Moxifloxacin replacement in contemporary tuberculosis drug regimens is ineffective against persistent *Mycobacterium tuberculosis*: Novel insights from the Cornell mouse model

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Running title: Moxifloxacin regimens do not kill culture filtrate-dependent *Mycobacterium tuberculosis*

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Abstract

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* remains a leading killer worldwide, and disease control is hampered by ineffective control of persistent infections. Substitution of moxifloxacin for isoniazid or ethambutol in standard TB regimens reduces treatment duration and relapse rates in animal studies and four-month regimens were not non-inferior in clinical trials. Resuscitation promoting factor (RPF) dependent bacilli have recently been implicated in *M. tuberculosis* persistence. We aimed to investigate the therapeutic effects of moxifloxacin substitution in the standard drug regimen for eradicating colony forming count (CFU) positive and RPF-dependent persistent *M. tuberculosis* using the Cornell murine model. *M. tuberculosis* infected mice were treated with regimens in which either isoniazid or ethambutol were replaced by moxifloxacin to the standard regimen. The efficacy of the regimens was compared to the standard regimen for bacterial CFU count elimination and removal of persistent tubercle bacilli evaluated using culture filtrate (CF) derived from *M. tuberculosis* strain H37Rv. We also measured disease relapse rates. Moxifloxacin-isoniazid substituted regimen achieved total organ CFU count clearance at 11 weeks post-treatment, faster than standard regimen (14 weeks), and with a 34% lower relapse rate. Moxifloxacin-ethambutol substituted regimen was similar to standard regimens in these regards. Importantly, neither moxifloxacin-substituted regimens nor the standard regimen could remove CF-dependent persistent bacilli. Evaluation of CF-dependent persistent *M. tuberculosis* requires confirmation in human studies, and has implications in future drug design, testing and clinical applications.

Key words: *Mycobacterium tuberculosis*, moxifloxacin, Resuscitation promoting factors, Cornell mouse model

INTRODUCTION

TB caused by *Mycobacterium tuberculosis* remains a leading cause of mortality worldwide (1). Current combination antimicrobial regimens require a prolonged 6-month treatment period. This long regimen leads to poor patient compliance which gives rise to the emergence of drug resistance and high relapse rates (2). Substitution of moxifloxacin (an 8-methoxy fluoroquinolone) for drugs in the contemporary anti-TB regimen has shown promise for improving treatment efficacy (3, 4). *In vivo*, replacement of isoniazid with moxifloxacin led to shortened treatment duration (5, 6), reduced relapse rates (6, 7) and favourable outcomes in BALB/c and granuloma-forming C3HeB/FeJ mice (8). In the recent REMox-TB trial, shorter (four-month) moxifloxacin-replacement regimens (either for isoniazid or ethambutol) in human clinical trials failed to achieve non-inferiority compared to standard regimens (3, 4), mainly due to higher relapse rates (3, 4, 9). Persistent bacteria that are tolerant to drug therapy may be implicated in the higher disease relapse (9).

M. tuberculosis persistence is the single most important hurdle hampering effective TB disease control (10). *M. tuberculosis* has the ability to survive in a dormant, non-multiplying and persistent state (11-14). These persistent bacteria do not grow on solid or liquid media and are undetectable using the conventional diagnostic methods, however can be resuscitated using resuscitation promoting factors (RPF) which are present in *M. tuberculosis* culture supernatant (15). Recently, we found that using culture filtrate (CF) containing RPF (15), could induce persistent bacteria to recommence multiplication, rendering them detectable once more in mice (16-18). Moreover, these CF-resuscitated tubercle bacilli could be completely eliminated using high-dose rifampicin regimens, shortening treatment duration with no disease relapses (16, 18). In this study, we used the Cornell mouse model (19, 20) to investigate the therapeutic impact of moxifloxacin replacement in standard TB regimen against both CFU count positive and CF-dependent bacteria. We compared the regimens in which either isoniazid or ethambutol were replaced by moxifloxacin to the standard regimen by measurements of the elimination rates of

CFU counts, the presence of CF-dependent *M. tuberculosis* in mouse organs and disease relapse rates.

MATERIALS AND METHODS

Bacterium and growth conditions. *M. tuberculosis* strain H37Rv was mouse-passaged and grown in 7H9 medium containing 0.05% Tween 80 and supplemented with 10% albumin dextrose complex (ADC; Becton and Dickinson, UK) at 37°C without disturbance for 15 days. The culture was stored at -70°C for subsequent animal infection. To determine the viable counts prior to infection, colony forming unit (CFU) counts were performed prior to freezing and once again after thawing. The CFU counting was carried out by plating serial 10-fold dilutions of the cultures on 7H11 agar medium supplemented with oleic albumin dextrose complex (OADC, Becton and Dickinson, UK). Colonies were counted after incubation of the plates at 37°C for 3 to 4 weeks. Viability was expressed as Log CFU/ml. The cultures were subsequently diluted in PBS and used for inoculations in mice.

Cornell mouse model. Moxifloxacin (M) substitution for either isoniazid (H) or ethambutol (E) in the standard TB drug regimen with rifampicin (R) and pyrazinamide (Z) was tested using the Cornell mouse model (19, 20). The model was conducted using the experimental design and procedure described previously (17).

Female BALB/c mice (6 to 8 weeks old, Harlan UK Ltd) were infected intravenously via the tail vein with 1.2×10^5 CFU of mouse-passaged *M. tuberculosis* strain H37Rv per mouse as described previously (16, 17, 20). The animal husbandry guidelines and all animal experiments were performed according to the Animals Scientific Procedures Act, 1986 (an Act of the Parliament of the United Kingdom 1986 c. 14) (Home Office Project licence Number 70/7077) with approval from St George's, University of London ethics committee.

As shown in Table 1, control group consisted of 8 infected and untreated mice. The treatment groups each contained 54 mice which were treated orally (0.2 ml) with RHZE, RHZM and RMZE regimens, respectively, 5 days per week for 14 weeks. The dosages for the drugs were R 10 mg/kg, H 25 mg/kg, Z 150 mg/kg, E 100 mg/kg and M 100 mg/kg. Rifampicin was administered 1 hour before the other drugs to avoid drug to drug interactions.

For assessment of treatment efficacy, a sample of 4 mice was sacrificed at 2, 4, and 6 weeks and 8 mice were sacrificed at 8, 11 and 14 weeks of treatment (Table 1). Mouse lungs and spleens were transferred into 2 ml tubes each containing 1 ml sterile distilled water and 2 mm diameter glass beads, followed by homogenizing using a reciprocal shaker (Thermo Hybaid Ltd) for 40 seconds at 6.5 speed. The CFU counts from each lung and spleen were performed using serial dilutions of the homogenates to plate on 7H11 agar plates.

At 11 and 14 weeks of treatment, the entire organ homogenates from the 8 mice were aliquoted equally into three tubes which were used 1. The CFU counting by plating out the organ homogenate suspension on 6 selective 7H11 agar plates. 2. culturing in 5 ml of selective Kirchner liquid medium (21) by the addition of polymyxin B 200 U/ml, carbenicillin 100 mg/l, trimethoprim 20 mg/l and amphotericin B 10 mg/l (Selectatab, Mast Diagnostica GmbH) for 4 weeks with subsequent sub-culturing of the entire culture onto Löwenstein-Jensen slopes for a further 4 weeks and 3. resuscitation of persistent bacteria by culture filtrate. Kirchner liquid medium was used to isolate different species of mycobacteria from human specimens. Mitchison *et al* (21) showed that liquid Kirchner medium, made selective by the addition of the antimicrobials, was more effective in the isolation of mycobacteria than other media tested. Culture negative organs were defined as no colonies grown on 7H11 agar plates and no growth in selective Kirchner liquid medium following inoculation on Löwenstein-Jensen slopes.

Immediately after termination of 14 weeks of chemotherapy, the remaining mice were administered 0.5 mg/mouse of hydrocortisone acetate by daily oral administration for 8 weeks to suppress host immunity, followed by CFU counting from lungs and spleens to determine disease relapse.

Resuscitation of *M. tuberculosis* in mouse lungs and spleens. For resuscitation of *M. tuberculosis* grown in mouse organs, culture filtrates containing RPFs were used as described previously (15-17).

M. tuberculosis H37Rv was grown in 7H9 medium without disturbance at 37°C for 15 to 20 days until an optical density of 1 to 1.5 was reached. The culture supernatants were collected by centrifugation at 3000 g for 15 minutes and sterilized by double filtration with 0.2 µm filters (Sartorius). The sterilized culture filtrates were made selective by the addition of polymyxin B 200 U/ml, carbenicillin 100 mg/l, trimethoprim 20 mg/l and amphotericin B 10 mg/l (Selectatab, Mast Diagnostica GmbH) and immediately used for broth dilution to count the most probable number (MPN) of the bacilli (22).

Broth counting of lungs and spleens was performed as serial 10-fold dilutions in which 0.5 ml of tissue homogenates were added to 4.5 ml of the culture filtrates. At 10-day intervals over a 2-month period of incubation at 37°C, the broth cultures were examined for visible turbidity changes. Growth of *M. tuberculosis* in turbid tubes was confirmed by colonial morphology on 7H11 agar plates. The MPN of viable bacilli was then estimated from the patterns of positive and negative tubes accosting to the method of US Food and Drug Administration (22). The absence of microorganisms other than mycobacteria from turbid tubes was confirmed by plating on blood agar medium (Oxoid) and Sabouraud dextrose agar (Oxoid). In order to assess the sterility of culture filtrates free of *M. tuberculosis*, tubes containing culture filtrates were incubated at 37°C for 2 months to ensure the absence of *M. tuberculosis* in the culture filtrates.

Statistical analysis. A simple model for mono-exponential bacterial growth and elimination (17, 23) was fitted to the profiles of CFU vs. time obtained experimentally. As simultaneously occurring exponential replication and death rates cannot be differentiated with this type of data, a "knet" exponential rate constant was estimated separately before treatment began ("knet_no_drug" where it would take a net positive value) and during treatment ("knet_with_drug" where it would take a net negative value). During therapy, knet is a 1^{st} order elimination rate constant which can be interpreted as the slope of the modelled line fit through the logarithmic-transform of the data (with units in these data of wk^{-1}). Parameter estimation

was carried out with nonlinear regression using the nonlinear least squares optimisation function “lsqnonlin” as part of the “pracma” package in the R statistical software language, with an objective function weighted by 1/(predicted value)^2. Standard errors of parameter estimates were calculated using the method descripted previously (24) with the Jacobian of model parameter sensitivities estimated using a numerical central difference method. The datasets comprised from multiple individual subject animals were treated as a naïve pool for data analysis purposes (25) rather than using the average of the data at each time-point. The significance of differences between model parameter estimates under different therapies was examined with pairwise Z-tests incorporating a Bonferroni correction of 3, where P values <0.017 would be considered significant. The significance of differences between the relapse rates was determined with pairwise Fisher’s exact tests also with a Bonferroni correction of 3, with P values <0.017 considered significant.

RESULTS

Treatment with moxifloxacin containing regimens in the Cornell mouse model. In the Cornell model, after three weeks of infection, mean CFU counts in the organs reached log 7.54 in lungs and 6.99 in spleens (Table 2).

When we investigated the substitution of moxifloxacin for either isoniazid or ethambutol in the current drug regimen on the rate of bacterial CFU elimination, we found that the early bactericidal activities were similar amongst the three drug regimens, which were 99% kill at 2.2 weeks for moxifloxacin replacing isoniazid regimen (RMZE), 2.7 weeks for standard regimen (RHZE) and 3 weeks for moxifloxacin replacing ethambutol regimen (RHZM). Treatment with RMZE increased the rate of bacterial elimination showing undetectable CFU counts at 11 weeks compared to 14 weeks for the standard regimen and moxifloxacin replacing ethambutol regimen (Table 2).

These observations coincided with bactericidal activities as assessed using the mono-exponential bacterial elimination rate constants (Fig 1, and Table 3) where the exponential rate constants (logarithmic base 10) for net bacterial elimination during treatment ($k_{net\_with\_drug}$) for standard, moxifloxacin replacing ethambutol and moxifloxacin replacing isoniazid regimens were -0.46, -0.50 and -0.65, in lungs and -0.46, -0.46 and -0.60 in spleens, respectively (Table 3). The higher the absolute value of this elimination rate constant (i.e. the steeper the slope of the elimination on the logarithmic scale with units of wk^{-1}), the quicker the exponential elimination rate of CFU counts in the organs These values indicate therefore that compared to the standard therapy, substitution of moxifloxacin for isoniazid gives a significant increase in bacterial elimination in both lungs and spleens while substitution of moxifloxacin for ethambutol makes a statistically indistinguishable difference.

In the CFU count free organs, no tubercle bacilli were recovered which was determined by negative cultures of the organ homogenates in selective Kirchner broth for 4 weeks followed by growth on Löwenstein–Jensen medium.

Post-treatment level of CF-resuscitated MPN in the Cornell model. In order to investigate the effect of moxifloxacin containing regimens on the post-treatment level of persistent bacilli through CF-induced resuscitation, lung and spleen homogenates at the weeks of treatment when CFU counts reached zero for each of the regimens were incubated with culture filtrates. As shown in Table 4, after 14 weeks of treatment with RHZE and RHZM, high levels of CF-resuscitated bacilli remained in both lungs and spleens. For RMZE treatment, at 11 weeks post-treatment, although CFU counts were zero, there were average 2.96 and 3.01 log of CF-resuscitated MPN of bacilli per lung and spleen, respectively. At 14 weeks of treatment, there were still 2-log MPN of the bacilli present (Table 4). The numbers of CF-dependent bacteria at 14 weeks amongst the three treatment groups were not significantly different ($p > 0.05$, $n = 8$).

Relapse rate of treatment with the moxifloxacin containing regimens in the Cornell model. After 8 weeks of high dosage steroid immunosuppression, disease relapse rates for the treatment with the three drug regimens were determined by the percentage of mice that developed positive *M. tuberculosis* cultures (CFU counts) in lungs, spleens or both. As shown in Table 5, treatment with the standard regimen RHZE gave rise to positive organs in 19 out of 21 mice (90% relapse rate) and RHZM led to 95% relapse rate after 14 weeks of treatment. In contrast, treatment with RMZE resulted in 59% of relapse ($P = 0.03$ vs. RHZE, $P = 0.009$ vs. RHZM, $P < 0.017$ significant at 0.05 level after Bonferroni correction for 3 pairwise comparisons).

We also measured CF-dependent bacilli using culture filtrates in the organs which showed CFU count negative after 8 weeks of steroid treatment. As shown in Table 5, the negative organs in each treatment group contained high numbers of CF-dependent cells. There are average 3.3, 3.51 and 3.05 logs of MPN per organ in the groups of RHZE, RHZM and RMZE, respectively.

DISCUSSION

This is the first study, using the reliable Cornell mouse model, to characterize the therapeutic efficacy of moxifloxacin-replacement *in vivo* against CF-dependent *M. tuberculosis* persistent cells. Compared to standard TB regimen, we found that moxifloxacin replacement for isoniazid: (i) failed to remove CF-dependent bacilli, despite having (ii) faster organ CFU count elimination rates and (iii) lower disease relapse rates. In contrast, moxifloxacin replacement for ethambutol failed to demonstrate any therapeutic benefits compared to the standard regimen. These results of CF resuscitation need to be confirmed in human studies, but may provide a novel mechanistic explanation for the results of moxifloxacin-replacement regimens in clinical trials. Findings in this study also have important future implications in TB novel drug design, diagnostic testing and clinical applications.

Moxifloxacin-replaced drug regimens are ineffective against CF-dependent tubercle bacilli. The greater therapeutic efficacy and lower relapse rates achieved with moxifloxacin-isoniazid replacement regimen, as compared to the standard regimen, in this study is consistent with previous reports (5-8). De Groote *et al* demonstrated that moxifloxacin replacing isoniazid in the standard regimen gave rise to 63% disease relapse (6). In other studies, Nuermberger and colleagues showed that the same drug regimen produced a lower disease relapse at 33.3% (7). Late studies using two pathologically distinct murine tuberculosis models demonstrated very similar low disease relapses (8). Our study confirmed this interesting observation showing 56% relapse, indicating the consistency of the drug regimen in different mouse models.

The underlining mechanisms that moxifloxacin replacing isoniazid was more efficacious than the standard or moxifloxacin replacing ethambutol regimens were unknown. It has been shown previously that when mice were treated with rifampicin-isoniazid-pyrazinamide, rifampicin-isoniazid or rifampicin-pyrazinamide for 6 months, rifampicin-pyrazinamide treated group demonstrated significantly lower relapse rates than the other two groups containing isoniazid

(26), suggesting that isoniazid antagonized the actions of rifampicin-pyrazinamide (26). It is possible that replacement of isoniazid with moxifloxacin eliminated the antagonistic drug interaction leading to the rapid organ CFU count clearance in mice.

The use of CFU counts as an end point reflects the clinical observations in patients to a large degree, which is related to clinical endpoints such as sputum culture conversion in patients (3, 27, 28). The improved efficacy with moxifloxacin replacement for isoniazid compared to the standard regimen reflected the clinical outcome in patients to some extent, for example, the moxifloxacin-isoniazid substitution regimen showed an effective bactericidal activity which was able to kill CFU count positive bacilli faster than the standard regimen, leading to the higher sputum culture conversion rate in humans (3, 27, 28).

Despite the improved performance, moxifloxacin-isoniazid replaced regimen was ineffective against CF-dependent bacterial cells, which has not been demonstrated previously. At 11 weeks and the end of the antibiotic therapy, despite the elimination of CFU count positive bacilli, considerable MPN of CF-dependent bacilli remained in all the mice, which were similar to those treated with the standard drug regimen or the moxifloxacin-ethambutol replaced regimen. This indicated that although the moxifloxacin-isoniazid replaced regimen was more bactericidal than the standard regimen, the drug regimen failed to show improved sterilizing activity against persistent bacteria. After 8 weeks of immunosuppression with steroid, there were 90% of standard regimen treated mice and 95% of moxifloxacin replacement for ethambutol treated mice with CF-dependent cells which became CFU count positive again. This high disease relapse rate can only be feasibly explained by the reactivation of CF-dependent cells since the mice were CFU count-zero before immunosuppression. In addition to a lower relapse rate, mice treated with moxifloxacin replacement for isoniazid regimen contained similar MPN counts in their negative organs to the other two groups. This indicated that certain drug regimens such as moxifloxacin replacement of isoniazid may induce heterogeneously more diverse bacterial populations, therefore not all CF-responding bacteria

regain their ability to form colonies on agar plates at the time we determined relapse, showing a lower disease relapse. Further studies are warranted on the induction of heterogeneity of bacterial populations using different anti-TB drug regimens.

Clinical trials and animal studies: different testbeds, same mechanism. None of the two moxifloxacin-replacement phase III clinical trials have demonstrated non-inferiority to standard regimens for treatment duration and disease relapses (3, 4); the underlying mechanism is unclear. An important finding of the REMoxTB trial is that there was a proportion of patients who showed sputum conversion quickly but continued to relapse after treatment with all three drug regimens (9). Our experiments suggest that the high relapse rates may have been due to CF-dependent bacilli (9) which, till now, have remained undetectable using conventional culture methods, including those used in the clinical trials (3, 4, 9). Persistent bacteria are established causes of prolonged chemotherapy and disease relapse (10). It has been repeatedly shown that in the Cornell mouse model, high relapse rate after treatment with the standard drug regimen was due to the presence of CF-dependent persistent bacteria (16, 17). Recently, we showed that drug regimens containing high doses of rifampicin (30 mg/kg or higher) could eliminate CF-dependent persistent bacilli, which led to shortened treatment duration from 14 weeks to just 6 weeks, without disease relapses (16, 18). The lesson learnt from the REMoxTB trial is that more rapid culture conversion in the short moxifloxacin containing regimens may not allow for shortened regimens due to the presence of persistent bacteria (9). A previous study also showed that early bactericidal activities of certain novel drug regimens were not necessarily predictive of any sterilizing effects (29). This may be attributed to the inability of the drug regimens to eliminate the persistent bacilli which were undetectable using the traditional microbiological methods. Therefore, in addition to the conventional microbiological methods, evaluation of anti-TB regimens by assessing their efficacies in eliminating RPF-dependent *M. tuberculosis* is important for providing a

comprehensive profile of novel drug regimens before proceeding to human clinical trials. The combined data sets on CFU counts, broth growth and persister counts will strengthen any claims to be made on a regimen, which will ultimately increase the confidence of advancing it into humans.

Clinical trials and animal studies: different testbeds and important considerations. The interpretation of the mouse results of moxifloxacin-replacement regimens compared with the results in clinical trials requires careful consideration. In the previous animal studies for example the burden of persistent bacteria had not been detected and assessed (5, 7) largely due to the undetectable feature of the persisters (16, 17). Importantly, there are clear differences in the pathophysiology of TB between humans and mice. Patients with active TB have persisters residing in a milieu of different pathogenic states, including necrotic/caseating lesions, central liquefactive lesions, open cavities, closed fibrotic granulomas (30, 31). Indeed, a patient over time may develop a combination of these lesions (10). Consequently, these heterogeneous persistent bacteria co-exist with fast growing bacteria at the time of the commencing antibiotic treatment (10). In contrast, mice do not form granuloma structures after *M. tuberculosis* infection (30) and persistent bacteria are generally low in absolute number (similar to the CFU counts) at the beginning of the treatment (16). In addition, an important study has shown that moxifloxacin does not diffuse into caseating lesions, which may also lead to reduced sterilizing activities against persisters in human TB (32).

Treatment of TB persisters is complex and future clinical trials require careful consideration of mechanistic *in vivo* studies, which can elucidate further insights into potential therapeutic targets. The study reported here represents an important step in the right direction by showing that RPF-dependent persisters may be a novel and important clinical therapeutic target.

In conclusion, moxifloxacin substitution in contemporary drug regimens was ineffective against resuscitation promoting factor dependent persistent *M. tuberculosis*, despite having favorable therapeutic efficacy against actively multiplying bacteria *in vivo*.

ACKNOWLEDGEMENTS

This work was supported by the Innovative Medicines Initiative Joint Undertaking resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution (grant number 115337). The publication reflects only the author's views. The European Commission is not liable for any use that may be made of the information herein. The financial support of MRC (MR/P011144/1) is gratefully acknowledged. We thank Alexander Liu for critical discussion.

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Figure legend

Figure 1. Treatment profiles of *M. tuberculosis* H37Rv with regimens RHZE, RMZE and RHZM in the Cornell mouse model. A. Elimination of CFU counts in lungs. B. Elimination of CFU counts in spleens. The solid arrow indicates the treatment starting at 3 weeks of post infection. The empty arrow indicates starting steroid treatment after the termination of 14 week therapy.

Table 1. Mouse tuberculosis experimental design

Treatment groups[a]	Total No. of mice[b]	D0	D21	2W	4W	6W	8W	11W	14W	22W[c]
Control	8	4	4							
RHZE	54			4	4	4	8	8	8	22
RHZM	54			4	4	4	8	8	8	22
RMZE	54			4	4	4	8	8	8	22

[a] Mice were intravenously infected at day 0. Treatment commenced at 21 days. Dosages for each drug were as follows: R 10 mg/kg, H 25 mg/kg, Z 150 mg/kg, E 100 mg/kg and M 100 mg/kg.

[b] Total mice were infected and treated excluding natural death of the mice during the course of treatment

[c] 8 weeks of hydrocortisone treatment post 14 weeks of treatment

Table 2. Organ CFU counts before and after treatment with experimental regimens

Time of infection and treatment	Mean Log CFU per lung ± SD				Mean Log CFU per spleen ± SD			
	Control	RHZE	RHZM	RMZE	Control	RHZE	RHZM	RMZE
D0[a]	4.80 ± 0.14				5.29 ± 0.03			
D21[b]	7.54 ± 0.03				6.99 ± 0.06			
2W[c]		6.09 ± 0.03	5.92 ± 0.14	5.70 ± 0.12		5.43 ± 0.08	5.54 ± 0.12	4.77 ± 0.10
4W		4.80 ± 0.07	4.52 ± 0.28	3.61 ± 0.16		4.05 ± 0.02	4.49 ± 0.08	3.31 ± 0.21
6W		4.08 ± 0.11	4.00 ± 0.09	2.53 ± 0.29		3.49 ± 0.16	3.81 ± 0.06	2.27 ± 0.20
8W		3.00 ± 0	3.25 ± 0.48	2.00 ± 0		2.44 ± 0.22	3.24 ± 0.13	1.57 ± 0.20
11W[d]		2.00 ± 0	2.00 ± 0	0		1.19 ± 0.29	2.00 ± 0	0
14W[d]		0	0	0		0	0	0

a. 2 hours post-infection. b. 21 days post-infection. c. week 2 post-treatment. d. CFU counts were derived from one third of tissue homogenate and limit detection was 3 CFU/organ.

Table 3. Elimination constant rates of different treatment groups

Treatment group	Elimination rate constant (wk-1)[a]			
	Lungs[b]		Spleens[c]	
	alpha	%RSE	alpha	%RSE
RHZE	-0.46	3.20	-0.46	4.76
RHZM	-0.50	8.57	-0.46	6.54
RMZE	-0.65	10.96	-0.57	6.40

a. Elimination rate constant equivalent to “knet_with_drug”
b. P = 0.008 RMZE vs. RHZE, P = 0.065 RMZE vs. RHZM, P = 0.384 RHZE vs. RHZM
c. P = 0.018 RMZE vs. RHZE, P = 0.011 RMZE vs. RHZM, P = 0.943 RHZE vs. RHZM
P < 0.017 significant at 0.05 level after Bonferroni correction for 3 pairwise comparisons.

Table 4. MPN of *M. tuberculosis* H37Rv in CFU count negative mouse lungs and spleens after treatment with different drug regimens

Treatment regimen	MPN/lung[a]				MPN/spleen[b]			
	11 week	95% confidence limits	14 week	95% confidence limits	11 week	95% confidence limits	14 week	95% confidence limits
RHZE	-		2.50 ± 0.19	2.35 - 2.67	-		2.56 ± 0.16	2.44 - 2.70
RHZM	-		2.55 ± 0.14	2.44 - 2.67	-		2.60 ± 0.09	2.52 - 2.69
RMZE	2.96 ± 0.15	2.86 - 3.08	2.30 ± 0.23	2.15 - 2.50	3.01 ± 0.14	2.92 - 3.11	2.35 ± 0.16	2.27 - 2.45

[a]determined by MPN of the diluted lung homogenates (n=8) with the culture filtrates. [b]determined by MPN of the diluted spleen homogenates (n=8) with the culture filtrates.

The CFU count zero organs showed no growth in Kirchner liquid medium following inoculation on Löwenstein-Jensen slopes.

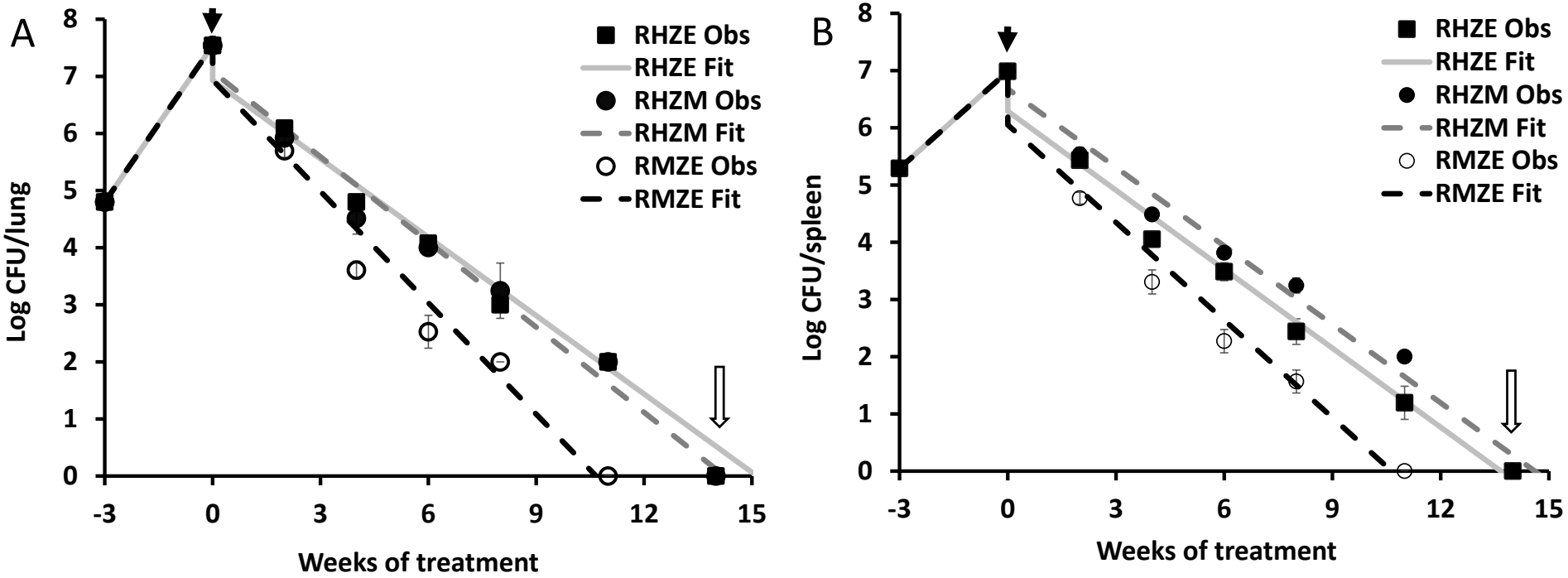
Broth counts were derived from one third of tissue homogenate and calculated to represent the MPN of entire organ. The limit of detection was 30 MPN/organ.

-, Colony count positive and MPN counts not performed organs. The limit of detection was 3 CFU/organ.

Table 5. Relapse rates of mice after treatment with different drug regimens

Positive culture from	RHZE	RHZM	RMZE
Spleen only	3	5	4
Lung only	0	2	5
Both organs	16	13	4
Neither organs	2	1	9
Total mice	21	21	22
Relapse rate pn/N (%)[a]	19/21 (90)	20/21 (95)	13/22 (59)
MPN in CFU count negative organs	3.30 ± 0.13	3.51 ± 0.11	3.05 ± 0.18

a, Relapse rates include all lungs or spleens or both organs positive for bacilli. N, total number of mice. pn, number of mice with CFU count positive organs.
P = 0.03 RMZE vs. RHZE, P = 0.009 RMZE vs. RHZM, P = 1 RHZE vs. RHZM
P < 0.017 significant at 0.05 level after Bonferroni correction for 3 pairwise comparisons


A
Log CFU/lung
Weeks of treatment
RHZE Obs
RHZE Fit
RHZM Obs
RHZM Fit
RMZE Obs
RMZE Fit
B
Log CFU/spleen
Weeks of treatment
RHZE Obs
RHZE Fit
RHZM Obs
RHZM Fit
RMZE Obs
RMZE Fit