Are moxifloxacin and levofloxacin equally effective to treat XDR tuberculosis?

Thomas Maitre, Grégoire Petitjean, Aurélie Chauffour, Christine Bernard, Najoua El Helali, Vincent Jarlier, Florence Reibel, Pascal Chavanet, Alexandra Aubry, Nicolas Veziris

Are moxifloxacin and levofloxacin equally effective to treat XDR tuberculosis?

Running tittle: Levofloxacin or moxifloxacin for XDR tuberculosis?

Thomas MAITRE[1,2], Grégoire PETITJEAN[3,4], Aurélie CHAUFFOUR[1,2], Christine BERNARD[1,2], Najoua EL HELALI[3], Vincent JARLIER[1,2], Florence REIBEL[1,2], Pascal CHAVANET[5,6], Alexandra AUBRY[1,2], and Nicolas VEZIRIS*[1,2]

[1] Sorbonne Université, UPMC Univ. Paris 06, CR7, Centre d'Immunologie et des Maladies Infectieuses, Team 13, INSERM U1135, Paris, France

[2] AP-HP, Hôpital Pitié-Salpêtrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, Bactériologie-Hygiène, Paris, France

[3] Groupe Hospitalier Paris Saint-Joseph, Unité de Microbiologie Clinique et Dosage des Anti-infectieux, Paris, France

[4] Université Paris Sud UFR Pharmacie, Laboratoire de Pharmacie Clinique, Chatenay Malabry, France

[5] Service de Maladies Infectieuses et Tropicales, Centre Hospitalier Universitaire, Dijon, France

[6] Université de Bourgogne, UMR1347, Dijon, France

Main text words: 3 438

*Corresponding author: Nicolas VEZIRIS (nicolas.veziris@upmc.fr)

SYNOPSIS

Background: Moxifloxacin retains partial activity against some fluoroquinolone-resistant mutants of *Mycobacterium tuberculosis*. Levofloxacin is presumed to be as active as moxifloxacin against drug-susceptible tuberculosis and to have a better safety profile.

Objectives: To compare the *in vivo* activity of levofloxacin and moxifloxacin against *M. tuberculosis* strains with various levels of fluoroquinolone resistance.

Methods: BALB/c mice were intravenously infected with 10^6 *M. tuberculosis* H37Rv and three isogenic mutants, GyrA A90V, GyrB E540A and GyrB A543V. Treatment with 50 or 100 mg/kg levofloxacin and 60 or 66 mg/kg moxifloxacin was given orally every 6 hours, for 4 weeks.

Results: Levofloxacin 50 and 100 mg/kg/6h and moxifloxacin 60 and 66 mg/kg/6h generated AUCs in mice equivalent to those of levofloxacin 750 and 1000 mg/day and moxifloxacin 400 and 800 mg/day, respectively, in humans. Moxifloxacin 60 and 66 mg/kg/6h had bactericidal activity against strain H37Rv (MIC ≤0.25 mg/L) and mutants GyrB E540A and GyrB A543V (MIC =0.5 mg/L). Against mutant GyrA A90V (MIC =2 mg/L), moxifloxacin 60 mg/kg/6h did not prevent bacillary growth whereas 66 mg/kg/6h had bacteriostatic activity. Levofloxacin 50 mg/kg/6h had bactericidal activity against H37Rv (MIC ≤0.25 mg/L) but not against the mutant strains. Levofloxacin 100 mg/kg/6h had bactericidal activity against H37Rv and mutants GyrB E540A (MIC = 0.5 mg/L) and GyrB A543V (MIC = 1 mg/L) but not against mutant GyrA A90V (MIC = 4 mg/L).

Conclusion: All mutations reduced fluoroquinolone activity, even those classified as susceptible according to phenotypic tests. High-dose levofloxacin is less effective than high-dose moxifloxacin against both fluoroquinolone-resistant and -susceptible *M. tuberculosis*

strains in mice.

INTRODUCTION

Misuse of antibiotics has led to the appearance of multidrug-resistant tuberculosis (MDR TB), defined as resistant to at least isoniazid and rifampin.[1] Since fluoroquinolones (FQs) and aminoglycosides have been used largely to treat these MDR TB cases, additionally FQ- and aminoglycoside-resistant MDR TB strains were selected, leading to extensively drug-resistant tuberculosis (XDR TB) strains. The World Health Organization (WHO) reported approximately 480 000 new cases of MDR TB in 2014, including 10% of XDR TB.[2] Prognosis of MDR and XDR TB is poor since the death rate increases from 10%, of drug-susceptible tuberculosis, to at least 17-24% and 23-61% of MDR and XDR TB, respectively.[3,4] FQ resistance appears to be the main factor explaining the poor prognosis of XDR TB.[5] The main mechanism of FQ resistance in *M. tuberculosis* relies on DNA gyrase mutations, which entail variable levels of resistance. We have shown in previous work in mice, that a human equivalent dose of 400 mg/day of moxifloxacin retains partial activity against FQ-resistant *M. tuberculosis* mutants.[6] We subsequently showed that this benefit was maintained against low-level FQ-resistant strains when moxifloxacin was used in combination with second-line drugs.[7] This concept of using an FQ despite *in vitro* resistance was validated in a clinical study in which gatifloxacin, as part of the 9-month Bangladesh regimen, was as active against low-level resistant strains as against FQ-susceptible strains.[8] Levofloxacin, another FQ with antituberculous activity, has shown higher early bactericidal activity (EBA) at 1000 mg/day than moxifloxacin and gatifloxacin at 400 mg/day.[9] When included in an anti-MDR TB regimen, levofloxacin at 750 mg/day has proven to be equivalent to moxifloxacin at 400 mg/day, with a 3-month sputum culture conversion rate taken as criterion of effectiveness.[10] More importantly, levofloxacin has a better safety profile than moxifloxacin and gatifloxacin.[9] In particular, levofloxacin prolongs the QT interval less than moxifloxacin, which makes it the preferred FQ for combination with drugs that do prolong the QT interval,

i.e., new anti-TB drugs such as bedaquiline or delamanid, or also clofazimine which is part of the short MDR TB treatment recently approved by the World Health Organization (WHO).[11] Therefore, WHO recommendations proposed levofloxacin as the preferred FQ to be included in an anti-MDR TB regimen. However, levofloxacin activity has never been evaluated against FQ-resistant strains.

Our objective was to compare the *in vivo* activities of levofloxacin and moxifloxacin against wild-type (WT) *M. tuberculosis* and strains harboring DNA gyrase mutations responsible for various levels of FQ resistance, using a murine model of infection.

MATERIALS AND METHODS

Antimicrobial agents

Solutions were prepared from tablets of moxifloxacin (400 mg; Bayer®) and levofloxacin (500 mg; Arrow Génériques®, Lyon, France). Tablets were crushed in a mortar and dissolved in sterile water at the desired concentration to provide a gavage solution. Levofloxacin and moxifloxacin powders (Sigma-Aldrich, France) were used to determine minimum inhibitory concentrations (MIC).

M. tuberculosis strains

Four *M. tuberculosis* strains were used, the WT reference strain H37Rv and three isogenic mutant strains harboring DNA gyrase substitutions: A90V in GyrA and two GyrB mutants selected *in vivo*. The latter two harbor the E540A and A543V mutations according to the numbering system used more frequently in the literature, or E501A and A503V according to the recently proposed consensus numbering system for GyrB.[12] *M. tuberculosis* strains were isolated from mice lungs and grown on Lowenstein-Jensen medium.[13] The mutation present in each strain was checked by sequencing the *gyrA* and *gyrB* QRDRs as previously described.[6]

Determination of minimum inhibitory concentrations

The MICs of ofloxacin, moxifloxacin, gatifloxacin, levofloxacin and enoxacin were determined using 7H11 agar supplemented with 10% OADC. MIC was defined as the lowest concentration that inhibited >99% of bacterial growth.

Murine model of tuberculosis

The study was approved by the Charles Darwin Research Ethics Committee (approval number 4568 2016031411142463 v3).

Five-week-old inbred BALB/c mice were purchased from the Janvier Breeding center (Le Genest Saint-Isle, France). Mice were inoculated in the tail vein with a 0.5-mL bacterial suspension that contained 5.7–6.0 $\log_{10}$ cfu of each *M. tuberculosis* strain. A first experiment aimed at comparing the virulence of *M. tuberculosis* H37Rv WT and mutant strains harboring substitutions in DNA gyrase. We inoculated three mice for each strain and monitored them during one month. A second experiment aimed at comparing the residual efficacies of moxifloxacin and levofloxacin against the FQ-resistant mutants and against the WT strain. We conducted this experiment twice, with 160 mice inoculated in each case and with two sets of FQ doses, *i.e.,* first with levofloxacin at 50 mg/kg/6h and moxifloxacin at 66 mg/kg/6h and second with levofloxacin at 100 mg/kg/6h and moxifloxacin at 60 mg/kg/6h. Mice were treated for 5 days per week during 4 weeks.

For each experiment and each *M. tuberculosis* strain (H37Rv WT and mutant strains GyrA A90V, GyrB E540A and GyrB A543V), 40 mice were inoculated: ten for determining the initial bacillary load in lungs (D0), ten for survival analysis and ten for assessing the treatment efficacy of moxifloxacin and levofloxacin. The moxifloxacin doses used aimed at mimicking the human equivalent 400 and 800 mg/day doses; the levofloxacin doses used aimed at mimicking the 750 and 1000 mg/day doses.[9,14–18]

Since the optimal $AUC_{0\text{-}24h}$/MIC ratio of levofloxacin against *M. tuberculosis* was unknown, we added a dose-ranging evaluation against H37Rv during the second experiment. We treated six H37Rv-infected mice with 25 mg/kg of levofloxacin and six with 35 mg/kg, given by oral gavage every 6 hours, 5 days per week during 4 weeks.

Assessment of efficacy

Treatment efficacy was measured in terms of survival rates and lung cfu counts. Ten mice from each treatment group were sacrificed one day after infection (D0). Surviving mice were sacrificed at the completion of treatment. The bacillary load was compared between end and start of treatment. When there was a statistically significant decrease, the activity was considered bactericidal. When the bacillary load was not statistically different from that at the start of treatment, the activity was considered bacteriostatic.

Levofloxacin and moxifloxacin dose-effect model

Nonlinear regression analysis using a sigmoid E_{max} effect model[19] was done based on bacterial concentrations in the lung after 4 weeks of treatment. Dose-effect sigmoid curves were drawn using the following Hill equation: bactericidal effect = E_{max} / [1 + 10 power [(logEC_{50}- x) x N]] where EC_{50} is the 50% effective exposure and N is the Hill coefficient. FQ exposure was expressed in C_{max}/MIC and $AUC_{0\text{-}24h}$/MIC ratios.

Statistical analysis

We compared cfu counts using the non-parametric Wilcoxon test and we evaluated survival data using the log-rank test. Statistical calculations were done using the website BiostaTGV (http://www.u707.jussieu.fr/biostatgv/). In the dose-effect model, levofloxacin and moxifloxacin effects were compared using the non-parametric Wilcoxon and Spearman tests. Calculations were done using SigmaPlot® software. Differences were considered statistically significant when p was <0.05.

Pharmacokinetic analysis in mice

We measured the pharmacokinetic parameters of each moxifloxacin and levofloxacin dose after the first dose was administered. Once the drug was administered orally, we anaesthetized the mice using halogenic gas (isoflurane). We collected blood by performing cardiac puncture in three mice for each time point. Blood was drawn 0, 10, 20, 30, 90, 240 and 360 minutes after gavage. The total fractions of levofloxacin and moxifloxacin were measured using a microbiological assay with *Escherichia coli* as assay organism.[20] We determined the maximum serum concentration (C_{max}) and area under the concentration (AUC) time curve in a compartmental and a non-compartmental model using Phoenix® software. Drug accumulation was assessed by determining the residual plasma concentrations in three mice after 7, 14 and 21 days of treatment.

RESULTS

Characteristics of FQ-resistant strains of *M. tuberculosis*

Levofloxacin and moxifloxacin MICs were, respectively, ≤0.25 and ≤0.25 mg/L for the WT strain H37Rv, 0.5 and 0.5 mg/L for the GyrB E540A mutant, 1 and 0.5 mg/L for the GyrB A543V mutant and 4 and 2 mg/L for the GyrA A90V mutant (Table 1).

Pharmacokinetic analysis in mice

Since the AUC/MIC ratio is believed to be the pharmacodynamic driver of FQ activity against *M. tuberculosis,*[21] 50 and 100 mg/kg/6h of levofloxacin in mice were considered to be equivalent to, respectively, 750 and 1000 mg/day in humans (Tables 2 and 3). For moxifloxacin, 60 and 66 mg/kg/6h in mice were considered to be equivalent to, respectively, 400 and 800 mg/day in humans. Weekly monitoring of residual moxifloxacin and levofloxacin concentrations did not show any accumulation of these drugs.

Virulence of FQ-resistant *M. tuberculosis* strains

Mice were infected with inocula ranging from 5.7 to 6.0 $\log_{10}$ cfu. Mortality of untreated H37Rv-infected mice was not different from that of mice infected with mutant strains ($p>0.22$) in the two experiments (data not shown).

Comparison of levofloxacin and moxifloxacin activities against FQ-resistant *M. tuberculosis*

Survival analysis

A 50 mg/kg/6h levofloxacin dose prevented mortality in GyrB E540A-infected mice ($p=0.02$) and delayed mortality in GyrB A543V-infected mice ($p=0.02$) but not in GyrA A90V- and

H37Rv-infected mice (Figure 1, supplementary data). A 100 mg/kg/6h levofloxacin dose prevented mortality in H37Rv-, GyrB E540A- and GyrB A543V-infected mice ($p<10^{-5}$) but not in GyrA A90V-infected mice (p=0.07) (Figure 2, supplementary data). A 60 mg/kg/6h moxifloxacin dose prevented mortality in H37Rv-, GyrB E540A-, GyrB A543V- and GyrA A90V-infected mice (p<0.0001) (Figure 3, supplementary data). A 66 mg/kg/6h moxifloxacin dose prevented mortality in GyrB E540A-, GyrB A543V- and GyrA A90V-infected mice (p=0.01, p=0.001 and p=0.01, respectively) (Figure 4, supplementary data). A 66 mg/kg/6h moxifloxacin dose was more effective than a 50 mg/kg/6h levofloxacin dose in GyrB A543V- and GyrA A90V-infected mice (p=0.004 and p=0.002, respectively) but not in GyrB E540A and H37Rv-infected mice. A 60 mg/kg/6h moxifloxacin dose was more effective than a 100 mg/kg/6h levofloxacin dose in GyrA A90V-infected mice (p=0.0001) but not in GyrB A543V-, GyrB E540A- and H37Rv-infected mice.

Lung cfu counts

Figure 1 shows lung cfu variations between the day before and 4 weeks after treatment initiation, depending on FQ dose and *M. tuberculosis* strain. Compared to D0, a 50 mg/kg/6h levofloxacin dose reduced lung cfu counts by 2.4 $\log_{10}$ cfu in H37Rv-infected mice (p=0.02), whereas the counts increased in GyrB E540A-, GyrB A543V- and GyrA A90V-infected mice (p=0.003, p=0.001 and p=0.0003, respectively). Compared to D0, a 100 mg/kg/6h levofloxacin dose reduced lung cfu counts by 3.0 $\log_{10}$ cfu in H37Rv-infected mice (p=0.0002), by 1.1 $\log_{10}$ cfu in GyrB E540A-infected mice (p=0.0002) and by 0.9 $\log_{10}$ cfu in GyrB A543V-infected mice (p=0.0003), whereas the counts increased in GyrA A90V-infected mice (p=0.001).

Compared to D0, a 60 mg/kg/6h moxifloxacin dose reduced lung cfu counts by 3.7 $\log_{10}$ in H37Rv-infected mice (p=0.0002), by 2.3 $\log_{10}$ cfu in GyrB E540A-infected mice (p=0.0002)

and by 2.7 $\log_{10}$ cfu in GyrB A543V-infected mice (p=0.0002), whereas the counts tended to increase in GyrA A90V-infected mice (p=0.07). Compared to D0, a 66 mg/kg/6h moxifloxacin dose reduced lung cfu counts by 3.4 $\log_{10}$ in H37Rv-infected mice (p=0.001), by 2.1 $\log_{10}$ cfu in GyrB E540A-infected mice (p=0.0003) and by 1.6 $\log_{10}$ cfu in GyrB A543V-infected mice (p=0.00002) but not in GyrA A90V-infected mice in which the cfu counts remained unchanged.

Mice infected with GyrB E540A, GyrB A543V and GyrA A90V and treated with 66 mg/kg/6h of moxifloxacin had final lung cfu counts lower than those treated with 50 mg/kg/6h of levofloxacin (p<0.0004). Mice infected with each *M. tuberculosis* strain and treated with 60 mg/kg/6h of moxifloxacin had final lung cfu counts lower than those treated with 100 mg/kg/6h of levofloxacin (p<0.01).

Bactericidal activities of levofloxacin and moxifloxacin against *M. tuberculosis* in a sigmoid-E_{max} effect model

Nonlinear regression analyses showed a good fit for moxifloxacin in the $AUC_{0\text{-}24h}$/MIC exposure model (r^2=0.95) and in the C_{max}/MIC exposure model (r^2=0.86) (Figure 2). For levofloxacin, nonlinear regression analyses showed a lesser fit in the $AUC_{0\text{-}24h}$/MIC exposure model (r^2=0.71) and in the C_{max}/MIC exposure model (r^2=0.79) (Figure 3). As shown, the levofloxacin dose-effect model exhibited an E_{max} of 5 $\log_{10}$ lung cfu reduction after 4 weeks with an EC_{50} $AUC_{0\text{-}24h}$/MIC ratio of 110 and a C_{max}/MIC ratio of 30. The moxifloxacin dose-effect model exhibited an E_{max} of 6.5 $\log_{10}$ lung cfu reduction after 4 weeks with an EC_{50} $AUC_{0\text{-}24h}$/MIC ratio of 90 and a C_{max}/MIC ratio of 10. The observed and calculated bactericidal effects of levofloxacin and moxifloxacin were statistically different in both the $AUC_{0\text{-}24h}$/MIC and the C_{max}/MIC exposure models (p<0.04).

DISCUSSION

Low-level resistance has been observed *in vitro* with many antibiotics for many years. In some cases, this low-level resistance allows successful use of antibiotics *in vivo,* as has been demonstrated with the tazobactam/piperacillin combination against extended-spectrum betalactamase-producing Enterobacteriacea.[22] Regarding antituberculous drugs, there have been descriptions of various levels of resistance against many antibiotics including the major compounds such as rifampin, isoniazid or FQs.[8,13]. Knowing the strong impact of FQ resistance on the prognosis of MDR TB,[5] and despite the risk of increasing FQ resistance by creating second-step mutants,[23] the use of FQs has been suggested in cases of low-level resistance.[11,24]

Data from clinical studies, including those on gatifloxacin or moxifloxacin, support the use of these drugs in cases of low-level resistance. In particular, it has been shown that a high-dose gatifloxacin-containing regimen (800 mg/d) is as active against gatifloxacin-susceptible strains as against strains with low-level gatifloxacin resistance (MIC<2 mg/L).[8] In a murine model of tuberculosis we demonstrated that moxifloxacin retained bactericidal activity against strains with low-level resistance (GyrB D500N; MIC, 0.5 mg/L) and displayed bacteriostatic activity in cases of intermediate-level resistance (GyrA A90V; MIC, 2 mg/L) but was not active in cases of high-level resistance (GyrA D94G; MIC, 4 mg/L).[6] We subsequently demonstrated that a similar gradual decrease in activity was also measurable when moxifloxacin was included in a very active second-line regimen containing pyrazinamide, ethionamide and amikacin.[7]

In the present work we wished to extend our previous studies to another FQ, levofloxacin, and to other low-level resistant mutants (GyrB E540A and GyrB A543V). Accumulating data on these low-level resistant strains is important since the choice to include a drug, although active *in vitro*, should be made with caution because of its possibly greater toxicity.

Moreover, with the increase in genotypic diagnoses of resistance, therapeutic choices will be made increasingly on the basis of the genotype only or they will be based initially on the genotype and subsequently adapted to the phenotype.[25] The murine model offers a unique opportunity to compare the activity of human equivalent doses of FQs against isogenic mutants of the WT reference strain H37Rv.

The present study contributes information on the use of FQs in cases of FQ-resistant tuberculosis. One important finding was that, against strains with the GyrA A90V mutation, which is the second most frequently encountered[13] and which entails intermediate-level resistance with MICs of moxifloxacin and levofloxacin of 2 and 4 mg/L, respectively, both FQs had, at most, bacteriostatic activity even when mimicking high human doses, *i.e.*, 800 mg/day of moxifloxacin and 1000 mg/day of levofloxacin. These results are concordant with the outcomes of patients infected with a strain harboring the GyrA A90V substitution that were unfavorable in three of five cases.[24] Thus, the benefit of adding an FQ to a regimen used against a strain harboring the GyrA A90V substitution is probably limited and, moreover, may increase FQ resistance by creating second-step mutants. [6,23]

Regarding the two GyrB mutants with low-level resistance (moxifloxacin and levofloxacin MICs = 0.5 and 1 mg/L), the efficacy of the drugs was variably dose-dependent. While moxifloxacin was bactericidal independent of the dose, levofloxacin did not prevent bacterial growth at 50 mg/kg/6h (the human equivalent of 750 mg/day) but displayed bactericidal activity at 100 mg/kg/6h (the human equivalent of 1000 mg/day). Interestingly, for both GyrB mutants against which moxifloxacin and levofloxacin have reduced activity at conventional dosing, the respective MICs fall in the susceptible range according to WHO criteria.[26] Thus, if only a phenotypic diagnosis of resistance is made without genotypic analysis, low-level resistance in strains such as these may be overlooked.

Taken together, these results suggest that the greatest benefit would be obtained with the use of moxifloxacin at 800 mg/day against strains with moxifloxacin MICs < 2mg/L. The possibility of using FQs despite *in vitro* resistance depends on the relative abundance of different DNA gyrase mutants in a given population and on their phenotypic susceptibility. The genotypic or phenotypic methods used to measure FQ susceptibility vary in the literature. We believe that the percentage of cases in which moxifloxacin could be active, *i.e.,* cases caused by strains for which the MIC is < 2 mg/l, ranges between 30% and 90% of ofloxacin-resistant MDR cases, depending on the methods used and on geographical variations.[13,24,27,28] Another limitation of the use of moxifloxacin against FQ-resistant strains is the inter-patient pharmacokinetic variability.[16,29]

Considering our results, levofloxacin appears to be a much less attractive option for the treatment of FQ-resistant strains. Indeed, based on our model, this FQ at the human equivalent dose of 750 mg/day should not have any activity, whereas at 1000 mg/day it would have activity against mutants of *M. tuberculosis* with levofloxacin MICs ≤1 mg/L. In particular, no activity is expected against the frequent GyrA A90V mutants. A higher dose of levofloxacin has been used recently (20 mg/kg) and may be an interesting option.[9,30]

A second important finding of this study concerns the difference in activity of moxifloxacin and levofloxacin against both FQ-resistant and FQ-susceptible strains. Moxifloxacin and levofloxacin have different pharmacokinetic profiles and *in vitro* activities. Moxifloxacin is more active than levofloxacin *in vitro* since its MICs for *M. tuberculosis* are usually one dilution lower.[31,32] Conversely, levofloxacin has a better pharmacokinetic profile in humans, with both AUC and C_{max} values 2- to 3-times higher than those determined for moxifloxacin.[14,18,33] Since the pharmacokinetic advantage of levofloxacin outweighed the MIC-related disadvantage, we expected that levofloxacin would have been more active than

moxifloxacin. Surprisingly, the opposite was observed, with moxifloxacin being more active than levofloxacin against both FQ-resistant and FQ-susceptible strains.

In order to compare the two drugs, we correlated the respective $AUC_{0\text{-}24h}$/MIC and C_{max}/MIC ratios to the cfu decrease (Table 3). These ratios abolish the pharmacokinetic and MIC differences, merging them into a unique indicator of activity. Our moxifloxacin dose-effect model exhibited an E_{max} of 6.5 $\log_{10}$ lung cfu reduction and an EC_{50} $AUC_{0\text{-}24h}$/MIC ratio of 90 after 4 weeks of treatment. These data are in accordance with the $AUC_{0\text{-}24h}$/MIC target ratio of at least 100 suggested by Shandil *et al.*[21] The levofloxacin dose-effect model exhibited a lower E_{max} (5 $\log_{10}$ lung cfu reduction) with a higher EC_{50} ($AUC_{0\text{-}24h}$/MIC ratio of 110). Thus, for the same exposure (*i.e,.* $AUC_{0\text{-}24h}$/MIC and C_{max}/MIC ratios) moxifloxacin was more bactericidal than levofloxacin.

This difference in activity between the two drugs could be explained in part by a difference in the effects of the anti-DNA gyrase activities of these two FQs. The FQ target is DNA gyrase, a ubiquitous enzyme that introduces negative supercoils into DNA and contributes to maintaining bacterial chromosome superstructure and integrity. Structural data revealed only few differences in levofloxacin– and moxifloxacin–enzyme interactions that involved the formation of a water/magnesium-ion bridge network between the enzyme and the C3/C4 keto acid of the FQs. On the other hand, cleaved-complex stability correlates well with *in vivo* efficacy and is likely to be related to the killing of *M. tuberculosis* cells. Thus, the better activity of moxifloxacin compared to levofloxacin could be correlated to its ability to promote the formation of more stable gyrase-DNA-FQ complexes.[3435]

Thus, these results suggest that moxifloxacin should be the preferred FQ in an MDR TB regimen against both FQ-susceptible and FQ-resistant strains. At first view, these results seem to contradict those of a clinical study that has shown the equivalence at 3 months of two regimens containing either moxifloxacin, 400 mg/day, or levofloxacin, 750 mg/day, for the

342 treatment of MDR TB.[10] However, previous studies in murine models of tuberculosis have
343 shown that, despite differences at late time points (≥6 months) there is no difference at early
344 time points (2 or 3 months) between levofloxacin- or moxifloxacin-containing regimens.[36,37]
345 Thus, the superior activity of moxifloxacin shown here may also be seen in humans if the
346 analysis were done at later time points.
347 This study had several limitations. First, FQ activities were assessed in mice during a 4-week
348 monotherapy regimen. The better activity of moxifloxacin against FQ-resistant strains should
349 be confirmed by further studies that assess it in a multidrug regimen for the treatment of XDR
350 TB with various levels of phenotypic FQ resistance. Second, we noted early mortality in
351 H37Rv-infected mice during the first study. This mortality was observed during the ten first
352 days among mice treated with levofloxacin at 50mg/kg/6h or moxifloxacin at 66mg/kg/6h. It
353 was induced by the intensive oral gavage regimen. These mice were included in an intent-to-
354 treat survival analysis in order not to underestimate the mortality of treated mice. These mice
355 were excluded from lung cfu count analysis in order not to overestimate cfu counts in lungs of
356 treated mice. Also BALB/c mice develop a TB disease that is characterized by larger
357 intracellular bacillary populations than those seen in humans.[38,39] This difference may favor
358 moxifloxacin over levofloxacin because of better intracellular penetration.[40, 41] Finally, it
359 should be recalled here that there are other drugs with the potential of circumventing common
360 quinolone-resistance mutations, *e.g.*, quinazolinediones (diones). [42]

361 In conclusion, the human equivalent high dose of moxifloxacin (800 mg/day) exhibited
362 greater bactericidal activity in mice than that of levofloxacin (1000 mg/day) against
363 susceptible or low-level FQ-resistant *M. tuberculosis* strains. In the absence of potential
364 toxicity limiting its use, moxifloxacin should be preferred to levofloxacin for the treatment of
365 MDR and XDR tuberculosis with low-level fluoroquinolone resistance.

366

FUNDING

This work was supported by a grant from Fondation pour la Recherche en Santé Respiratoire.

TRANSPARENCY DECLARATIONS

None to declare

REFERENCES

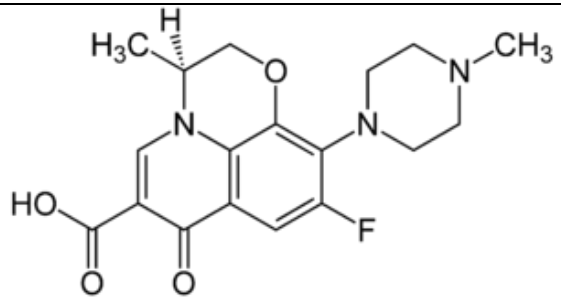
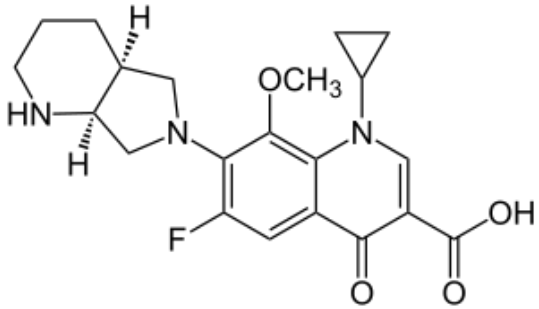
1. Mahmoudi A, Iseman MD. Pitfalls in the care of patients with tuberculosis. Common errors and their association with the acquisition of drug resistance. *JAMA* 1993; **270**: 65–8.
2. World Health Organization. *Global tuberculosis report 2015*. [S.l.]: World Health Organization; 2015.
3. Shah N, Pratt R, Armstrong L, Robison V, Castro KG, Cegielski J. EXtensively drug-resistant tuberculosis in the united states, 1993-2007. *JAMA* 2008; **300**: 2153–60.
4. Schnippel K, Shearer K, Evans D, Berhanu R, Dlamini S 'celo, Ndjeka N. Predictors of mortality and treatment success during treatment for rifampicin-resistant tuberculosis within the South African National TB Programme, 2009 to 2011: a cohort analysis of the national case register. *Int J Infect Dis* 2015; **39**: 89–94.
5. Kim DH, Kim HJ, Park S-K, *et al.* Treatment Outcomes and Survival Based on Drug Resistance Patterns in Multidrug-resistant Tuberculosis. *Am J Respir Crit Care Med* 2010; **182**: 113–9.
6. Poissy J, Aubry A, Fernandez C, *et al.* Should Moxifloxacin Be Used for the Treatment of Extensively Drug-Resistant Tuberculosis? An Answer from a Murine Model. *Antimicrobial Agents and Chemotherapy* 2010; **54**: 4765–71.
7. Fillion A, Aubry A, Brossier F, Chauffour A, Jarlier V, Veziris N. Impact of Fluoroquinolone Resistance on Bactericidal and Sterilizing Activity of a Moxifloxacin-Containing Regimen in Murine Tuberculosis. *Antimicrobial Agents and Chemotherapy* 2013; **57**: 4496–500.
8. Aung KJM, Van Deun A, Declercq E, *et al.* Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. *The International Journal of Tuberculosis and Lung Disease* 2014; **18**: 1180–7.
9. Johnson JL, Hadad DJ, Boom WH, *et al.* Early and extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2006; **10**: 605–12.
10. Koh W-J, Lee SH, Kang YA, *et al.* Comparison of Levofloxacin versus Moxifloxacin for Multidrug-Resistant Tuberculosis. *Am J Respir Crit Care Med* 2013; **188**: 858–64.
11. The World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis (2016 update). 216AD.
12. Maruri F, Sterling TR, Kaiga AW, *et al.* A systematic review of gyrase mutations associated with fluoroquinolone-resistant Mycobacterium tuberculosis and a proposed gyrase numbering system. *J Antimicrob Chemother* 2012; **67**: 819–31.
13. Bernard C, Aubry A, Chauffour A, Brossier F, Robert J, Veziris N. In vivo Mycobacterium tuberculosis fluoroquinolone resistance emergence: a complex phenomenon poorly detected by current diagnostic tests. *J Antimicrob Chemother* 2016; **71**: 3465–72.

14. Peloquin CA, Hadad DJ, Molino LPD, *et al.* Population Pharmacokinetics of Levofloxacin, Gatifloxacin, and Moxifloxacin in Adults with Pulmonary Tuberculosis. *Antimicrobial Agents and Chemotherapy* 2008; **52**: 852–7.
15. Démolis JL, Kubitza D, Tennezé L, Funck-Brentano C. Effect of a single oral dose of moxifloxacin (400 mg and 800 mg) on ventricular repolarization in healthy subjects. *Clin Pharmacol Ther* 2000; **68**: 658–66.
16. Nijland HMJ, Ruslami R, Suroto AJ, *et al.* Rifampicin reduces plasma concentrations of moxifloxacin in patients with tuberculosis. *Clin Infect Dis* 2007; **45**: 1001–7.
17. Nicolle L, Duckworth H, Sitar D, Bryski L, Harding G, Zhanel G. Pharmacokinetics/pharmacodynamics of levofloxacin 750mg once daily in young women with acute uncomplicated pyelonephritis. *International Journal of Antimicrobial Agents* 2008; **31**: 287–9.
18. Piscitelli SC, Spooner K, Baird B, *et al.* Pharmacokinetics and safety of high-dose and extended-interval regimens of levofloxacin in human immunodeficiency virus-infected patients. *Antimicrobial agents and chemotherapy* 1999; **43**: 2323–2327.
19. Drusano GL. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. *Nat Rev Microbiol* 2004; **2**: 289–300.
20. Courvalin P, Leclercq R, Rice LB, Kitzis, M. D. *Antibiogram (edition 3), Antibiotic dosings.* Portland, Or.; [Washington, DC: Eska publishing ; ASM Press; 2010.
21. Shandil RK, Jayaram R, Kaur P, *et al.* Moxifloxacin, Ofloxacin, Sparfloxacin, and Ciprofloxacin against Mycobacterium tuberculosis: Evaluation of In Vitro and Pharmacodynamic Indices That Best Predict In Vivo Efficacy. *Antimicrob Agents Chemother* 2007; **51**: 576–82.
22. Retamar P, López-Cerero L, Muniain MA, Pascual Á, Rodríguez-Baño J, Group the E-R. Impact of the MIC of Piperacillin-Tazobactam on the Outcome of Patients with Bacteremia Due to Extended-Spectrum-β-Lactamase-Producing Escherichia coli. *Antimicrob Agents Chemother* 2013; **57**: 3402–4.
23. Drlica K, Zhao X. Mutant selection window hypothesis updated. *Clin Infect Dis* 2007; **44**: 681–8.
24. Chien J-Y, Chien S-T, Chiu W-Y, Yu C-J, Hsueh P-R. Moxifloxacin Improves Treatment Outcomes in Patients with Ofloxacin-Resistant Multidrug-Resistant Tuberculosis. *Antimicrobial Agents and Chemotherapy* 2016; **60**: 4708–16.
25. Farhat MR, Sultana R, Iartchouk O, *et al.* Genetic Determinants of Drug Resistance in Mycobacterium tuberculosis and Their Diagnostic Value. *Am J Respir Crit Care Med* 2016. Available at: http://www.atsjournals.org.gate2.inist.fr/doi/abs/10.1164/rccm.201510-2091OC. Accessed July 11, 2016.
26. Anon. *Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*. Geneva: World Health Organization; 2014. Available at: http://www.ncbi.nlm.nih.gov/books/NBK247420/. Accessed September 21, 2016.
27. Rigouts L, Coeck N, Gumusboga M, *et al.* Specific gyrA gene mutations predict poor treatment outcome in MDR-TB. *J Antimicrob Chemother* 2016; **71**: 314–23.
28. Bernard C, Veziris N, Brossier F, *et al.* Molecular diagnosis of fluoroquinolone resistance in Mycobacterium tuberculosis. *Antimicrob Agents Chemother* 2015; **59**: 1519–24.
29. Ruslami R, Ganiem AR, Dian S, *et al.* Intensified regimen containing rifampicin and moxifloxacin for tuberculous meningitis: an open-label, randomised controlled phase 2 trial. *The Lancet infectious diseases* 2013; **13**: 27–35.
30. Heemskerk AD, Bang ND, Mai NTH, *et al.* Intensified Antituberculosis Therapy in Adults with Tuberculous Meningitis. *N Engl J Med* 2016; **374**: 124–34.
31. Nosova EY, Bukatina AA, Isaeva YD, Makarova MV, Galkina KY, Moroz AM. Analysis of mutations in the gyrA and gyrB genes and their association with the resistance of

Mycobacterium tuberculosis to levofloxacin, moxifloxacin and gatifloxacin. *Journal of Medical Microbiology* 2013; **62**: 108–13.
32. Rodríguez JC, Ruiz M, Climent A, Royo G. In vitro activity of four fluoroquinolones against Mycobacterium tuberculosis. *International Journal of Antimicrobial Agents* 2001; **17**: 229–31.
33. Stein GE, Schooley SL, Nicolau DP. Urinary bactericidal activity of single doses (250, 500, 750 and 1000mg) of levofloxacin against fluoroquinolone-resistant strains of Escherichia coli. *International Journal of Antimicrobial Agents* 2008; **32**: 320–5.
34. Aubry A, Pan X-S, Fisher LM, Jarlier V, Cambau E. Mycobacterium tuberculosis DNA Gyrase: Interaction with Quinolones and Correlation with Antimycobacterial Drug Activity. *Antimicrob Agents Chemother* 2004; **48**: 1281–8.
35. Blower TR, Williamson BH, Kerns RJ, Berger JM. Crystal structure and stability of gyrase–fluoroquinolone cleaved complexes from *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences* 2016; **113**: 1706–13.
36. Ahmad Z, Tyagi S, Minkowski A, Peloquin CA, Grosset JH, Nuermberger EL. Contribution of Moxifloxacin or Levofloxacin in Second-Line Regimens with or without Continuation of Pyrazinamide in Murine Tuberculosis. *Am J Respir Crit Care Med* 2013; **188**: 97–102.
37. Veziris N, Truffot-Pernot C, Aubry A, Jarlier V, Lounis N. Fluoroquinolone-Containing Third-Line Regimen against Mycobacterium tuberculosis In Vivo. *Antimicrob Agents Chemother* 2003; **47**: 3117–22.
38. Driver ER, Ryan GJ, Hoff DR, *et al.* Evaluation of a mouse model of necrotic granuloma formation using C3HeB/FeJ mice for testing of drugs against Mycobacterium tuberculosis. *Antimicrob Agents Chemother* 2012; **56**: 3181–95.
39. Grosset J. Mycobacterium tuberculosis in the extracellular compartment: an underestimated adversary. *Antimicrob Agents Chemother* 2003; **47**: 833–6.
40. Garraffo R, Lavrut T, Durant J, *et al.* In vivo comparative pharmacokinetics and pharmacodynamics of moxifloxacin and levofloxacin in human neutrophils. *Clin Drug Investig* 2005; **25**: 643–50.
41. Michot J-M, Seral C, Van Bambeke F, Mingeot-Leclercq M-P, Tulkens PM. Influence of efflux transporters on the accumulation and efflux of four quinolones (ciprofloxacin, levofloxacin, garenoxacin, and moxifloxacin) in J774 macrophages. *Antimicrob Agents Chemother* 2005; **49**: 2429–37.
42. Drlica K, Mustaev A, Towle TR, Luan G, Kerns RJ, Berger JM. Bypassing fluoroquinolone resistance with quinazolinediones: studies of drug-gyrase-DNA complexes having implications for drug design. *ACS Chem Biol* 2014; **9**: 2895–904.

M. tuberculosis strain	MIC (mg/L)			
	Enoxacin	Ofloxacin	Levofloxacin	Moxifloxacin
H37Rv (WT)	8	≤0.5	≤0.25	≤0.25
Gyr B E540A	16	1	0.5	0.5
Gyr B A543V	16	2	1	0.5
Gyr A A90V	>32	8	4	2

Table 1: MICs of FQ for H37Rv WT and GyrA A90V, GyrB E540A and GyrB A543V mutant strains of *M. tuberculosis*

Drug	Structure	Mice			Humans*		
		Dose (mg/kg/6h)	C_{max} (mg/L)	$AUC_{0\text{-}24h}$ ** (mg.h/l)	Dose (mg/kg/day)	C_{max} (mg/L)	$AUC_{0\text{-}24h}$ (mg.h/l)
Levofloxacin		50	15	68	750	7-12	63-93
		100	18	148	1000	12-16	129-137
Moxifloxacin		60	6	56	400	3-6	48-58
		66	8	70	800	6-7	60-87

AUC: area under the concentration time curve; C_{max}: maximum concentration;

* human pharmacokinetic data were obtained from [9,14,17,18,33] for levofloxacin and [14,16,29] for moxifloxacin.

** the $AUC_{0\text{-}24h}$ was obtained by multiplying by 4 the AUC measured after 1 dose .

Table 2: Pharmacokinetic parameters for levofloxacin and moxifloxacin in mice after a single dose by gavage compared to those in humans

503

Strain	Levofloxacin					Moxifloxacin				
	MIC (mg/L)	50 mg/kg/6h		100 mg/kg/6h		MIC (mg/L)	60 mg/kg/6h		66 mg/kg/6h	
		C_{max}/MIC	AUC $_{0\text{-}24h}$ /MIC*	C_{max}/MIC	AUC $_{0\text{-}24h}$ /MIC*		C_{max}/MIC	AUC $_{0\text{-}24h}$ /MIC*	C_{max}/MIC	AUC $_{0\text{-}24h}$ /MIC*
H37Rv (WT)	≤0.25	60	272	72	592	≤0.25	24	224	44	280
GyrB E540A	0,5	30	136	36	296	0,5	12	112	22	140
GyrB A543V	1	15	68	18	148	0,5	12	112	22	140
GyrA A90V	4	3.75	17	4.5	37	2	3	28	5.5	35

AUC $_{0\text{-}24h}$: area under the concentration time curve during 24h; MIC: minimum inhibitory concentration; C_{max}: maximum concentration

* AUC $_{0\text{-}24h}$ was obtained by multiplying by 4 the AUC measured after 1 dose.

Table 3: AUC/MIC and C_{max}/MIC ratios of levofloxacin and moxifloxacin for H37Rv, GyrA A90V, GyrB E540A and GyrB A543V mutant strains of *M. tuberculosis*

510

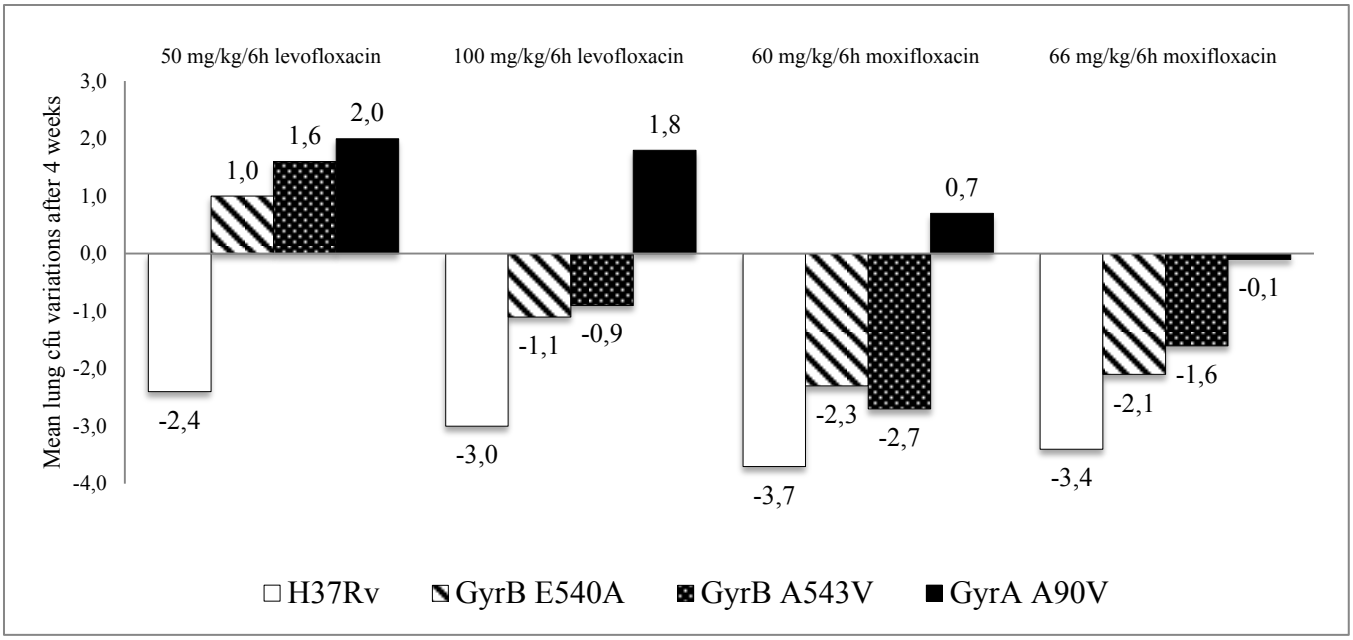


Figure 1: Lung cfu count variations in the interval between D0 (before) and 4 weeks after treatment initiation according to FQ dose and *M. tuberculosis* strain

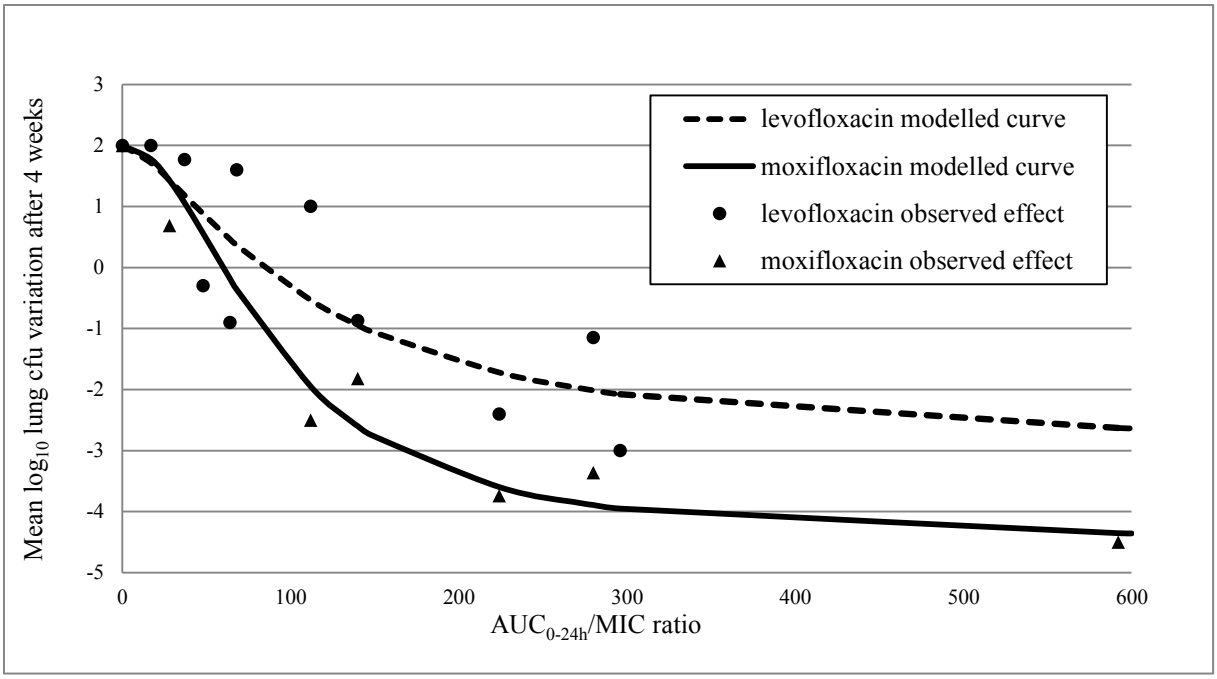


Figure 2: Effects after a 4-week treatment of increasing $AUC_{0\text{-}24h}$/MIC ratios on levofloxacin and moxifloxacin *in vivo* activities against *M. tuberculosis* strains in a sigmoid-E_{max} effect model.

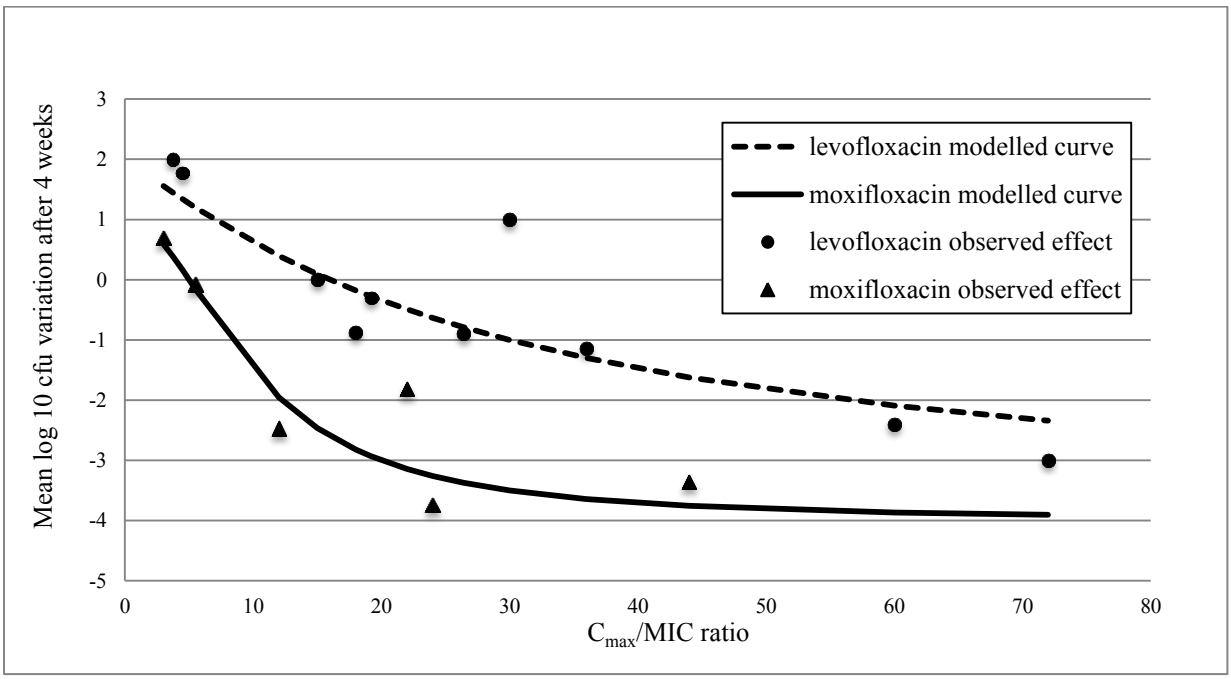


Figure 3: Effects after a 4-week treatment of increasing C_{max}/MIC ratios on levofloxacin and moxifloxacin *in vivo* activities against *M. tuberculosis* strains in a sigmoid-E_{max} effect model