Modeling In Vivo Pharmacokinetics and Pharmacodynamics of Moxifloxacin Therapy for Mycobacterium tuberculosis Infection by Using a Novel Cartridge System

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To study the efficacy of moxifloxacin treatment for tuberculosis, we utilized a novel cartridge system to simulate in vivo pharmacokinetics. We found this system to be a robust method for modeling in vivo pharmacokinetics and present data supporting the utility of intermittent moxifloxacin treatment as a component of antituberculosis chemotherapy.

Tuberculosis (TB) continues to be a leading cause of morbidity and mortality worldwide. The current treatment for active TB requires a lengthy course of medication, adherence to which is often limited by clinical, social, financial, and behavioral factors. Although supervised chemotherapy is recommended by the World Health Organization, it remains to be completely implemented, particularly in resource-poor countries. The cost of supervision frequently exceeds the cost of the drugs themselves. If a more intermittent treatment regimen could be developed, supervision costs would be reduced. The maximum interval between doses for an antibiotic is limited both by the in vivo half-life (maximum interval between doses for an antibiotic is limited) and by the postantibiotic effect (PAE), the continued inhibition of bacterial replication after an antibiotic has been withdrawn (4, 12). Moxifloxacin (MXF) is an 8-methoxy quinolone with in vitro and in vivo activities against Mycobacterium tuberculosis (9, 11, 22).

In this study, we examined the bactericidal effects and PAEs of MXF against M. tuberculosis using a novel cartridge system designed to closely simulate in vivo parameters for pharmacokinetics (PK) and pharmacodynamics (PD) (10). The cartridge system (Fig. 1A) consists of a hollow-fiber cartridge (BioVest International, Minneapolis, Minn.), a central reservoir, an input reservoir, an output (waste) reservoir, a fast (perfusion) pump, a slow (dilation) pump, and a dosing pump (to simulate oral dosing). The cartridge (Fig. 1B) consists of semipermeable capillaries (70-kDa molecular mass cutoff) which divide the cartridge into an intracapillary compartment (ICC), for perfusion of the growth medium, and an extracapillary compartment (ECC), in which bacteria can be inoculated. The perfusion pump circulates medium between the central reservoir and the cartridge. The input reservoir contains medium that is delivered to the central reservoir by the dilation pump. Excess medium is bled from the central reservoir to the waste reservoir in such a way that the volume of medium in the system (central reservoir, tubing, ICC, and ECC) is maintained at a constant 52 ml. The dosing pump delivers drug to the central reservoir at a programmed rate for a programmed interval of time. The flow rate from the input reservoir to the central reservoir determines the drug t_{1/2} in the system.

To simulate the PK parameters of a 400-mg dose of MXF in humans (maximum concentration of the drug in serum [C_{max}], 3.1 to 4.5 μg/ml; area under the concentration-time curve, 31 to 48 μg·h/ml; t_{1/2}, 12 to 13 h [12]), the required dose of MXF administered to the cartridge system was calculated to be 100 μg. This dosage takes into account 50% protein binding in human serum (12) and thus was designed to achieve a C_{max} of 1.6 to 2.3 μg/ml, equivalent to the free-drug C_{max} in human serum. However, it should be noted that we could find no experimental evidence to either support or invalidate the assumption that the degree of protein binding impacts the clinical efficacy of the quinolones. We set up separate dosing regimens for each of the five cartridges: (i) no drug given; (ii) 100 μg given at time zero; (iii) 100 μg given at time zero followed by 100 μg at 168 h; (iv) 100 μg given at time zero and 96 and 192 h; and (v) 33 μg given at time zero and 96 and 192 h. In all cases, drug was administered by the dosing pump in a total volume of 1 ml over 15 min.

A model describing the PK properties of the cartridge system is shown below:

$$X_1 \xrightarrow{Q} X_2 \xrightarrow{k_{elim}}$$

where $X_1$ is the mass of drug (in micrograms) at any time (t) in the infusion pump, $X_2$ is the mass of drug at time t in the system, Q is the zero-order rate constant of administration of the drug, and $k_{elim}$ is the first-order elimination rate constant. Since in our studies the drug was administered over the course of 15 min, the value of Q is 4 h⁻¹ (there are four 15-min intervals per h). From basic PK, the $t_{1/2}$ in hours of the drug in the system is defined by

$$t_{1/2} = 0.693/k_{elim}$$

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where $k_{\text{elim}}$ is the rate constant of elimination of the drug from the system. Solving for the desired $t_{1/2}$ of 12 h gives a $k_{\text{elim}}$ of $0.05775 \text{ h}^{-1}$. Again, from pharmacokinetics, drug clearance (CL) from the system is defined by

$$CL = k_{\text{elim}} \cdot V$$

where $V$ is the volume of distribution. Thus, a $k_{\text{elim}}$ of 0.05775 h$^{-1}$ and a $V$ of 52 ml (see the description of the system above) gives a clearance rate of 3.003 ml/h. In order to achieve a $t_{1/2}$ of 12 h, the flow rate of the dilution pump was therefore set at 3.003 ml/h. Assuming that distribution is instantaneous, the amount of drug in the system during the infusion of a dose can be described by the following differential equation:

$$\frac{dX_2}{dt} = Q \cdot X_1 - k_{\text{elim}} \cdot X_2$$

which, when subjected to the LaPlace transformation (18), becomes

$$X_2 = A(1 - e^{-0.05775t}) + 0.05775$$

where $A$ is four times the dose of drug (in micrograms) and $X_2$ is the mass of drug (in micrograms) in the system at $t$ (in hours). Following the 15-min infusion, $X_2$ is given by

$$X_2 = X_{\text{post}}e^{-0.05775t}$$

where $X_{\text{post}}$ is the mass of drug (in micrograms) in the system following the infusion (i.e., equation 4 is solved for a $t$ of 0.25 h).

In this study, the growth medium was Middlebrook 7H9 broth with 10% oleic acid-albumin-dextrose-catalase supplement (both from BD Diagnostic Systems, Sparks, Md.), 0.5% glycerol (Mallinckrodt Baker, Phillipsburg, N.J.), and 0.05% Tween 80 (Sigma-Aldrich, St. Louis, Mo.). A total of 10^8 CFU of $M. tuberculosis$ H37Ra were inoculated into the ECCs of each of five cartridge systems and allowed to equilibrate overnight in a 37°C, 5% CO2 incubator. Gas exchange was provided by a 0.2-μm-pore-size filter venting the central reservoir. The five cartridge systems were run in parallel.

The culture in the untreated control cartridge demonstrated steady exponential growth, with a doubling time of approximately 33 h for the first 300 h of the experiment, at which point the number of viable bacteria stabilized at between 10^7 and 10^8 CFU (Fig. 2A). This demonstrated the feasibility of establishing bacterial populations within the cartridge. In the cartridges receiving 100-μg doses of MXF, there was a significant decline in the number of viable bacilli (Fig. 2B to D). The CFU counts in the cartridge receiving 33-μg doses of MXF did not vary significantly during the course of the experiment (Fig. 2E). These results demonstrate the bactericidal effects of MXF in the environment within the cartridge.

Of particular interest was the PAE, which, for the purposes of this study, was defined as the time required for the CFU count in a treated cartridge to increase 1 log_{10} unit above the nadir count observed after dosing. The schedule for intermittent chemotherapy is constrained by the duration of the PAE. Specifically, dosing should be spaced such that no resumption of bacterial multiplication occurs between doses (5). Thus, while a single 100-μg dose in the cartridge system demonstrated a PAE of 195 h (8.1 days), it is clear that bacterial multiplication resumed within 140 h (5.8 days) after administration of the drug (Fig. 2B). In addition, once bacterial growth resumed, the culture exhibited a doubling time nearly equal to that of the control (approximately 37 h, compared to 33 h for the untreated culture). Even weekly dosing (Fig. 2C) allowed some bacterial replication to occur. In contrast, dosing with 100 μg of MXF every 4 days resulted in a continual decline in bacterial counts (Fig. 2D). Dosing with only 33 μg of MXF every 4 days resulted in neither a significant decline nor an increase in bacterial load (Fig. 2E). Based on the results of this in vitro study, and with the assumption that the same PK properties would occur in humans, 400-mg doses of MXF...
could be spaced 3 to 4 days apart without allowing a significant rebound of bacterial multiplication. However, spacing doses 1 week apart is not advised, as an appreciable amount of bacterial growth was seen to occur (Fig. 2C).

Several in vitro PK and PD models for testing antibiotic activity have been described previously (1, 2, 6, 7, 20, 21). In contrast to what occurs with conventional methods for antibiotic testing, bacteria in these models are exposed to drug concentrations that change over time to better simulate the in vivo situation. While these in vitro models have been used to assess the PK and PD parameters of antimicrobials, including fluoroquinolones, against other bacteria (8, 13–17), to our knowledge they have not been used to evaluate the PAE properties of fluoroquinolones against *M. tuberculosis*. By simulating PK parameters in humans, our in vitro PK-PD system determined the PAE of a single 400-mg dose of MXF on *M. tuberculosis* to be over 1 week. This result is compared to a PAE of greater than 15 days in a traditional static in vitro study of MXF against *M. tuberculosis* H37Rv after an exposure period of 24 h (A. S. Ginsburg et al., unpublished data). Recently, Chan et al. reported the in vitro PAE of a 2-μg/ml concentration of MXF against *M. tuberculosis* to be 0.3 h (3). However, this study used a radiometric assay to measure the cumulative growth of control and treated cultures, and the PAE was defined in a different manner, such that the results of that study and our study cannot be directly compared. Because the cartridge-based dynamic PK-PD system better simulates the PK properties of MXF in humans, the clinically relevant PAE is most likely better approximated in this system than in the traditional in vitro model. Our finding that bacillary growth resumes less than 1 week following a single 100-μg dose of MXF in the cartridge model is in good agreement with another study in which weekly doses of up to 400 mg of MXF per kg of body weight, a dose approximating the area under the concentration-time curve of a 400-mg dose in humans (19), had no effect on bacterial growth in infected mice. However, daily
administration of 400 mg of MXF/kg resulted in a >5-log reduction in lung bacillary count compared to the count in untreated mice after 28 days of therapy (23). The cartridge-based in vitro system utilized in this study has wide-ranging potential in the study of antimicrobials, encompassing more than the study of a single drug-pathogen interaction. The design of this apparatus may facilitate the study of multidrug regimens, mixed infections, and the dynamics of selection for antibiotic-resistant mutants.

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ADDENDUM IN PROOF


REFERENCES