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Dose optimization of moxifloxacin and linezolid against tuberculosis using mathematical modeling and simulation

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ABSTRACT

Introduction: There is an urgent need for new anti-tuberculosis (TB) drugs and optimization of current TB treatment. Moxifloxacin and linezolid are valuable options for the treatment of drug-resistant TB; however, it is crucial to find a dose at which these drugs not only show high efficacy but also suppress the development of further drug resistance.

Methods: Activity of moxifloxacin and linezolid against *Mycobacterium tuberculosis* was studied in the hollow-fiber infection model system in log-phase growth under neutral pH and slow growth in an acidic environment. Doses that achieved maximum bacterial kill while suppressing the emergence of drug resistance were determined. Through Monte Carlo simulations the quantitative output of this in vitro study was bridged to the human patient population to inform optimal dosage regimens while accounting for clinical minimum inhibitory concentration (MIC) distributions.

Results and Discussion: Moxifloxacin activity was significantly decreased in an acidified environment. The loss of activity was compensated by accumulation of the drug in TB lung lesions; therefore, moderate efficacy can be expected. Moxifloxacin 800 mg/day is the dose that most likely leads to resistance suppression while exerting maximum bacterial kill. Linezolid demonstrated very good activity even at a reduced pH. Linezolid 900 mg once-daily (QD) is likely to achieve a maximum killing effect and prevent the emergence of drug resistance; 600 mg QD in a robust drug regimen may have similar potential.

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1. Background

Tuberculosis (TB) is the leading infectious disease killer globally, resulting in 1.8 million deaths a year [1]. A third of the world population is currently infected with TB [2]. Global emergence of multidrug-resistant (MDR) TB (resistant to at least isoniazid and rifampicin) makes the TB epidemic an even greater problem as treatment outcomes for MDR-TB patients are substantially worse than those for drug-susceptible TB patients [1,2]. The World Health Organization (WHO) reports approximately half a million new cases of MDR TB per year [2]. These MDR-TB patients require prolonged therapy with second-line drugs that are costly, less effective and often highly toxic, while successful treatment outcome can be expected in only about 50% of these patients [1]. Extensively drug-resistant (XDR) TB is defined as resistance to isoniazid, rifampicin, and at least one fluoroquinolone and an injectable agent. Treatment failure is experienced in at least two-thirds of XDR TB patients [1]. There is an urgent need for new anti-TB drugs and optimization of current TB treatment.

Moxifloxacin and linezolid are key second-line and third-line agents, respectively, and are valuable options for the treatment of MDR TB. Considering the limited amount of potent drug candidates against drug-resistant TB, it is crucial to find a dose at which these drugs not only show high efficacy but also suppress the development of further drug resistance. Toxicity presents a limiting factor; moxifloxacin can cause QT interval prolongation and linezolid is known to cause neurotoxicity and thrombocytopenia in some patients. To find the right dose and to avoid the development of bacterial resistance to these potent drugs requires a better understanding of the relationship between target site

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pharmacokinetics (PK) and pharmacodynamics (PD). There is also a lack of clarity on whether linezolid exhibits a time-dependent or concentration-dependent effect, and whether it is bacteriostatic or bactericidal. Although some consider linezolid to be a time-dependent antibacterial agent, particularly against *Staphylococcus aureus* [3], linezolid has been shown to kill *M. tuberculosis* in an exposure-dependent manner [4,5], with toxicity driven by trough concentrations [5].

The pH measured in TB-diseased lung tissue of patients with progressive drug-resistant TB and severe lung lesions is approximately 5.5 (median, range 5.0-7.2) [6]. pH has a significant influence on the activity of many antibiotics, including moxifloxacin [7–9], and affects mycobacterial growth [10].

Using the hollow-fiber infection model, we investigated the interplay of lung tissue pH as measured in patients with drug-resistant TB [6], clinical target site drug exposure [11], and PD response. Using mechanism-based mathematical models and simulations, we determined the dose required to kill the bacilli during both log-phase growth and slow growth in an acidic environment ('acidic phase') while suppressing emergence of resistance.

2. Methods

2.1. Antimicrobial agents

Moxifloxacin hydrochloride powder (potency 91.7%, LOT: M280) was purchased from Matrix Scientific (Columbia, SC, USA). The drug was dissolved in sterile water to a stock solution of 2 mg/mL. Sterile infusion bags of linezolid (ZYVOX®, 600 mg/300 mL, LOT: 15B06U94) were purchased from Pfizer (Morrisville, NC, USA). Stock solutions were serially diluted to the desired concentrations with Middlebrook 7H9 broth (Becton Dickinson).

2.2. Microorganism

Mycobacterium tuberculosis strain H37Ra (ATCC 25177) was purchased from American Type Culture Collection (Manassas, VA, USA) and stored at -80°C. Bacterial stocks were incubated in 7H9 broth with 10% OADC supplement and 0.2% glycerol (7H9-OADC) at 37°C, 5% CO_2 with shaking conditions for 4 days to achieve exponential growth phase.

2.3. Susceptibility studies and mutation frequencies

The minimum inhibitory concentration (MIC) at neutral pH was performed as described by the Clinical and Laboratory Standards Institute (CLSI) [12]. At acidified pH, MIC was determined as described by Heifets and Sanchez [13]. MIC was defined as the lowest drug concentration that allowed less than 1% growth compared with drug-free controls. Mutation frequencies were determined by plating approximately 5×10^6 colony-forming units (CFU)/mL (200 µL) of mycobacteria on Middlebrook 7H10 agar plates with and without drug supplementation at 3-times the MIC. Fifteen plates for each drug were incubated for 25 days at 37°C in a humidified incubator with 5% CO_2 atmosphere. A total bacterial population of approximately 1.5×10^7 CFU was evaluated for each drug.

2.4. Hollow-fiber infection model

The concept of the hollow-fiber infection model system has been previously described [14,15]. Briefly, mycobacteria in the extra-capillary space of a hollow-fiber cartridge (peripheral compartment) were exposed to dynamically changing drug concentrations over time. Clinically relevant doses and corresponding concentration-time profiles as they had been observed in TB patients were simulated in the hollow-fiber infection model system (based on maximum concentration [C_{max}], time to C_{max} [t_{max}], and an elimination half-life [$t_{1/2}$]) (Table 1) [16,17]. With the use of computer-programmed peristaltic pumps, drug solution was infused over 2 h directly into the central compartment, mimicking a t_{max} of 2 h, as observed in patients taking oral doses of moxifloxacin and linezolid. A pump maintained a constant circulation of fluid between the central and peripheral compartment and enabled drugs to quickly distribute throughout the entire system. The peripheral compartment was separated from the central compartment by semipermeable hollow fibers that cannot be crossed by bacteria. These membranes allow free diffusion of drug, nutrients and bacterial metabolites based on a concentration gradient. Other computer-programmed peristaltic pumps created a gradual dilution of the drug concentration in the system by infusing drug-free fresh 7H9 broth supplemented with 10% ADC and 0.2% glycerol (7H9-ADC) into the system and isovolumetrically withdrawing drug-containing medium from the central compartment, similar to a first-order elimination process.

2.5. Experimental set-up

Mycobacterium tuberculosis was grown to log-phase growth in 7H9-OADC. Bacterial density was determined via optical density (OD) measurement at a wavelength of 600 nm (calibration curve bacterial density [CFU/mL] versus log(OD): $y = 0.9253x + 8.3411$, $R^2 = 0.9796$). 10 mL of the bacterial suspension (1×10^6 CFU/mL, total inoculum: 10^7 CFU) was then inoculated into the peripheral compartment of 14 hollow-fiber cartridges that had been preconditioned with 7H9-ADC for 3 days at 37°C. The bacteria were allowed to adapt to the hollow-fiber system environment for another 3 days to ensure the exponential-growth phase was reached when the first dose was administered at day 0.

The first two arms were left untreated (A and B); the pH of the 7H9-ADC for one growth control (B) was acidified with citric acid (Sigma Aldrich) to a final pH of 5.8. According to experiments conducted by Gumbo et al., a pH of 5.8 still allows bacterial net growth, although at rates lower than those of bacilli in log-phase growth at pH 6.8; no net growth can be expected at a pH as low as 5.5 [10]. Arms C, D and E were treated with simulated moxifloxacin daily doses of 400, 600 and 800 mg (Table 1). To evaluate the drug effect under conditions similar to those observed in TB lung lesions of patients with progressive disease, the same moxifloxacin doses were tested under acidified pH and a previously reported lesion penetration coefficient of 3.2 was taken into account, in that doses were multiplied by this coefficient (arms F, G, H) [11].

For linezolid, dosing regimens of 600 mg q24h, q12h and q8h were simulated under both neutral pH (arms I, J, K) and acidified pH (arms L, M, N) conditions. Linezolid 600 mg q8h is higher than prescribed to humans but was included in this study to delineate where on the dose-response curve the two clinically-prescribed linezolid dosages were located.

Human half-lives with respect to drug exposure were simulated; 7 h for moxifloxacin [16] and 3 h for linezolid [17]. Unbound or free drug concentration-time profiles were simulated in the in-vitro system accounting for 50% and 31% protein binding for moxifloxacin and linezolid, respectively (Table 1) [18,19].

2.6. Pharmacokinetic validation

Serial samples from the central compartment of each infection model were drawn at 2, 4, 7.8, 23.8, 26, 28, 31.8, 47.8, 50, 71.8, 599.8 and 602 h after the start of the first 2-h infusion. Moxifloxacin and linezolid concentrations in these samples were measured using validated liquid chromatography-tandem mass spectrometry (LC-MS-MS) methods as described below. A one-compartment PK model with zero-order input and first-order

Table 1
Time-kill study design moxifloxacin and linezolid.

Arm[a]	Drug	pH[b]	Simulated dosage regimen [mg]	Free fraction	Actual dose infused [mg]	Lesion/serum ratio[c]
C	**moxifloxacin**	6.8	400 q24h	0.50	200 q24h	
D			600 q24h		300 q24h	
E			800 q24h		400 q24h	
F		5.8	400 q24h	0.50	200 q24h	3.2
G			600 q24h		300 q24h	3.2
H			800 q24h		400 q24h	3.2
I	**linezolid**	6.8	600 q24h	0.69	414 q24h	
J			600 q12h		414 q12h	
K			600 q8h		414 q8h	
L		5.8	600 q24h	0.69	414 q24h	1.0[d]
M			600 q12h		414 q12h	1.0[d]
N			600 q8h		414 q8h	1.0[d]

[a] arms A and B were untreated growth controls at neutral and acidified pH, respectively.
[b] acidified pH was measured inside severe lung lesions of multidrug-resistant tuberculosis (MDR-TB) patients[29].
[c] accounting for moxifloxacin accumulation in lung lesions (penetration coefficient: 3.2)[11].
[d] unknown and assumed to be 1.

Table 2
Measured moxifloxacin and linezolid exposures.

Arm[a]	Dose [mg]	fCmax$_{SS}$[b] [mg/L]	fAUC$_{24hr,SS}$[b] [mg*h/L]	fCmin$_{SS}$[b] [mg/L]
C	400	1.35	12.87	0.21
D	600	2.03	19.07	0.17
E	800	2.60	26.81	0.31
F	400[c]	4.02	41.77	0.50
G	600[c]	6.46	65.18	0.73
H	800[c]	8.99	91.43	1.04
I	600 q24h	8.57	33.69	0.003
J	600 q12h	8.74	81.76	0.62
K	600 q8h	9.88	123.63	1.85
L	600 q24h	8.91	41.05	0.02
M	600 q12h	8.10	87.33	0.99
N	600 q8h	10.27	127.87	1.89

[a] moxifloxacin was administered in arms C-H, linezolid was administered in arms I-N; arms A and B were untreated growth controls.
[b] f: free (protein-unbound), SS: at steady state.
[c] due to the accumulation of moxifloxacin in tuberculosis (TB) lesions,[11] doses in arms F, G and H resulted in higher peak and trough concentrations and drug exposures compared with the respective doses in arms C, D and E.

elimination was fitted to the data (see Equation 1 below); the 24-h free area under the concentration-time curve (AUC) at steady-state (fAUC$_{24hr,ss}$) was calculated for each treatment arm using the individual PK parameter estimates. PK measures are shown in Table 2.

2.7. LC-MS-MS assays

The collected PK samples were stored in a -80°C freezer until quantification at the University of Florida (UF) Infectious Disease Pharmacokinetics Laboratory (IDPL). Drug concentrations were measured using validated LC-MS-MS assays on a DIONEX UltiMate 3000 RS pump and a DIONEX UltiMate 3000 RS autosampler (Thermo Scientific), column compartment and diode array detector, a TSQ Endura LC-MS-MS system, a Dell Dimension computer and Xcalibur 2.2 SP1.48 analytical software (Thermo Scientific). The lower limit of quantification was 0.2 µg/mL for moxifloxacin and 0.3 µg/mL for linezolid. The moxifloxacin and linezolid recoveries from 7H9 broth were 99.89% and 100%, respectively. The overall inter-batch precision for quality control samples ranged from 0.72 to 5.64% for moxifloxacin, and from 1.34 to 3.57% for linezolid.

2.8. Microbiological response

The mycobacteria-containing hollow-fiber cartridge of each infection model was sampled at baseline (day 0) and on days 1, 2, 3, 7, 15, 18, 22, 25, and 28 just before the next scheduled drug dose. 10-fold serial dilutions were quantitatively plated onto drug-free 7H10 agar supplemented with 10% OADC and 0.2% glycerol and incubated as described above. To detect and quantify a less susceptible subpopulation, bacterial suspensions were also quantitatively plated onto 7H10 agar plates containing drug at 3-times the MIC. To evaluate the microbiological response to different drug exposures, time-kill curves were obtained by plotting the change in total bacterial density (CFU/mL) over time.

2.9. Pharmacokinetic-pharmacodynamic modeling

Non-parametric adaptive grid program (Big NPAG) [20] was used to simultaneously analyse measured drug concentrations, total bacterial population counts (i.e., susceptible + less susceptible subpopulation) and counts of a less susceptible subpopulation ('resistant population' in Table 3) of all drug regimens. Several PK-PD models were fit to the data. Parameter estimates were calculated by maximal a posteriori probability (MAP) Bayesian techniques. The equations of the final mathematical model are shown below [15]:

$$dX_1/dt = R_0 - (CL/V_c) \bullet X_1 \quad (1)$$

$$dN_S/dt = K_{gmax-S} \bullet N_S \bullet E - K_{kmax-S} \bullet M_S \bullet N_S - K_{nat-S} \bullet N_S \quad (2)$$

$$dN_R/dt = K_{gmax-R} \bullet N_R \bullet E - K_{kmax-R} \bullet M_R \bullet N_R - K_{nat-R} \bullet N_R \quad (3)$$

$$E = 1 - (N_R + N_S)/POPMAX \quad (4)$$

$$M_S = (X_1/V_c)^{H-S}/\left[(X_1/V_c)^{H-S} + EC_{50-S}{}^{H-S}\right] \quad (5)$$

$$M_R = (X_1/V_c)^{H-R}/\left[(X_1/V_c)^{H-R} + EC_{50-S}{}^{H-R}\right] \quad (6)$$

Table 3
Final parameter estimates pharmacokinetic-pharmacodynamic (PK-PD) model.

Parameter	Moxifloxacin log-phase growth		Moxifloxacin acidic phase growth*		Linezolid log-phase growth		Linezolid acidic phase growth	
	Estimate	SD	Estimate	SD	Estimate	SD	Estimate	SD
Vc [L]	140.879	8.784	145.541	10.841	29.834	10.761	31.171	12.604
CL [L/hr]	15.378	0.294	14.604	0.482	9.297	2.788	9.414	0.636
Kgmax-s [log_{10} CFU/mL*h^{-1}]	0.290	0.162	0.405	0.088	0.367	0.258	0.346	0.147
Kkmax-s [log_{10} CFU/mL*h^{-1}]	0.897	0.003	0.561	0.684	2.644	2.166	0.249	0.166
EC_{50}k-s [mg/L]	0.542	0.136	1.709	0.193	4.130	3.127	5.738	2.987
Hk-s	7.909	6.555	2.943	3.281	12.519	5.087	11.025	5.500
Knat-s [log_{10} CFU/mL*h^{-1}]	0 FIX	–	0.353	0.057	0 FIX	–	0.323	0.179
Kgmax-r [log_{10} CFU/mL*h^{-1}]	0.017	0.018	0 FIX	–	0.125	0.171	0 FIX	–
Kkmax-r [log_{10} CFU/mL*h^{-1}]	1.335	0.118	0 FIX	–	1.939	0.493	0 FIX	–
EC_{50}k-r [mg/L]	3.907	2.283	0 FIX	–	6.842	2.498	0 FIX	–
Hk-r	15.025	4.096	0 FIX	–	11.400	5.834	0 FIX	–
Knat-r [log_{10} CFU/mL*h^{-1}]	0 FIX	–	0 FIX	–	0 FIX	–	0 FIX	–
POPMAX [log_{10} CFU/mL]	7.432	7.625	6.001	0.666	7.879	7.604	8.674	8.638
Total population [log_{10} CFU/mL]	4.780	2.398	4.544	0.100	4.628	3.636	4.455	3.757
Resistant population [CFU/mL]	0.907	0.789	0 FIX	–	1.851	1.272	0 FIX	–

Vc – volume of central compartment; CL - clearance; Kgmax – maximum growth rate; Kkmax – maximum kill rates; EC_{50} – drug concentration that produces half maximum bacterial kill; Knat – naturally occurring death rate constant; POPMAX – maximum bacterial density based on the growth control; CFU – colony-forming units; SD – standard deviation

* population medians are reported for moxifloxacin acidic phase growth model; population means are reported for the remaining three models

Where dX_1/dt represents the change in drug amount in the central compartment over time, R_0 represents the infusion rate, and CL and V_c the clearance and volume of the central compartment, respectively. N_S and N_R represent the bacterial density of a susceptible and a less susceptible subpopulation, respectively. K_{gmax} and K_{kmax} are the maximum growth and kill rates, respectively. K_{nat}, the naturally occurring death rate constant, was included to describe slow bacterial growth under acidified pH (suffixes $_{-S}$ and $_{-R}$ represent the susceptible and less susceptible subpopulation). POPMAX in the logistic growth term E is the maximum bacterial density based on the growth control. M_S and M_R represent the killing effect of the drug on the susceptible and less susceptible subpopulation, modeled as a saturable Michaelis-Menten–type kinetic event. H is the Hill coefficient (slope factor) and EC_{50} is the drug concentration that produces half-maximum bacterial kill.

2.10. Simulations and probability of target attainment (PTA)

Simulations were run using the mathematical modeling software package Berkeley Madonna version 8.3.23 (University of California, Berkeley, CA, USA). Final PK-PD model parameter estimates were used to simulate microbiological outcome for varying drug exposures. We identified the exact drug exposure (relative to MIC) required to achieve a specific PD target. The objectives for both moxifloxacin and linezolid treatment were resistance suppression during log-phase growth while maximizing antimicrobial kill of the susceptible bacterial subpopulation [4,5,15], and a 1.0 log_{10} kill relative to baseline after one month of treatment in the acidic milieu (taking into account the slowed growth of mycobacteria in an acidified environment, the long duration of TB treatment of at least 6-9 months and the fact that no resistance was observed in the acidic milieu). Monte Carlo simulations were performed to evaluate how many patients of a virtual clinical trial would achieve the drug exposure breakpoints related with the defined targets at different doses. The simulated clinical trials consisted of 10 000 virtual patient concentration-time profiles per dosing regimen based on literature values for clearance, volume of distribution and absorption rate constants (7.7 L/h, 76.4 L and 0.529 h^{-1}, respectively, for moxifloxacin and 6.0 L/h, 47.0 L and 0.583 h^{-1}, respectively, for linezolid) [16,21], and accounted for inter-patient variability in these PK parameters (30% CV). We calculated the probability of target attainment (PTA) for each dose and at each clinically relevant MIC [22].

3. Results

3.1. Microbiology

At neutral pH the MIC was 0.25 μg/mL for moxifloxacin and 1.0 μg/mL for linezolid. The MIC in acidified pH was 0.5 μg/mL for both drugs. The mutation frequency (MF) for moxifloxacin was 2.02×10^{-7} CFU and for linezolid was $<6.73 \times 10^{-8}$ CFU.

3.2. Time-kill curves

Time-kill curves for moxifloxacin and linezolid are shown in Figs. 1 (a) and (b). While the growth control at neutral pH (open circles, solid line) grew by approximately 2 log_{10} CFU/mL, there was a reduction in bacterial count in the control arm at acidified pH (six-point stars, dashed line).

3.2.1. Moxifloxacin

A clear dose-response was observed for the different moxifloxacin doses at neutral pH (solid lines). A dose of 800 mg q24h caused a rapid kill and did not demonstrate regrowth whereas a completely resistant bacterial population grew back at the lowest dose of 400 mg q24h (Fig. 4). Moxifloxacin 600 mg q24h did not sterilize the system. Under acidified pH, moxifloxacin activity was significantly decreased (dashed and dotted lines). Bacteria did not regrow and resistance was not detected, likely due to the unfavorable pH conditions.

3.2.2. Linezolid

In the least frequent dosage regimen (600 mg q24h) and at neutral pH, linezolid showed bacteriostatic activity (four-point stars, solid line); here, a resistant subpopulation emerged (Fig. 4). Two doses a day eradicated the bacteria within 25 days – an additional third dose per day did not increase the efficacy, which indicates the maximum drug effect had been achieved at 1200 mg per day. An acidified pH did not seem to affect linezolid activity; reduction in log_{10} CFU/mL compared to control appeared to be similar for study arms under neutral pH and acidified pH. A dose of 600 mg once-daily did not eradicate the bacteria. No considerable difference in bacterial response was observed between two and three daily doses of 600 mg. Subpopulations with reduced susceptibilities to linezolid or moxifloxacin did not arise in either of the two control arms.

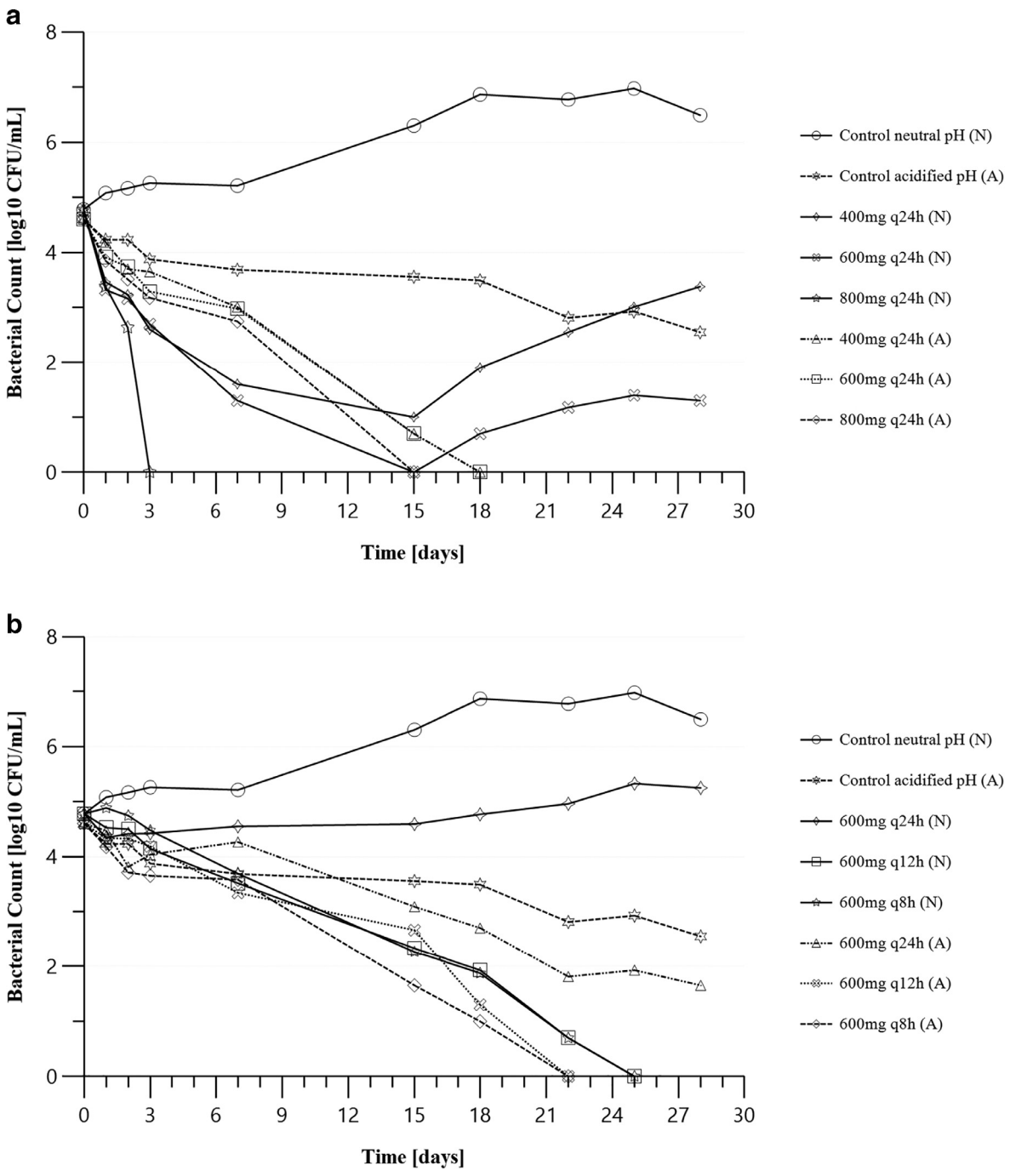

Fig. 1. Time-kill plots moxifloxacin (a) and linezolid (b) on a semi-logarithmic scale.

3.3. PK-PD modeling and simulation

Free drug exposures, including free peak and trough levels, for each treated hollow-fiber study arm are shown in Table 2. Final PK-PD model parameter estimates and model diagnostics are shown in Tables 3 and 4. No resistance emergence was observed for linezolid and moxifloxacin in the acidic phase; therefore, all model parameters related to a resistant subpopulation were fixed to 0 in this particular scenario. The moxifloxacin concentration that produced 50% of the maximum killing effect in the sensitive bacterial subpopulation (EC_{50}k-s) was significantly higher at acidified pH (1.71 mg/L +/- 0.19) compared with at neutral pH (0.54 mg/L +/- 0.14), indicating there is a considerable loss of activity against slowly replicating TB bacilli in the acidic phase (Table 3). Similarly, the moxifloxacin maximum kill rate (K_{kmax}) was higher at neutral pH compared with acidified pH. A slight but non-significant increase in linezolid EC_{50}k-s was observed in acidified medium compared with neutral pH. The 24-h free moxifloxacin AUC at steady-state ($fAUC_{24hr,ss}$) required to suppress resistance and maximize antimicrobial killing during log-phase growth was 24.03 mg*h/L. This resulted in a $fAUC_{24hr,ss}$/MIC ratio of 96.12 (MIC of *M. tuberculosis* strain H37Ra was 0.25 mg/L at neutral pH). At acidified pH the $fAUC_{24hr,ss}$/MIC ratio needed to achieve 1.0 $\log_{10}$ CFU/mL kill relative to baseline after one month of treatment was 132.88 (MIC: 0.5 μg/mL on acidified agar). Linezolid $fAUC_{24hr,ss}$/MIC ratios of 35.56 and 88.80 (neutral and acidic pH, respectively) were

Table 4
Model diagnostics: regression line characteristics of plots of predicted vs. observed values for moxifloxacin and linezolid concentrations, the resultant change in the total bacterial population, and the changes in the resistant subpopulation, as well as bias and precision measures.

Drug	Growth	DV	a	b	R^2	*P*-value	MWE	BAMWSE
Moxifloxacin	Log-phase	Concentrations	0.05	0.97	0.967	<<0.001	−0.126	0.466
		Total bact. Pop.	0.00	0.99	0.967	<<0.001	0.152	1.606
		Resist. Subpop.	−1.50	1.58	0.982	<0.005	−0.076	0.367
	Acidic phase	Concentrations	0.07	0.98	0.987	<<0.001	−0.039	0.179
		Total bact. Pop.	1.14	0.68	0.870	<<0.001	−0.257	11.973
Linezolid	Log-phase	Concentrations	0.15	1.00	0.976	<<0.001	−0.440	1.103
		Total bact. Pop.	0.00	1.01	0.983	<<0.001	−0.192	0.727
		Resist. Subpop.	−0.69	1.36	0.798	0.053	0.051	0.512
	Acidic phase	Concentrations	0.08	1.01	0.973	<<0.001	−0.238	1.129
		Total bact. Pop.	0.00	1.00	0.949	<<0.001	0.002	0.876

DV, dependent variable; a, intercept of the best least squares line YOBS = a + b * YPRED; b, slope of the regression line; MWE, mean weighted error (PRED - OBS); BAMWSE, bias-adjusted mean weighted squared error

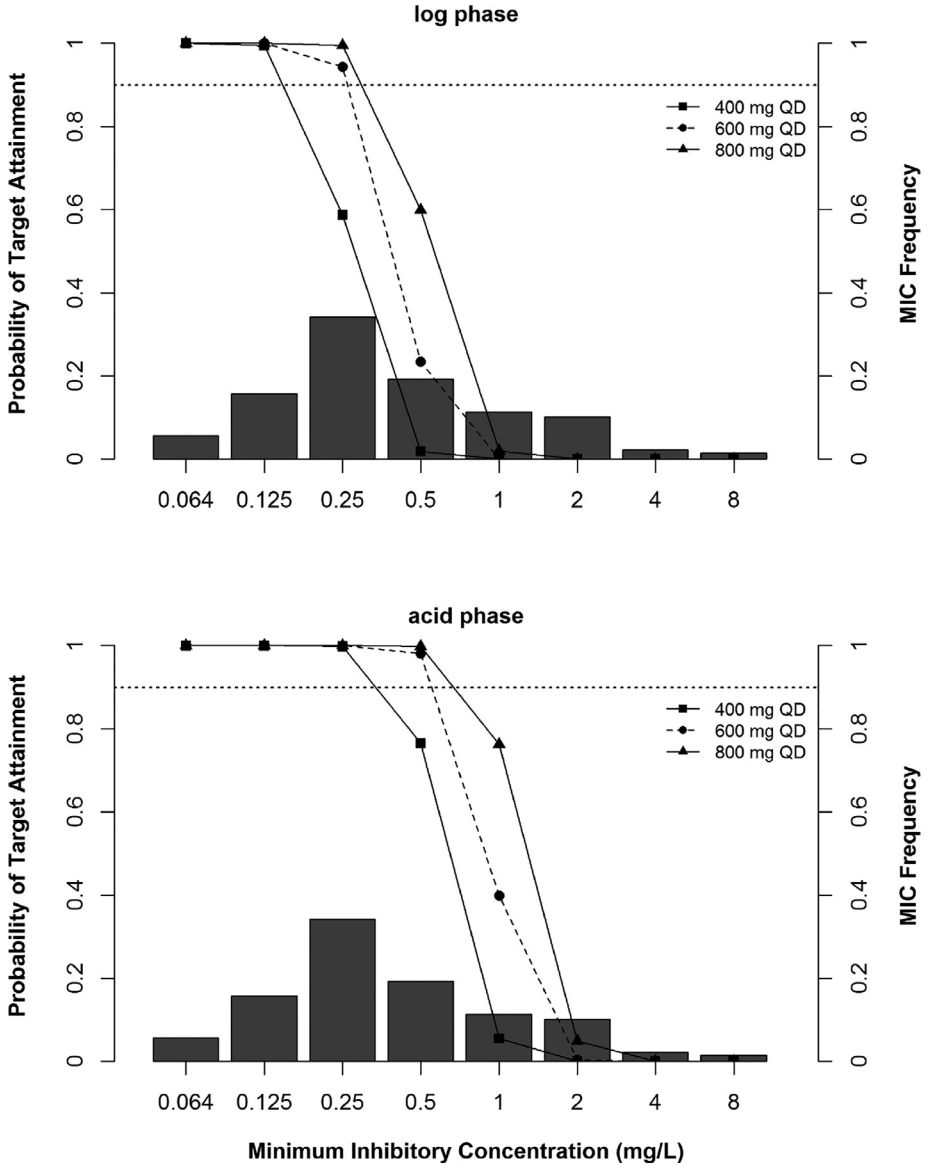

Fig. 2. Probability of target attainment for moxifloxacin doses in log-phase and acidic phase growth, taking into account the accumulation of moxifloxacin in lung lesions. The targets for log-phase growth and acidic phase growth were, respectively, resistance suppression and 1.0 $\log_{10}$ kill relative to baseline. European Committee on Antimicrobial Susceptibility Testing (EUCAST) minimum inhibitory concentration (MIC) distribution included 1467 observations.

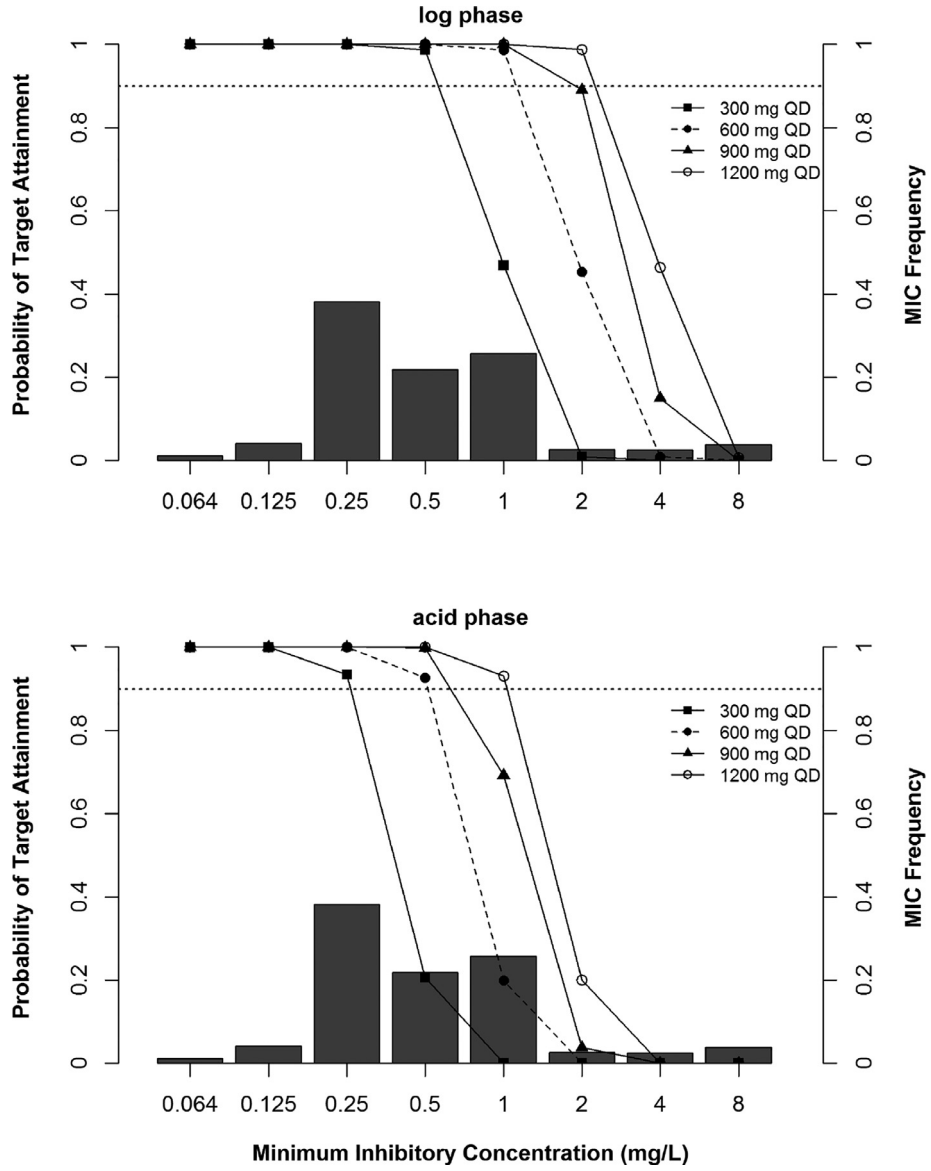

Fig. 3. Probability of target attainment for linezolid doses in log-phase and acidic phase growth. The targets for log-phase growth and acidic phase growth were, respectively, resistance suppression and 1.0 $\log_{10}$ kill relative to baseline. European Committee on Antimicrobial Susceptibility Testing (EUCAST) minimum inhibitory concentration (MIC) distribution included 828 observations.

needed to achieve the same targets, i.e., resistance suppression during log-phase growth and a 1.0 $\log_{10}$ CFU/mL kill relative to baseline in the acidic phase.

In simulated clinical trials, the PTA during bacterial exponential growth phase was 58.7% for patients taking the currently approved moxifloxacin dose of 400 mg QD, versus 94.4% at 600 mg and 99.5% at 800 mg (at an MIC of 0.25 mg/L that represented the mode of a clinical MIC distribution). MIC distributions were reported by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [22]. At MIC of 0.5 mg/L, which is relatively close to the susceptibility breakpoint of 1.0 mg/L [12], resistance suppression occurred in only 1.9% at 400 mg vs. 23.4% at 600 mg and 59.9% at 800 mg. In the acidic phase, 600 mg and 800 mg once-daily performed equally well up to an MIC of 0.5 mg/L; 98.1% vs. 99.8% PTA (Fig. 2).

Another clinical trial was simulated with four linezolid study arms (300, 600, 900 and 1200 mg QD) each consisting of 1000 virtual patients. For log-phase growth, no differences were observed between the dosing arms at the modal value of the MIC distribution (MIC: 0.25 mg/L, PTA: 100% for all doses). 600 mg QD performed well up to an MIC of 1.0 mg/L (98.7% PTA). Clear differences in outcome between various doses were observed for clinical isolates wherein linezolid MIC against these isolates were as high as 2.0 mg/L: 0.9% at 300 mg, 45.3% at 600 mg, 89.1% at 900 mg and 98.8% at 1200 mg QD. In the acidic phase, 600 mg QD performed well up to an MIC of 0.5 mg/L. Further results are shown in Fig. 3.

4. Discussion

We studied the activity of moxifloxacin and linezolid against *M. tuberculosis* in different physiological conditions, in exponential phase growth under neutral pH and acidic phase where slowly growing bacilli are found, and determined the doses that achieved maximum bacterial kill while suppressing the emergence of drug resistance. The hollow-fiber infection model system of TB is a non-clinical drug development tool with predictive accuracy for clinical and microbiological outcomes [23], advanced by the Critical Path to TB Drug Regimens (CPTR) Initiative, and has been endorsed by leading global regulatory authorities [24]. Through Monte Carlo

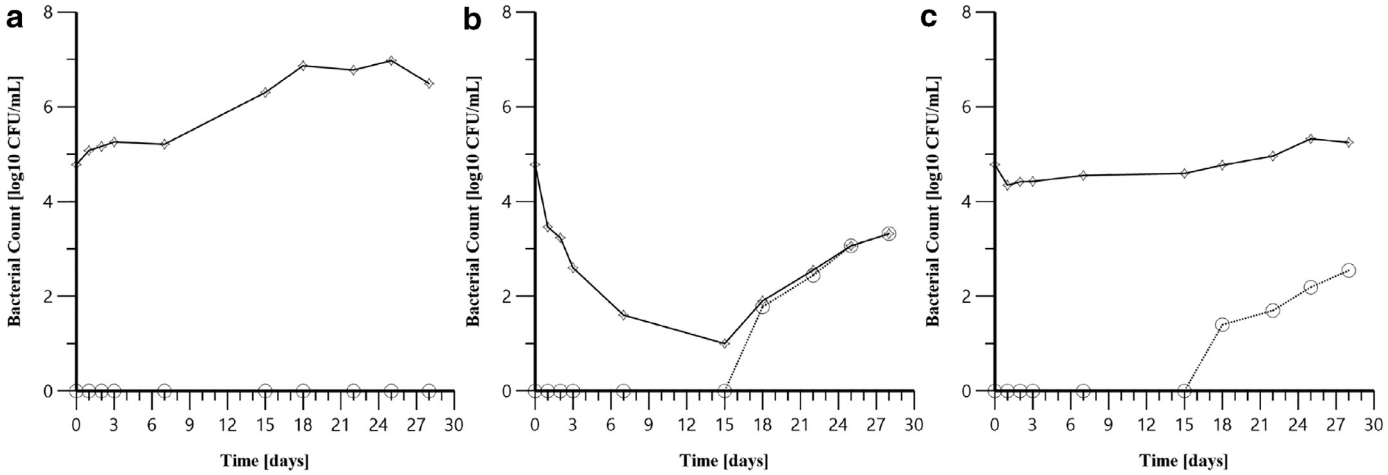

Fig. 4. The responses of the total *Mycobacterium tuberculosis* population (four-point stars, solid lines) and the drug-resistant subpopulation (open circles, dotted lines) over the course of 28 days of moxifloxacin and linezolid exposure ([a] growth control at neutral pH, [b] moxifloxacin 400 mg q24h at neutral pH, [c] linezolid 600 mg q24h at neutral pH). Resistant clones were not detected until day 18. At day 28, the drug-resistant subpopulation represented 100% of the total *Mycobacterium tuberculosis* population after treatment with moxifloxacin 400 mg once-daily (b), and 0.2% after treatment with linezolid 600 mg once-daily (c) (0% in the growth control arm). Resistance was not detected at acidified pH, nor in any of the other treatment arms at neutral pH.

simulations the quantitative output of our in vitro study could be bridged to the human patient population to inform optimal dosage regimens.

Our results indicate that a moxifloxacin dose of 600-800 mg per day would have sufficient efficacy against *M. tuberculosis* in an acidified environment, under the condition that the drug accumulates in TB lung lesions as shown by Heinrichs et al. [11]. To kill *M. tuberculosis* in log-phase growth and to prevent the emergence of drug resistance, a daily dose of 800 mg is likely required. These findings are in agreement with previously published work by Gumbo et al., who recommended a daily moxifloxacin dose of 600-800 mg [15]. Further evaluation of tolerability of such a high dose is needed. Serious side effects of moxifloxacin include severe diarrhea, dizziness, tendonitis that can lead to tendon rupture, joint problems, and a less arrhythmogenic prolongation of the QTc interval [25]. Higher moxifloxacin doses of 600 and 800 mg a day are currently being investigated in a large phase III trial, "the evaluation of a standard treatment regimen of anti-tuberculosis drugs for patients with MDR-TB (STREAM)", ClinicalTrials.gov Identifier: NCT02409290. Although the estimated primary completion date of the trial is April 2021, rates of serious adverse drug effects have been low so far for moxifloxacin.

For isolates with the most frequently observed linezolid MIC of 0.25 mg/L, a linezolid dose of 300 mg QD is predicted to have high target attainment rates during both log-phase growth and in the acidic phase. For less susceptible isolates (MIC of 1.0 mg/L and above), however, 600-1200 mg once a day would be needed to suppress resistance and maximize antimicrobial kill. At high doses, however, there will be some trade-off in terms of adverse drug events, particularly at a dose of 1200 mg QD. Linezolid can decrease platelet count within the first few weeks of treatment. Peripheral neuropathy and bone marrow suppression are serious adverse effects that were observed in some patients during long-term use of linezolid. Therefore, toxicity is a limiting factor for linezolid use in MDR-TB patients who are usually treated for multiple months. A compromise may be an initial daily dose of 600-900 mg allowing for dose reductions to be made when exposure-related side effects are observed. Brown et al. showed that linezolid toxicity is driven by trough levels [5]. As a consequence, we strongly recommend administering the entire daily dose all at once instead of dividing it into multiple daily doses (e.g. 600 mg QD versus 300 mg BID), because this would result in increased trough levels and thereby in a higher risk of toxicity. As resistance suppression is also achieved through combination therapy, a daily dose of 600 mg of linezolid might be sufficient if implemented in a robust drug regimen. This hypothesis can be tested in clinical trials. Our results conform with the outcome of another linezolid hollow-fiber study conducted by Brown et al., who showed that with linezolid monotherapy, a dose higher than 600 mg is likely needed to prevent drug resistance [5].

Our findings also stress the need for improved and more cost-efficient TB diagnostics. The only information usually available on a clinical isolate is whether or not it is susceptible to a certain drug based on susceptibility breakpoints. Information on the individual MIC of a patient isolate would be very useful as doses could be adjusted accordingly. This, of course, would lead to a significant increase in TB treatment costs.

Our study has certain limitations. The absence of an immune system in the hollow-fiber infection model system may have led to an underestimation of microbial kill inside the human body. On the other hand, the activity against non-replicating persistent bacilli was not addressed, and this may actually result in higher drug exposure breakpoints that require higher doses. For safety reasons, we used the attenuated *M. tuberculosis* strain H37Ra in this study. Similarity to the virulent strain H37Rv with respect to drug susceptibility and log-phase growth has been shown previously [26–28]. As hollow-fiber study experiments are prohibitively expensive, the 14 arms of this large hollow-fiber study were not done in duplicate, which led to somewhat higher standard deviations on few parameter estimates. We chose to conduct one relatively large study investigating more different conditions (e.g. acidified pH vs. neutral pH for two drugs) rather than replicating all arms of a smaller experiment (only one drug or one pH level). Yet, one could argue that when having multiple exposures and an exposure response is established, this is exactly replication; more exposure produces more effect. Furthermore, in the clinic, resistance suppression can be achieved through combination therapy, although there is room for improvement considering the increasing numbers of drug-resistant cases. In our study, we aimed to determine the dose that maximizes activity of moxifloxacin and linezolid. This information is pivotal when designing and testing new combination therapy regimens against MDR TB in clinical trials or in future in vitro experiments.

In summary, we have shown that moxifloxacin activity significantly decreased in an acidified environment as measured inside severe lung lesions of MDR-TB patients [6]. The loss of activity,

however, is to some extent compensated by the accumulation of the drug in TB lung lesions, therefore, moderate efficacy can be expected. Moxifloxacin 800 mg/day is the dose that most likely leads to resistance suppression during log-phase growth while exerting maximum bacterial kill.

Linezolid was shown to have very good activity against *M. tuberculosis* even at a decreased pH. It is therefore a vital option for kill of bacilli in the acidic phase, particularly if the isolate is also resistant to pyrazinamide. Linezolid 900 mg QD is very likely to achieve a maximum killing effect and prevent the emergence of drug resistance. Linezolid 600 mg QD in a robust drug regimen may have similar potential.

Declarations

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Competing interests

No author has a commercial or other association that might pose a conflict of interest.

Ethical approval

Not required.

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