at (A) 200 mg every 12 h (Q12h) with half-lives ($T_{1/2}$ or $t_{1/2S}$) of 12, 8, and 4 h, (B) 400 mg every 24 h (QD) with half-lives of 12, 8, and 4 h, and (C) 800 mg every 48 h (QOD) with half-lives of 12, 8, and 4 h. $fAUC_{24h}$, area under the concentration-time curve at 24 h for free, unbound fraction of drug.

TABLE 1 Parameter values (mean parameter vector) for the population pharmacokinetic/pharmacodynamic analysis^a

Parameter	V_c (liters)	CL (liters/h)	K_{g-s} (h ⁻¹)	K_{g-r} (h ⁻¹)	K_{kill-s} (h ⁻¹)	K_{kill-r} (h ⁻¹)	C_{50-s} (mg/liter)	C_{50-r} (mg/liter)	H_s	H_r	Pop _{max} (CFU/ml)	IC ₂ (CFU/ml)	IC ₃ (CFU/ml)
Mean	310.2	27.6	0.123	0.035	0.262	0.296	0.374	5.59	9.24	3.15	1.22×10^8	4.67×10^7	5.61
Median	346.5	25.9	0.136	0.038	0.187	0.297	0.055	5.41	8.01	1.19	1.01×10^8	4.61×10^7	4.13
SD	111.8	3.75	0.038	0.007	0.239	0.105	0.873	0.523	6.31	5.52	4.13×10^7	1.94×10^7	5.37

^a V_c , volume of the central compartment; CL, clearance; K_{g-s} , growth rate constant for the susceptible population; K_{g-r} , growth rate constant for the less-susceptible population; K_{kill-s} , kill rate constant for the susceptible population; K_{kill-r} , kill rate constant for the less-susceptible population; C_{50-s} , drug concentration at which effect is half-maximal for the susceptible population; C_{50-r} , drug concentration at which effect is half-maximal for the less-susceptible population; H_s , Hill's constant for the susceptible population; H_r , Hill's constant for the less-susceptible population; Pop_{max}, maximal total population size; IC₂, initial total population size; IC₃, initial moxifloxacin less-susceptible population size.

The clearances represent free-drug clearance, and the protein binding of moxifloxacin employed was 50% (11). The growth rate constants identified for the susceptible and less-susceptible populations were 3- to 4-fold different, compatible with the hypothesis that the less-susceptible population was somewhat less biofit. While the kill rate constants for the two populations were comparable, the impact of resistance can be seen in the estimates of C_{50-s} and C_{50-r} (i.e., the drug concentrations at which the effect is half-maximal for the susceptible and less-susceptible populations, respectively). In both the mean and median estimates, they differed by greater than 10-fold.

Figure 2 displays the pre-Bayesian (population) and Bayesian (individual) predicted-observed regressions for each system output (drug concentration, total bacterial burden, and less-susceptible bacterial burden). The fit of the model to the data was acceptable. In the pre-Bayesian regressions, r^2 values were 0.731, 0.839, and 0.824 for the MXF concentrations, total bacterial burden, and less-susceptible bacterial burden, respectively. For the Bayesian regressions, these values were 0.983, 0.943, and 0.946, respectively. Measures of bias and precision were acceptable.

Determination of the impact of different pharmacodynamic drivers on the susceptible less-susceptible *M. tuberculosis* burdens and delineation of the linked pharmacodynamic drivers. The median parameter vector was employed to calculate the impact of the MXF concentration-time profile for each regimen and half-life. The impact on the susceptible bacterial burden is displayed in Fig. 3. In the 48-h dosing interval group, the 8- and 4-h half-life arms are saw-toothed. This is because of the mismatch between dosing interval and half-life, allowing some regrowth before the end of the dosing interval. The regrowth is more apparent for the 4-h half-life, as would be expected. Nonetheless, the amount of bacterial load decline is substantial for all dosing intervals and half-lives. Figure 3 also displays the achieved half-lives and C_{avg} (the latter is a transform of AUC as AUC/dosing interval). The achieved exposures and half-life were acceptable.

In Fig. 4, the bacterial load reduction is plotted against different independent variables (peak concentration [C_{peak}], C_{avg} , and C_{min}). A sigmoid maximum effect (E_{max}) function was fit to the data. Both C_{avg} and C_{min} generate r^2 values that are similar. Given the very small values of the EC_{50} (0.000215 mg/liter) and Hill coefficient (0.283) for the C_{min} regression and the prior finding that C_{avg} was the dynamically linked index for bacterial load decline (2), we chose this index for the linkage to bacterial kill.

In Fig. 5, the relationships between the number of less-susceptible *M. tuberculosis* isolates at the end of the experiment and the different independent variables (C_{peak} , C_{avg} , and C_{min}) are displayed. While bacterial load decline is a monotonic function, our laboratory has described that the relationship between the number of less-susceptible isolates and a measure of drug exposure is a nonmonotonic function (12, 13). The C_{avg} does not have an inverted U form. C_{peak} and C_{min} do have the form of an inverted U, but the C_{min} has a much clearer relationship, with increasing C_{min} leading to lower numbers of less-susceptible isolates. It is also clear that there are still greater than 3 log₁₀ CFU/ml less-susceptible isolates when the trough MXF approaches 0.5 mg/liter. While C_{min} is likely the dynamically linked index for resistance suppression, it is clear that standard MXF dosing is highly unlikely to achieve resistance suppression over a 28-day span.

FIG 2 Predicted-observed plots for drug (moxifloxacin) concentrations (top panels), total bacterial burdens (middle panels), and less-susceptible bacterial burden (bottom panels) for *Mycobacterium tuberculosis* H37Rv from the pre-Bayesian regression (left panels) and the Bayesian regression (right panels).

FIG 3 Comparison of the impact of 12-, 8-, and 4-h half-lives (T_{1/2}) of moxifloxacin upon the sensitive population bacterial kill, as seen in every-48-h dosing (Q 48 hr DI) with an 800-mg dose (A), every-24-h dosing (Q 24 hr DI) with a 400-mg dose (B), and every-12-h dosing (Q 12 hr DI) with a 200-mg dose (C). In panel D, the achieved C_{avg} values and half-lives are displayed.

DISCUSSION

MXF is a repurposed agent that has achieved an important place in the physician's armamentarium for the treatment of tuberculosis. Understanding the linkage of different exposure indices to the ability of the drug to cause maximal bacterial load reduction and to suppress amplification of a less-susceptible subpopulation when employed as a single agent is central to identifying the optimal dose and schedule for administration.

Another important issue is the recognition that true between-patient variability exists in pharmacokinetic parameter values in a population. Such differences may lead to subpopulations of patients that do not respond to therapy in the expected way (14–16). An example is the population of patients with ventilator-associated bacterial pneumonia who have higher glomerular filtration rates (14, 15). In some instances, such a subpopulation may result in driver switching, leading to failure of therapy (8, 9). For these reasons, we decided to restudy (3) MXF for the dynamic driver for bacterial cell kill as well as for resistance suppression and to include subpopulations with a range of half-lives within each dosing interval.

In Fig. 1, the change in total bacterial counts over time is displayed for three different dosing intervals and for three half-lives within each dosing interval. There are some modest differences as a function of dosing interval and half-life. An issue with

FIG 4 Relationship between pharmacodynamic drivers C_{peak} (A), C_{avg} (B), and C_{min} (C) and moxifloxacin-sensitive bacterial load reduction in a sigmoid E_{max} model. Q 48 hr, Q 24 hr, and Q 12 hr represent dosing intervals of every 48, 24, and 12 h, respectively.

interpreting the results rests in the confounding effect of amplification of the less-susceptible subpopulation. In all instances, a less-susceptible subpopulation emerged and completely replaced the susceptible population over the 28-day experimental period (Fig. S1).

In order to have the deepest understanding of the impact of interval and half-life on the replication dynamics, it is important to model all the data simultaneously, as we have done previously (2–7, 10).

The fit of the model to the data for all three system outputs is displayed in Fig. 2. The predicted-observed plots for both pre-Bayesian (population) and Bayesian (individual) analyses as well as measures of bias and precision indicate that the model fit the data acceptably well. The mean and median parameter vectors as well as standard deviations (SD) are displayed in Table 1.

The fully parametric analyses allowed us to strip out the confounding effects of resistance amplification and to quantify the impact of the regimen/half-life on the susceptible subpopulation. This is shown in Fig. 3. The median Bayesian parameter vector allowed calculation of the susceptible population over time. Little difference was seen across half-lives in the 12- and 24-h dosing intervals. In the 48-h interval with the 4-h

FIG 5 Relationship between pharmacodynamic drivers C_{peak} (A), C_{avg} (B), and C_{min} (C) and mofloxacin-resistant bacterial load. In panel D, the half-lives ($T_{1/2}$) with each dosing interval are displayed to clarify the effect on the less-susceptible population. Q 48 hr, Q 24 hr, and Q 12 hr represent dosing intervals of every 48, 24, and 12 h, respectively.

half-life, a sawtooth pattern emerges that results from regrowth at the back end of the 48-h interval. As discussed below, this will also interact with the less-susceptible subpopulation.

In Fig. 4, the sensitive subpopulation bacterial load decline is plotted against measures of MXF exposure. Both C_{avg} and C_{min} do a reasonable job of describing the change in susceptible bacterial burden in a sigmoid E_{max} model. C_{avg} (AUC) has a slightly better r^2 . Given that Shandil et al. (3) previously identified this driver as being linked to overall bacterial load decline in a murine model, we chose this descriptor as the dynamically linked index.

Bacterial kill for a susceptible population is a monotonic function (i.e., the greater the dynamic index, the greater the bacterial load decline up to a maximum). Resistance suppression follows an “inverted-U” pattern (12, 13, 17). Figure 5 displays the resistant bacterial burden at the end of therapy (day 28) as a function of the different exposure indices employed in Fig. 4. Here, C_{min} provides the clearest linkage to the behavior of the less-susceptible subpopulation. The largest value of C_{min} is associated with the smallest less-susceptible subpopulation. Of special interest (Fig. 5D), we see that the every-48-h dosing interval is associated with the largest less-susceptible subpopulation and that the shortest half-life produces the lowest C_{min} values (as would be expected) and that is associated with the largest less-susceptible *M. tuberculosis* population at the end of therapy.

In summary, MXF can obtain near-optimal bacterial kill of the susceptible subpopulation on any of the three intervals examined. This is likely because C_{avg} is the dynamically linked index for *M. tuberculosis* kill of the susceptible population. However, because C_{min} is linked to resistance suppression and because of the between-patient differences in pharmacokinetic parameter values, every-48-h dosing leads to the largest less-susceptible population when the drug is used as monotherapy. Further, MXF concentrations are linked to QT_c prolongation and C_{max} appears to be the pharmacodynamic driver of this adverse effect (18). Using the 800-mg dose every 48 h would drive the highest C_{max} . This would place the patient at the highest risk for arrhythmias and would be an improvident choice. Balancing bacterial kill, resistance suppression, and regimen adherence, our data indicate that the dose of 400 mg daily is the optimal choice. We would also point out that therapy for *M. tuberculosis* is almost always a multidrug regimen. It is important to optimize doses and schedules to suppress resistance emergence for each agent. This is because of true between-patient variability in pharmacokinetic parameter values. It is equally important to recognize that therapy in this disease requires multiple agents. For well-chosen regimens by dose and schedule, the resistance suppressive effect may not be of major significance.

MATERIALS AND METHODS

Much about the methods employed here is detailed in references 2, 10, and 19.

Bacteria. *M. tuberculosis* strain H37Rv (ATCC 27294) was used. Stocks of the bacteria were stored at -80°C . For each experiment, an aliquot of the bacterial stock was inoculated into filter-capped T-flasks containing 7H9 Middlebrook broth that was supplemented with 0.05% Tween 80 and 10% albumin, dextrose and catalase (ADC). The culture was incubated at 37°C in 5% CO_2 on a rocker platform for 4 to 5 days to achieve log-phase growth.

Susceptibility testing and mutation frequency determination. Susceptibility studies for MXF were conducted with log-phase-growth H37Rv using the agar proportional method described by the CLSI (20) and the absolute serial dilution method on 7H10 agar plus 10% OADC (ADC with oleic acid). Briefly, 10^4 CFU of H37Rv in log-phase growth were plated on Middlebrook 7H10 agar (Becton, Dickinson Microbiology Systems, Sparks, MD) supplemented with 10% OADC (Becton, Dickinson Microbiology Systems) containing 2-fold dilutions of MXF. The cultures were incubated at 37°C and 5% CO_2 . After 4 weeks of incubation, the MICs were determined by identifying the lowest drug concentration at which there was no bacterial growth on the agar plate. For the agar proportional method, the lowest concentration of a drug that provided a 99% reduction in the bacterial density relative to the no-drug control was read as the MIC. For the absolute serial dilution method, the MIC was read as the lowest concentration of drug for which there was no growth on the agar plate.

The mutation frequency of the H37Rv strain was evaluated using methods that are described elsewhere (10). Briefly, H37Rv cultures in log-phase growth were inoculated onto plates containing Middlebrook 7H10 agar plus 10% Middlebrook OADC with MXF at a concentration equivalent to $3.0\times$ the MIC. The mutation frequency was identified after 4 weeks of incubation at 37°C and 5% CO_2 .

HFIM 28-day studies using clinically relevant drug exposures for log-phase-growth *M. tuberculosis*. The goals of the study were to identify the exposure index (C_{peak} , C_{avg} , and C_{min}) most linked to two metrics: reduction of susceptible bacterial burden and suppression of amplification of less-susceptible organisms. Because of true between-patient variability in pharmacokinetic parameter values, we also wished to determine the impact on the pharmacodynamic index linkage at various clinically relevant half-lives, including 12, 8, and 4 h.

The experimental arms for the studies using log-phase-growth *M. tuberculosis* H37Rv were as follows: (A) No-treatment growth control. (B to D) MXF at 800 mg administered every 48 h with half-lives of 12, 8, and 4 h. (E to G) MXF at 400 mg administered every 24 h with half-lives of 12, 8, and 4 h. (H to J) MXF at 200 mg administered every 12 h with half-lives of 12, 8, and 4 h.

Pharmacokinetic and protein binding data. Protein binding values for MXF were obtained from the article of Stass and Kubitzka (11), and human pharmacokinetic parameters from reference 19 were employed.

Detection of resistance amplification. Serial bacterial specimens were collected from the hollow fiber system arms over the course of the 28-day experiments. The samples were washed and then quantitatively plated onto antibiotic-free agar and antibiotic-supplemented agar to characterize the effect of each treatment regimen on the total bacterial and less-susceptible bacterial populations. A volume of 500 μl was removed from the peripheral compartment and washed. A 200- μl subsample was streaked onto the zero-dilution plate. Another 200 μl was serially diluted and quantitatively cultured onto MXF-free agar. Aliquots of the bacterial suspension were also quantitatively cultured on agar supplemented with $3\times$ the baseline MIC for MXF. These cultures were used to determine the effect of each MXF regimen on the total and less-susceptible *M. tuberculosis* populations.

Achievement of target exposure profiles. Serial samples of media were collected from the hollow fiber treatment arms for assay of drug content by liquid chromatography-tandem mass spectrometry

(LC-MS/MS) by a previously published technique (2) to confirm that the targeted concentration-time profiles were achieved.

Population pharmacokinetic/pharmacodynamic mathematical model. We simultaneously modeled 3 system outputs for the analysis of the log-phase *M. tuberculosis* data. The system outputs were concentration of MXF, total *M. tuberculosis* burden, and burden less susceptible to MXF. Population modeling was performed employing the Non-Parametric Adaptive Grid (NPAG) program of Leary et al. (21) and Neely et al. (22). Modeling choices (weighting, etc.) and goodness-of-fit evaluations were as previously published (9). As the initial size of the total bacterial burden for the no-treatment control was very near the maximal size and contained little system information, the no-treatment control was not included in the analysis. Simulation was performed with the ADAPT V program of D'Argenio et al. (23) using Bayesian posterior parameter estimates.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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