Impact of Spore Biology on the Rate of Kill and Suppression of Resistance in Bacillus anthracis

G. L. Drusano,¹* O. O. Okusanya,² A. O. Okusanya,² B. van Scoy,¹ D. L. Brown,¹ C. Fregeau,¹ R. Kulawy,¹ M. Kinzig,³ F. Sörgel,³,⁴ H. S. Heine,⁵ and A. Louie¹

Ordway Research Institute¹ and Institute for Clinical Pharmacodynamics, Ordway Research Institute,² New York; IBMP—Institute for Biomedical and Pharmaceutical Research, Nürnberg-Heroldsberg,³ and Department of Pharmacology, University of Duisburg—Essen, Essen,⁴ Germany; and United States Army Research Institute for Infectious Diseases, Fort Detrick, Maryland⁵

Received 16 June 2009/Returned for modification 7 August 2009/Accepted 11 August 2009

Bacillus anthracis is complex because of its spore form. The spore is invulnerable to antibiotic action. It also has an impact on the emergence of resistance. We employed the hollow-fiber infection model to study the impacts of different doses and schedules of moxifloxacin on the total-organism population, the spore population, and the subpopulations of vegetative- and spore-phase organisms that were resistant to moxifloxacin. We then generated a mathematical model of the impact of moxifloxacin, administered by continuous infusion or once daily, on vegetative- and spore-phase organisms. The ratio of the rate constant for vegetative-phase cells going to spore phase (Ksv) to the rate constant for spore-phase cells going to vegetative phase (Ksv) determines the rate of organism clearance. The continuous-infusion drug profile is more easily sensed as a threat; the Ksv/Ksv ratio increases at lower drug exposures (possibly related to quorum sensing). This movement to spore phase protects the organism but makes the emergence of resistance less likely. Suppression of resistance requires a higher level of drug exposure with once-daily administration than with a continuous infusion, a difference that is related to vegetative-to-spore (and back) transitioning. Sperone biology has a major impact on drug therapy and resistance suppression. These findings explain why all drugs of different classes have approximately the same rate of organism clearance for Bacillus anthracis.

Bacillus anthracis remains a major threat for intentional release and would be expected to cause considerable mortality and morbidity. Both ciprofloxacin and levofloxacin have gained FDA approval for the postexposure prophylaxis of anthrax infections. Moxifloxacin is also a fluoroquinolone (9), but with greater potency for gram-positive pathogens. This makes it an appealing choice for evaluation.

Fluoroquinolones are also highly active against other bioterror pathogens, such as Yersinia pestis (6, 7). Bacillus anthracis remains a special case because it forms spores. This protects this pathogen from rapid death due to drug therapy as well as from other environmental challenges. We have studied the Δ-Sterne strain and its isogenic mutant (CR4) that lacks the ability to form spores. In our original levofloxacin evaluation, the non-spore-forming mutant was eradicated from the system very quickly (3). We have seen this repeatedly for drugs of different classes (data not shown). The spore form provides a ready refuge from the onslaught of chemotherapy.

In this investigation, we wished to evaluate moxifloxacin against Bacillus anthracis. Since we have previously demonstrated that the schedule of administration has an impact on the likelihood of the emergence of resistance to linezolid (8), we wished to examine the impact of once-daily administration of moxifloxacin versus continuous-infusion administration. Finally, we recognized how complex the spore form makes this model system. Consequently, we wished to model multiple system outputs simultaneously, and we generated a mathematical model examining the total population (sensitive plus resistant organisms, both vegetative and spore phase), the total spore population as determined after heat shock (antibiotic-sensitive plus antibiotic-resistant spore-phase organisms), the total antibiotic-resistant population (vegetative plus spore phase), and the resistant population after heat shock (which measures antibiotic-resistant spores). We also measured moxifloxacin concentrations to determine how they had an impact (or not) on each of the populations measured.

MATERIALS AND METHODS

Bacteria, media, susceptibility testing, and frequency of mutation to resistance. The Δ-Sterne strain of Bacillus anthracis was evaluated. This strain lacks the pXO1 and pXO2 virulence plasmids containing the toxin and capsule genes, respectively. Moxifloxacin powder was kindly supplied by Bayer Pharmaceuticals (Wuppertal, Germany).

MICs of moxifloxacin were determined simultaneously by the macrobroth and agar dilution methods in Mueller-Hinton II broth (MHB) and Mueller-Hinton II agar (MHA) using the methods outlined by the CLSI (2). MICs were read after 24 h of incubation at 35°C. Trailing endpoints were observed. After discussion with H. Heine, our coinvestigator at USAMRIID and a member of the CLSI advisory committee, the MIC was defined as the lowest moxifloxacin dilution that resulted in a ≥80% reduction in growth compared to the growth controls. Minimal bactericidal concentrations (MBCs) were determined using standard methods (1). The frequencies of mutation to resistance in the presence of moxifloxacin concentrations equivalent to 2.5 times the MIC (2.5 MIC) were determined in three trials.

In vitro HF pharmacodynamic infection model. The hollow-fiber (HF) infection model described previously (3, 7, 8) was used to study the responses of B. anthracis to moxifloxacin exposures, simulating human pharmacokinetics. HF cartridges (FiberCell Systems, Frederick, MD) consist of bundles of HF capillaries encased in a plastic housing. The fibers have numerous pores that permit...
FIG. 1. Impact of a no-treatment control and of moxifloxacin administered at different continuous-infusion exposures on total colony counts (A), spore colony counts (after heat shock) (B), moxifloxacin-resistant colony counts (C), and moxifloxacin-resistant spore colony counts (after heat shock) (D) of the Δ-Stere strain of *Bacillus anthracis*. Inf, infusion; HS, heat shock.

the passage of nutrients and low-molecular-weight species, such as antibiotics, but exclude bacteria. Approximately 15 ml of extracapillary space lies between the fibers and the cartridge housing. The medium within the central reservoir was continuously pumped through the HFs, and low-molecular weight compounds rapidly equilibrated across the fibers with the extracapillary space. Thus, microorganisms that were inoculated into the extracapillary space were exposed to conditions approximating those that prevailed in the central reservoir.

Antibiotic was infused over 1 h into the central reservoir at predetermined time points by syringe pumps. Antibiotic-containing medium was isovolumetrically replaced with drug-free medium, simulating a half-life of 12 h. The rate of fresh medium infusion was used for the evaluation of chromatograms.

For each experiment, 15 ml of a *B. anthracis* suspension (10^7 CFU/ml) was inoculated into the extracapillary space of multiple HF cartridges, and the experiment was initiated by infusing antibiotic. At predetermined time points, an 800-μl sample of bacteria was collected from each HF system. Samples were centrifuged twice, resuspended, and quantitatively cultured onto drug-free MHA and onto MHA containing 2.5 μg/ml (for total spore plus vegetative organisms) and onto MHA containing moxifloxacin at 2.5× MIC (for resistant spore and vegetative organisms). The media taken from the central reservoir over the first 48 h were assayed for moxifloxacin concentrations to confirm that the desired pharmacokinetic profiles were achieved. The drug concentrations measured were within 10% of the targeted values as measured by the area under the concentration-time curve from 0 to 24 h (AUC_0–24) (data not shown). The experiment was duplicated.

**Determination of moxifloxacin concentrations.** Samples obtained from each treatment arm were stored at −80°C until they were assayed for their moxifloxacin concentrations.

**Antibiotic administration regimens** producing the same AUC_0–24 as administration by the once-daily regimen were studied (exposures equivalent to doses of 100 mg, 150 mg, 200 mg, and 250 mg). All drug regimens simulated the moxifloxacin free-drug concentrations. Samples were taken from each HF system over the 14-day experiment. Over the course of the study, bacterial samples collected from each HF system were divided into two aliquots. The first aliquot was washed twice to prevent drug carryover before it was quantitatively plated onto drug-free MHA and onto MHA containing 2.5× MIC of moxifloxacin for enumeration of the total (spore plus vegetative) *B. anthracis* population and of the spore-plus-vegetative *B. anthracis* subpopulation that was resistant to >2× baseline MIC, respectively. The second aliquot of the bacterial suspension was heat shocked at 65°C for 30 min in order to kill the vegetative-phase organisms. After heat...
FIG. 2. Impact of a no-treatment control and of moxifloxacin administered at different daily administration exposures on total colony counts (A), spore colony counts (after heat shock) (B), moxifloxacin-resistant colony counts (C), and moxifloxacin-resistant spore colony counts (after heat shock) (D) of the Δ-Sterne strain of Bacillus anthracis. Q24h, every 24 h; HS, heat shock.

shock, the aliquot was washed to prevent drug carryover and was then quantitatively cultured on antibiotic-free and moxifloxacin-supplemented agar plates in order to characterize the effect of each moxifloxacin regimen on the total spore population and the spore population that was resistant to >2.5× MIC of moxifloxacin.

Mathematical modeling of drug exposure-response relationships of the total bacterial population and the resistant bacterial subpopulation for both vegetative-phase organisms and spore forms. In order to understand mathematically the spore- plus vegetative-phase organism system with susceptible and resistant populations for each, five simultaneous parallel inhomogeneous differential equations (seven equations overall), shown below, were used to describe the time course of moxifloxacin concentrations and the total populations and resistant subpopulations for all counts and for spore counts only.

The different drug treatment regimens were simultaneously comodeled in a population sense using the BigNPAG population modeling program (6). Bayesian estimates were generated for each regimen.

\[ \frac{dX_1}{dt} = R(1) - (CL/V) \times X(1) \]  

where \( X_1 \) is the moxifloxacin amount in the central compartment, \( R(1) \) is the piecewise constant infusion rate of drug, \( CL \) is the moxifloxacin clearance, and \( V \) is the virtual volume of the central compartment.

\[ \frac{dX_2}{dt} = K_{s} \times \left( 1 - \frac{X(2) + X(3) \times \text{POPMAX}}{X(2) + K_{s} \times X(4) - K_{s} \times X(2)} \right) - \frac{X(2) \times M_{s}}{X(2) + K_{s} \times X(4) - K_{s} \times X(2)} \]  

\[ \frac{dX_3}{dt} = K_r \times \left( 1 - \frac{X(2) + X(3) \times \text{POPMAX}}{X(2) + K_{r} \times X(5) - K_{r} \times X(3)} \right) - \frac{X(3) \times M_{r}}{X(3) + K_{r} \times X(5) - K_{r} \times X(3)} \]  

\[ M_{s} = \frac{[X(1)/V]^3 \times (C_{s0}^M)^3 + [X(1)/V]^2}{(C_{s0}^M)^3} \]  

where \( X_s \) and \( X_r \) are the total counts (vegetative plus spore phases) of Bacillus anthracis for the sensitive and resistant populations, respectively; \( K_{s0} \) and \( K_{r0} \) are the first-order growth rate constants for the sensitive and resistant populations, respectively; POPMAX is the maximal population of total counts; \( K_{s} \) and \( K_{r} \) are the first-order kill rate constants for the sensitive and resistant populations; \( M_{s} \) and \( M_{r} \) are the sigmoid Emax kill functions for the sensitive and resistant populations, wherein \( H_{s} \) and \( H_{r} \) are the Hill constants for the sensitive and resistant populations; and \( C_{s0} \) and \( C_{r0} \) are the moxifloxacin concentrations at which the kill rate is half-maximal for the sensitive and resistant populations.

\[ \frac{dX_4}{dt} = \left[ K_{s} \times X(2) \right] - \left[ K_{s} \times X(4) \right] \]  

\[ \frac{dX_5}{dt} = \left[ K_{r} \times X(3) \right] - \left[ K_{r} \times X(5) \right] \]  

where \( X_s \) and \( X_r \) are the populations of drug-sensitive and drug-resistant spores present after heat shock, respectively, and \( K_{s} \) and \( K_{r} \) are the first-order rate constants of vegetative-phase cells going to spore phase and spore-phase cells going to vegetative phase, respectively.

System outputs. System output 1, calculated as \( X(1)/P(1) \), where \( P(1) \) is the volume of the control compartment, is the moxifloxacin drug concentration. System output 2, calculated as \( \log_{10} \left( \frac{X(2) + X(3)}{X(2)} \right) \), is the total population of all cells (vegetative plus spore phase, sensitive plus resistant) placed on drug-free plates. System output 3, calculated as \( \log_{10} X(3) \), is the total population of resistant cells (vegetative plus spore phase), where subculture volumes are grown on moxifloxacin-containing plates. System output 4, calculated as \( \log_{10} [X(4) + X(5)] \), is the total counts after heat shock with culture on drug-free plates. System output 5, calculated as \( \log_{10} X(5) \), is the counts after heat shock with culture on moxifloxacin-containing plates.
RESULTS

MIC/MBC of moxifloxacin for *B. anthracis* strain ∆-Sterne.

The modal MIC of moxifloxacin for the Δ-Sterne strain was 0.06 mg/liter, and the modal MBC for this strain was 1.0 mg/liter (performed in duplicate on three occasions).

Total population and total spore colony counts for the ∆-Sterne strain of *B. anthracis*. The total (vegetative plus spore, sensitive plus resistant) counts of *Bacillus anthracis* grown in HF units exposed to a continuous infusion of moxifloxacin (free AU(0→24) exposures equivalent to doses of 100 mg, 150 mg, 200 mg, and 250 mg per day) plus a no-treatment control are displayed in Fig. 1A; those for once-daily administration (50 mg, 100 mg, 150 mg, 200 mg, and 250 mg per day) are displayed in Fig. 2A. The corresponding data for heat-shock (moxifloxacin-resistant spore counts) for continuous-infusion and daily administration failed due to resistance emergence at 150 mg, but not at 150 mg for continuous-infusion administration. Once-daily administration failed due to resistance emergence at 150 mg, but not at 200 mg.

### TABLE 1. Parameter values for the two modes of administration of moxifloxacin

| Parametera (unit) | q24h b administration | Continuous infusion |
|------------------|------------------------|---------------------|
|                  | Mean | Median | SD   | Mean | Median | SD   |
| Vc (liters)      | 87.0 | 91.2   | 9.86 | 168  | 188    | 51.2 |
| CL (liters/h)    | 12.6 | 13.0   | 1.33 | 12.2 | 12.0    | 1.36 |
| Krs (h−1)        | 6.61 | 8.24   | 3.30 | 3.88 | 2.40    | 2.68 |
| Ksv (h−1)        | 0.419| 0.338  | 0.371| 0.943| 0.151   | 0.635|
| Kcs (h−1)        | 7.28 | 8.24   | 2.00 | 7.94 | 6.41    | 1.16 |
| Kva (h−1)        | 15.5 | 1.86   | 0.453| 16.4 | 19.8    | 4.43 |
| CS(0) (h−1)      | 0.513| 0.460  | 0.262| 0.751| 0.667   | 0.575|
| CS(0) (h−1)      | 2.29 | 2.08   | 1.02 | 2.05 | 1.91    | 1.15 |
| CVa (h−1)        | 23.4 | 29.8   | 9.31 | 12.3 | 5.93    | 12.6 |
| CVa (h−1)        | 6.09 | 2.63   | 6.71 | 10.7 | 7.38    | 5.11 |
| POPMAX            | 3.56 × 10^8| 1.39 × 10^8| 3.65 × 10^8| 1.32 × 10^6| 2.49 × 10^7| 2.32 × 10^8 |
| Kva (h−1)        | 3.32 | 4.96   | 2.26 | 0.928| 1.20    | 0.889 |
| Ksv (h−1)        | 7.01 | 7.31   | 2.79 | 6.29 | 2.41    | 8.87 |
| IC(2) CFU/ml     | 7.36 × 10^7| 4.12 × 10^7| 1.04 × 10^8| 7.46 × 10^7| 3.85 × 10^7| 9.65 × 10^7 |
| IC(3) CFU/ml     | 193 | 169    | 140  | 331  | 99      | 269  |
| IC(4) CFU/ml     | 1.88 × 10^6| 1.74 × 10^6| 1.78 × 10^4| 2.05 × 10^6| 1.88 × 10^6| 2.43 × 10^2 |
| IC(5) CFU/ml     | 28  | 32     | 9    | 30   | 25      | 10   |

a Parameters are defined in Materials and Methods. IC(2) to IC(5) are the initial conditions in the respective pharmacokinetic compartments. IC(2), total sensitive organism counts; IC(3), total resistant organism counts; IC(4), total spore counts; IC(5), resistant spore counts. The initial condition for drug concentration is zero.

b q24h, every 24 h.
Impact of the administration mode on the emergence of resistance. Aside from the fact that the organisms stay in the vegetative phase longer, the once-daily mode of administration also has an impact on the emergence of resistance. The integrated impact can be seen most clearly by examining Fig. 4A and B. By simply plotting the number of resistant mutants at the end of the experiment (h 366) as a function of the moxifloxacin AUC (except for the no-treatment control, which also has an impact on the emergence of resistance. The integration of the biology of *Bacillus anthracis* is how and how it affects our attempts at chemotherapy.

The behavior of the spore population is particularly intriguing. The control spore population (Fig. 1B and 2B controls are the same data) declines continuously after the first 48 h of growth in a nutrient-rich medium. No threat is detected, and the organism stays mostly in vegetative phase (about 10,000-fold more vegetative-phase organisms than spores at day 14). With drug pressure (Fig. 1A and B and 2A and B), if the danger is sensed, the number of spores starts to increase. However, if the pressure is sufficient to suppress or kill the resistant organisms, then the total population and the spore population will fall. It is the ratio of the rate constants governing the transition from the vegetative to the spore phase and from the spore phase back to the vegetative phase (K_out/K_in ratio) that determines the final rate of clearance of the total population (determined by extensive simulation from Bayesian estimates). It would appear that there is no sensor for threats for the transition back from the spore phase to the vegetative phase, since this parameter (K_out) always remains high, irrespec-

**DISCUSSION**

The examination of total populations and total resistant populations (vegetative phase plus spore phase) and of the spore-only populations (total and resistant) demonstrated how complicated the biology of *Bacillus anthracis* is and how it affects our attempts at chemotherapy.

### TABLE 2. Fit of the model to the data for all system outputs after the Bayesian step

| Variable | Regression equation | $r^2$ | $P$ |
|----------|---------------------|-------|-----|
| **Continuous-infusion administration** | | | |
| Moxifloxacin concn | $Y = 0.998 \times X - 0.002$ | 0.986 | $< 0.0001$ |
| Total-population counts | $Y = 1.011 \times X - 0.092$ | 0.874 | $< 0.0001$ |
| Resistant total-population counts | $Y = 1.150 \times X - 0.337$ | 0.792 | $< 0.0001$ |
| Total spore counts | $Y = 1.140 \times X - 0.722$ | 0.769 | $< 0.0001$ |
| Resistant spore counts | $Y = 0.968 \times X + 0.054$ | 0.818 | $< 0.0001$ |
| **Daily administration** | | | |
| Moxifloxacin concn | $Y = 0.957 \times X - 0.002$ | 0.968 | $< 0.0001$ |
| Total-population counts | $Y = 0.938 \times X + 0.423$ | 0.889 | $< 0.0001$ |
| Resistant total-population counts | $Y = 1.062 \times X - 0.099$ | 0.911 | $< 0.0001$ |
| Total spore counts | $Y = 1.153 \times X - 0.892$ | 0.736 | $< 0.0001$ |
| Resistant spore counts | $Y = 0.939 \times X - 0.047$ | 0.935 | $< 0.0001$ |

### TABLE 3. Bayesian parameter values for moxifloxacin administered as a continuous infusion

| Parameter* (unit) | Value for parameter at the following dose-equivalent exposure: |
|-------------------|---------------------------------------------------------------|
| $V_c$ (liter)     | Control 100 mg 150 mg 200 mg 250 mg                          |
| CL (litrers/h)    | 97.9 75.0 95.3 91.4                                         |
| $K_{out}$ (h$^{-1}$) | 0.0101 8.26 8.25 8.25                                         |
| $K_{in}$ (h$^{-1}$) | 0.0111 0.276 1.12 0.353                                         |
| $K_v$ (h$^{-1}$)  | 3.28 8.28 8.28 8.28                                         |
| $K_s$ (h$^{-1}$)  | 9.84 17.9 25.0 16.9                                         |
| $C_{max}$ (h$^{-1}$) | 0.992 0.246 0.315 0.462                                         |
| $C_{ava}$ (h$^{-1}$) | 4.26 1.63 1.37 2.11                                         |
| $H_{1}$ (h$^{-1}$) | 6.17 20.9 30.0 30.0                                         |
| $H_{2}$ (h$^{-1}$) | 19.5 2.74 2.74 2.73                                         |
| POPMAX            | $2.29 \times 10^7$ $1.00 \times 10^9$ $5.19 \times 10^8$ $9.58 \times 10^7$ $1.40 \times 10^8$ |
| $K_{out}$ (h$^{-1}$) | 0.0296 1.17 4.94 5.30                                         |
| $K_{in}$ (h$^{-1}$) | 2.02 1.1 6.65 7.23                                         |
| $K_v$ (h$^{-1}$)  | 0.722 0.769 0.722 0.769                                         |
| $K_s$ (h$^{-1}$)  | 0.054 0.818 0.792 0.874                                         |
| $IC(2)$ CFU/ml   | 2.82 $\times 10^8$ 2.06 $\times 10^7$ 2.35 $\times 10^7$ 2.06 $\times 10^7$ 2.16 $\times 10^7$ |
| $IC(3)$ CFU/ml   | 445 50 169 222 80                                         |
| $IC(4)$ CFU/ml   | 1.91 $\times 10^6$ 1.86 $\times 10^6$ 1.88 $\times 10^6$ 1.87 $\times 10^6$ 1.86 $\times 10^6$ |
| $IC(5)$ CFU/ml   | 39 15 32 34 22                                         |

* Parameter definitions are given in Materials and Methods. IC(2) to IC(5) are initial conditions in the respective pharmacokinetic compartments. IC(2), total sensitive organism counts; IC(3), total resistant organism counts; IC(4), total spore counts; IC(5), resistant spore counts. The initial condition for drug concentration is zero.
The emergence of resistance, which drives this process, occurs only for the 100-mg-equivalent regimen in the continuous-infusion group but occurs in the 50-mg-, 100-mg-, and 150-mg-equivalent regimens in the daily administration group. Also, resistance emerges after day 3 in the continuous-infusion group but earlier, by 2 days, in the once-daily administration group. Thus, spore formation and the emergence of resistance together influence the outcome of therapy, and the mode of administration alters both spore formation rates and the ease of resistance emergence. The modeling process provides insight here and leads to the question of how to achieve optimal (best cell kill, no resistance emergence) moxifloxacin therapy for *Bacillus anthracis*.

Optimal chemotherapy can be achieved with moxifloxacin at relatively low exposures. Exposures equivalent to 200 to 250 mg of moxifloxacin per day, administered either once daily or as a continuous infusion with the same daily AUC, produce near-maximal cell kill and suppress the emergence of resistance. For instance, a 200-mg-equivalent exposure to moxifloxacin  

$$K_{vs}$$  

$$K_{sv}$$  

$$c_{sv}$$  

$$c_{sr}$$  

$$h_s$$  

$$h_r$$  

$$p_{omax}$$  

$$K_v$$  

$$K_r$$  

$$C_{50-s}$$  

$$C_{50-r}$$  

$$C_{g-s}$$  

$$C_{g-r}$$  

$$K_{k-s}$$  

$$K_{k-r}$$  

$$H$$  

$$V_c$$  

$$C_L$$

| Parametera | Unit | Control | 50 mg | 100 mg | 150 mg | 200 mg | 250 mg |
|------------|------|---------|-------|--------|--------|--------|--------|
| $V_c$ (liter) | 188  | 201  | 186  | 190  | 188  |
| $C_L$ (liter/h) | 12.1  | 10.8  | 11.1  | 12.7  | 11.9  |
| $K_{vs}$ (h⁻¹) | 0.0050 | 5.14  | 6.57  | 7.42  | 1.81  | 2.31  |
| $K_{sv}$ (h⁻¹) | 0.0056 | 0.478 | 1.82  | 0.0407 | 0.152 | 0.161 |
| $K_{c_{sv}}$ (h⁻¹) | 6.55  | 6.24  | 7.48  | 6.58  | 4.29  | 4.48  |
| $K_{c_{sr}}$ (h⁻¹) | 7.87  | 20.0  | 13.9  | 19.9  | 20.0  | 16.9  |
| $C_{c_{sv}}$ (h⁻¹) | 1.98  | 0.393 | 0.516 | 0.227 | 0.719 | 0.667 |
| $C_{c_{sr}}$ (h⁻¹) | 4.26  | 0.984 | 0.707 | 2.16  | 1.89  | 2.27  |
| $H_{s}$ (h⁻¹) | 6.17  | 5.84  | 30.0  | 30.0  | 1.00  | 1.00  |
| $H_{r}$ (h⁻¹) | 19.5  | 6.77  | 16.0  | 7.60  | 6.91  | 7.29  |
| POPMAX | 1.53 × 10⁷ | 6.37 × 10⁷ | 4.81 × 10⁸ | 6.50 × 10⁶ | 9.22 × 10⁶ | 8.25 × 10⁶ |
| $K_{c_{50-s}}$ (h⁻¹) | 0.0657 | 0.0657 | 1.38  | 0.0657 | 2.09  | 1.91  |
| $K_{c_{50-r}}$ (h⁻¹) | 4.56  | 2.91  | 25.9  | 2.27  | 1.01  | 1.04  |
| IC(2) CFU/ml | 2.89 × 10⁸ | 1.96 × 10⁷ | 2.80 × 10⁷ | 2.15 × 10⁷ | 5.36 × 10⁷ | 3.60 × 10⁷ |
| IC(3) CFU/ml | 471  | 677  | 71  | 629  | 96  | 45  |
| IC(4) CFU/ml | 1.86 × 10⁶ | 1.86 × 10⁶ | 1.90 × 10⁶ | 1.89 × 10⁶ | 2.42 × 10⁶ | 2.36 × 10⁶ |
| IC(5) CFU/ml | 40  | 41  | 21  | 40  | 24  | 15  |

TABLE 4. Bayesian parameter values for moxifloxacin administered once daily

---

**FIG. 3.** Relationship between increasing moxifloxacin exposure, as indexed to the AUC (mg · h/liter), when moxifloxacin is administered as a continuous infusion (A) or once daily (B) and the change in the parameter ratio $K_v/K_r$ (■). Both parameters were identified by Bayesian estimation.
Moxifloxacin as a continuous infusion generates a 14-day cell kill of 3.23 log_{10} CFU/ml, while a 250-mg-equivalent exposure produces a cell kill of 4.02 log_{10} CFU/ml. These are kills of 0.23 log_{10} and 0.29 log_{10} CFU/ml/day, respectively. These exposures as once-daily administration produce kills of 3.49 log_{10} and 3.98 log_{10} CFU/ml, with daily kills of 0.25 log_{10} and 0.28 log_{10} CFU/ml/day, respectively. With both doses and both administration modes, resistance was suppressed. Consequently, one can say that at relatively low exposures, optimal cell kill and resistance suppression are achievable, irrespective of the mode of administration.

It is important to examine the Bayesian estimates by dose for the rate constants associated with the vegetative-phase-to-spore-phase transition ($K_{vs}$) and with the transition back to vegetative phase ($K_{sv}$). $K_{sv}$ is clearly sensitive to drug pressure, indexed to AUC. In Fig. 3A and B, the ratio of $K_{sv}$ to $K_{vs}$ is plotted as a function of the moxifloxacin AUC. For the continuous-infusion administration group, the $K_{sv}/K_{vs}$ ratio transitions from quite low values to values approximately fivefold higher after a moxifloxacin AUC of approximately 8 mg · h/liter and before an AUC of about 10.6 mg · h/liter. For the once-daily administration group, these values are 13.6 and 15.7 mg · h/liter, indicating that the once-daily administration mode requires a level of drug exposure about 50 to 70% higher than that for continuous infusion to cause the sensing of a threat and faster transition into spore phase. This also implies that the continuous-infusion mode results in more pressure and is more easily sensed. Because of this, the vegetative-phase cells go into spore phase under lower pressure and are able to avoid the threat posed by the drug, while the cells exposed to the once-daily regimen stay in vegetative phase longer and are easier to kill. However, bacteria in the spore phase are metabolically inactive and cannot become resistant (but, as we demonstrated earlier, organisms acquiring resistance in vegetative phase can enter spore phase). The spore-phase organisms eventually reenter vegetative phase and are able to be killed, if the regimen is resistance suppressive.

The two administration modes also have an impact on the emergence of resistance per se. Administration as a continuous infusion shuts off the emergence of resistance in Bacillus anthracis at an AUC0–24 of approximately 9.6 mg · h/liter (Fig. 4A), while the once-daily mode of administration requires a moxifloxacin AUC0–24 of 14.8 mg · h/liter, a value about 50% greater, to accomplish this (Fig. 4B). Therefore, the success or failure of a regimen can be said to rely explicitly on drug exposure, but this is modulated through the presence of a spore form, which can shield organisms, as well as by the mode of drug administration, which affects the vegetative/spore transition and the ease with which resistance amplification is suppressed.

In summary, moxifloxacin is able to kill Bacillus anthracis at relatively low dose-equivalent exposures. The success of the regimens is dependent on the mode of administration. Continuous-infusion administration is more easily sensed as a threat by Bacillus anthracis and, at a lower exposure, causes a transition to the spore phase. Continuous-infusion administration also suppresses resistance more efficiently (the time for which the concentration of the drug is above the MIC is the pharmacodynamically linked variable for resistance suppression). The spore phase makes the identification of optimal regimens considerably more complex. Since adherence to a regimen is related to the frequency of administration, moxifloxacin for therapy of Bacillus anthracis should be administered once daily, but at a dose that maximizes the attainment of an AUC/MIC ratio of 253 (AUC, 15.2 mg · h/liter; MIC, 0.06 mg/liter) for wild-type strains (a standard dose of 400 mg produces this MIC 99.7% of the time in a 9,999-subject Monte Carlo simulation).

Further research into the interaction of drug therapy with the quorum-sensing mechanisms in Bacillus anthracis needs to be undertaken (5) in order to ascertain whether this is the mechanism behind the observed change in the transition rate constants.

ACKNOWLEDGMENTS

This work was supported by P01AI060908, a grant from NIAID to the Emerging Infections and Pharmacodynamics Laboratory. The con-
tent of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health. We have no conflicts to disclose.

REFERENCES
1. Clinical and Laboratory Standards Institute. 1999. Document M26–A. Methods for determining bactericidal activity of antimicrobial agents; approved guidelines. Clinical and Laboratory Standards Institute, Wayne, PA.
2. Clinical and Laboratory Standards Institute. 2006. Document M7–A7. Methods for dilutional antimicrobial susceptibility testing for bacteria that grow aerobically; approved standard, 7th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
3. Deziel, M., H. Heine, A. Louie, M. Kao, W. R. Byrne, J. Bassett, L. Miller, K. Bush, M. Kelley, and G. L. Drusano. 2005. Identification of effective antimicrobial regimens for use in humans for the therapy of Bacillus anthracis infections and post-exposure prophylaxis. Antimicrob. Agents Chemother. 49:5099–5106.
4. Heine, H. S., A. Louie, F. Sorgel, J. Bassett, L. Miller, L. J. Sullivan, M. Kinzig-Schippers, and G. L. Drusano. 2007. Comparison of two different protein synthesis inhibitor antibiotics for the therapy of Yersinia pestis delivered by aerosol challenge in a mouse model of pneumonic plague. J. Infect. Dis. 196:782–787.
5. Jones, M. B., R. Jani, D. Ren, T. K. Wood, and M. J. Blaser. 2005. Inhibition of Bacillus anthracis growth and virulence-gene expression by inhibitors of quorum sensing. J. Infect. Dis. 191:1881–1888.
6. Leary, R., R. Jelliffe, A. Schumitzky, and M. Van Guilder. 2001. An adaptive grid non-parametric approach to pharmacokinetic and dynamic (PK/PD) models, p. 389–394. In Proceedings of the 14th IEEE Symposium on Computer-Based Medical Systems. IEEE Computer Society, Bethesda, MD.
7. Louie, A., M. R. Deziel, W. Liu, and G. L. Drusano. 2007. Impact of resistance selection and mutant growth fitness on the relative efficacies of streptomycin and levofloxacin for plague therapy. Antimicrob. Agents Chemother. 51:2661–2667.
8. Louie, A., H. S. Heine, K. Kim, D. L. Brown, B. VanScoy, W. Liu, M. Kinzig-Schippers, F. Störgel, and G. L. Drusano. 2008. Use of an in vitro model of Bacillus anthracis infection to derive a linezolid regimen that optimizes bacterial kill and prevents emergence of resistance. Antimicrob. Agents Chemother. 52:2386–2396.
9. Sullivan, J. T., M. Woodruff, J. Lettieri, V. Agarwal, G. J. Kroll, P. T. Leese, S. Watson, and A. H. Heller. 1999. Pharmacokinetics of a once-daily oral dose of moxifloxacin (Bay 12-8039), a new enantiomerically pure 8-methoxy quinolone. Antimicrob. Agents Chemother. 43:2793–2797.