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Optimal doses of rifampicin in standard drug regimen to shorten tuberculosis treatment duration and reduce relapse by eradicating persistent bacteria

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Running title: High dose rifampicin to treat murine tuberculosis

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Abstract

OBJECTIVE: Although high-dose rifampicin holds promise for improving tuberculosis disease control by eradication of persistent bacteria, the optimal dose of rifampicin which kills persistent bacteria and shortens the treatment duration is unknown.

METHODS: The Cornell mouse model was used to test the efficacy of rifampicin in elevated dose combined with isoniazid and pyrazinamide to kill actively growing and persistent bacilli and to measure relapse rate. Persistent bacteria were evaluated using *Mycobacterium tuberculosis* culture supernatant containing resuscitation promoting factors. Pharmacokinetic parameters and dose-dependent activity on cultivable and persistent bacilli were determined.

RESULTS: Increasing doses of rifampicin in combination with isoniazid and pyrazinamide resulted in dose-dependent faster bacterial clearance. Evaluated both on solid media and in culture filtrate containing resuscitation promoting factors, a regimen containing a standard dose of rifampicin at 10 mg/kg over 14 weeks failed to achieve organ sterility. In contrast, higher doses of rifampicin achieved organ sterility in a much shorter time of 8 to 11 week. Disease relapse, which occurred in 86% of mice treated with the standard regimen for 14 weeks, was completely prevented by rifampicin doses of 30 mg/kg and above.

Conclusions: In the treatment of murine tuberculosis, a rifampicin dose of 30 mg/kg was sufficient to eradicate persistent *M. tuberculosis*, allowing shorter treatment duration without disease relapse.

Introduction

TB remains one of the most prevalent and lethal infectious diseases worldwide, despite of the advent of anti-TB drugs and global healthcare initiatives, leading to approximately 2 million deaths annually.[1] Although the current drug regimen is effective, 6 months of chemotherapy are necessary to achieve a cure. The long duration of therapy leads to poor patient compliance which gives rise to high relapse rates (7-13%) and the emergence of drug-resistant strains.[2] Thus, shortening the duration of chemotherapy is of significant clinical benefit. Unfortunately, under the current paradigm, it takes more than 6 years to bring a new drug from bench to bedside, and more than 20 years for novel drug combinations to emerge.[3] This problem is amplified by the fact that tubercle bacilli can become dormant and persistent, undetectable by conventional tests. The persistent bacteria are tolerant to current TB drugs and difficult to eradicate using the dose levels in the current drug regimen.[4, 5] Therefore, to bridge the gap, there is an urgent need to optimize the doses of the drugs that are already used in the standard treatment regimen to maximize their bactericidal and sterilizing activities.[6]

Of the current anti-tuberculous drugs, rifampicin was introduced at suboptimal doses.[6, 7] Rifampicin exhibits bactericidal activity, killing actively growing organisms and sterilizing activity, killing the persisting bacilli that are responsible for relapse.[6, 8-10] It can be used at higher doses without serious adverse effects.[11-14] Previous studies showed that high-dose rifampicin therapy up to 35 mg/kg is well-tolerated in man[14-16] and increases the rate of tuberculosis clearance.[15] Similar observations were made in mice [13, 17-19] with a maximum tolerable dose of 160 mg/kg per day.[19] Recent results of a randomized clinical trial in South Africa and Tanzania by the PanACEA consortium suggested that rifampicin at 35 mg/kg was more efficacious than the standard rifampicin dose regimen by increasing culture conversion time in liquid medium.[20] However, it is not known if rifampicin at 35 mg/kg is able to shorten the treatment duration and provides a low relapse rate. We have showed that *M. tuberculosis*

forms persistent bacteria which are dependent on culture filtrate (CF) containing resuscitation promoting factors [21] to recommence multiplication. We demonstrated for the first time that a high-dose rifampicin drug regimen was able to kill CF-dependent persistent bacteria, enabling a shortened treatment duration in mice without disease relapse.[13] However, in our previous study, we only used one high dose of the drug (50 mg/kg). It is therefore crucial to find the minimum dose size of rifampicin capable of killing persistent bacteria with a favorable toxicity profile to patients.

Herein, we studied the therapeutic effects of incremental doses of rifampicin in combination with isoniazid and pyrazinamide in the Cornell mouse model. We measured the rate of elimination of bacterial cfu counts and relapse rates. We detected and quantified persistent bacilli in cfu count-free organs using *M. tuberculosis* culture filtrates.

Materials and methods

Bacterial strains and growth conditions

M. tuberculosis strain H37Rv was mouse-passaged and grown in 7H9 medium supplemented with 10% albumin dextrose complex (ADC; Becton and Dickinson, UK) and containing 0.05% Tween 80 at 37°C without disturbance for 15 days. The culture was subsequently stored at -70°C for animal infection. To determine the viable counts prior to infection, colony forming unit (cfu) counting was performed prior to freezing and once again after thawing. 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 and viability was expressed as Log cfu/mL. The cultures were subsequently diluted in PBS and used for inoculations in mice. All culture media 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). Human medicines of

rifampicin (Rifadin capsules, Sanofi Aventis), isoniazid (isoniazid tablets, Focus) and pyrazinamide (Zinamide tablets, Genus Pharmaceuticals) were used in this study.

Cornell mouse model

Rifampicin at different dose sizes in combination with isoniazid and pyrazinamide was tested using the Cornell mouse model.[22, 23] The model was conducted using the experimental design and procedure described previously.[24] Briefly, as shown in Table 1, at 3 weeks after *M. tuberculosis* H37Rv infection, treatment was given to female BALB/c mice for 14 weeks with 150 mg/kg pyrazinamide, 25 mg/kg isoniazid combined with 10, 20, 30, 40 and 50 mg/kg rifampicin by daily oral administration for 5 days per week. A sample of 4 mice was sacrificed at the beginning of the treatment and 8 mice was sacrificed 2nd, 4th, 6th, 8th, 11th and 14th week of treatment to monitor cfu counts. The organ homogenates from 6th to 14th week were cultured in selective Kirchner liquid medium for 4 weeks with subsequent sub-culturing onto selective Löwenstein-Jensen slopes for a further 4 weeks.

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 their host immunity, cfu counts from lungs and spleen were performed to determine disease relapse.

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.

Pharmacokinetics of rifampicin in BALB/c mice

Pharmacokinetic (PK) profiles of rifampicin were determined in uninfected and infected mice in a dose-ranging study with regimens matching those used in the Cornell mouse model which were administered orally by gavage. There were three BALB/c mice in 2 parallel

uninfected or infected groups (total n=12 each). The infected group was treated previously with each of these drug regimens for 8 weeks. After both groups were given the drug regimens, serial venous blood samples (20 µL) were collected at time points 1, 2, 3, 4, 5, 6, 8 and 24 hours post-dose by tail puncture and mixed with 40 µl of water. The blood samples were stored at -80°C and subsequently transported in dry ice to GlaxoSmithKline Tres Cantos for bioanalysis. The concentrations of rifampicin in the blood were determined by UPLC-MSMS assay. PK parameters were calculated using a noncompartmental analysis model (NCA) in the R software package (v 3.3.2).

Resuscitation of *M. tuberculosis* in mouse lungs and spleens

For resuscitation of *M. tuberculosis* grown in mouse organs, culture filtrates containing resuscitation promoting factor (RPF) were used as described previously.[13, 21, 24] *M. tuberculosis* H37Rv was grown in 7H9 medium for 15 to 20 days until an optical density of 1 to 1.5 was reached. The cultures were harvested by centrifugation at 3000 g for 15 minutes and sterilized by filtration with 0.2 µm filter (Sartorius) twice. The culture filtrates were made selective by addition of polymyxin B 200000 U/L, carbenicillin 100 mg/L, trimethoprim 20 mg/L and amphotericin B 10 mg/L (Selectatab, Mast Diagnostica GmbH). Broth counting of lungs and spleens was performed as serial 10-fold dilutions in triplicate 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. The patterns of positive and negative growth tubes will be used to calculate the most probable number (MPN) of the bacilli.[25]

Growth of *M. tuberculosis* in turbid tubes was confirmed by colonial morphology on 7H11 agar plates. 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*.

Statistical analysis

A simple model for monoexponential bacterial growth and elimination was used.[24, 26] Standard errors of parameter estimates were calculated using the method outlined by Landaw *et al.* [27] 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 [28] 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 15 (including a comparison versus 50 mg/kg rifampicin monotherapy from previous data) [13], where P values <0.0033 would be considered significant. The significance of differences between the relapse rates was determined with pairwise Fisher's exact tests with a Bonferroni correction of 15, with P values <0.0033 considered significant.

RESULTS

Treatment with regimens containing different dose sizes of rifampicin in the Cornell mouse model

We investigated the effect of rifampicin at 10, 20, 30, 40 and 50 mg/kg in combination regimens with fixed standard doses of isoniazid and pyrazinamide on the rate of bacterial eradication and relapse in the Cornell mouse model. As shown in Table 2 and Fig 1a, there was a rifampicin dose-dependent increase in the rate of eradication of cfu counts in the lungs. At rifampicin 10 and 20 mg/kg regimens, the rate of pulmonary bacterial eradication was slow showing 99% kill at 3.5 weeks and at 2.5 weeks, respectively. Treatment with rifampicin 30, 40 and 50 mg/kg increased the rate of bacterial eradication (99% kill at 1.8, 1.6 and 1.4 weeks, respectively). Undetectable *M. tuberculosis* cfu counts were achieved in

mouse lungs after 14 weeks treatment for 10 mg/kg, 11 weeks for 20 mg/kg, 8 weeks for 30 and 40 mg/kg and 6 weeks with 50 mg/kg of rifampicin containing regimens (Table 2). A similar dose response trend was observed in spleens except cfu count free organs were achieved at 6 weeks for both 40 and 50 mg/kg rifampicin regimens (Table 2 and Fig 1b). These activities were confirmed by the estimates of the exponential rate constants (logarithmic base 10) for net bacterial elimination during treatment ($k_{net\_with\_drug}$) in both lung and spleen cfu count profiles versus time (Table 3). The elimination rate constants become faster (i.e. greater in magnitude) with increasing dose, in a linear relationship in both lungs and spleens (Fig. 1c and 1d). In the cfu count free organs, no tubercle bacilli were recovered as confirmed by negative cultures of the organ homogenates in selective Kirchner medium. No outward signs of toxicity or abnormal behavior were observed in any of the mice treated with all doses of rifampicin containing regimens.

Pharmacokinetics of rifampicin in combination with isoniazid and pyrazinamide

Rifampicin blood concentrations after administration of rifampicin containing regimens with isoniazid and pyrazinamide were examined over a period of 24 hour in both *M. tuberculosis* infected and uninfected BALB/c mice. As shown in Fig 2, there was a linear, dose-proportional increase in the exposure of rifampicin as indicated by both maximal concentration of rifampicin (Cmax) (Fig 2a) and the overall drug exposure (AUC) (Fig 2b) in both uninfected and infected mice. The dose linearity of the rifampicin PK in this range of doses was further supported by a plot of clearance versus dose from each regimen (Fig 2c). Clearance (equal to Dose/AUC) was shown to be approximately constant at ~0.04 L/h/kg in both infected and uninfected animals at each dose level. Both AUC and Cmax of rifampicin were similar between infected and uninfected animals at all the doses examined (< 30% difference in either measure at all doses uninfected versus infected).

Post-treatment level of persisters in the Cornell mouse model

In order to investigate the effect of different rifampicin dose regimens on the post-treatment level of persisters through RPF-induced resuscitation, lung and spleen homogenates at the weeks of treatment when cfu counts reached zero for each of the regimens were incubated with CF containing RPFs. As shown in Table 4, after 14 weeks of treatment with the rifampicin 10 mg/kg regimen, despite cfu cultures being negative, the number of RPF-dependent persisters was still high. At 11 weeks post-treatment, there were significant levels of CF-resuscitated bacilli in lungs and spleens for the rifampicin 20 mg/kg regimen, whilst reduced numbers of persisters were present at 14 weeks of treatment. At 8 weeks of treatment, there were low numbers of persisters present after treatment with rifampicin 30 mg/kg regimen, complete persister eradication was seen at 11 weeks. There were no persistent bacteria at 8, 11 and 14 week for rifampicin 40 mg/kg treatment. The regimen containing 50 mg/kg rifampicin, although failed to clear persisters at 6 week, showed no CF-resuscitated bacilli in both lungs and spleens at 8, 11 and 14 weeks of treatment (Table 4).

Relapse rate of treatment with the regimens containing different doses of rifampicin in the Cornell model

The organ cfu counts are shown in Table 5. The treatment with the regimen containing 10 mg/kg of rifampicin gave rise to *M. tuberculosis* positive organs in 19 out of 23 mice (86.3% relapse rate). 20 mg/kg rifampicin containing regimen led to 33% relapse rate after 14 weeks of treatment. In contrast, treatment with the regimens containing 30, 40 and 50 mg/kg of rifampicin resulted in zero counts in the organs showing relapse free ($P < 0.001$).

DISCUSSION

TB drug regimens capable of eradicating persistent bacilli likely have the greatest clinical value to shorten the treatment duration and reduce relapse rate. In this study, the efficacy of a

dose range for rifampicin in the standard drug regimen was studied and CF-dependent persisters were quantified at the time points when cfu count free organs were reached in the Cornell mouse model. We intended to define if we could utilize CF-dependent persistent *M. tuberculosis* as a biomarker for assessment of TB treatment outcome. The Cornell model is a reliable surrogate for efficacy in tuberculosis focused on disease relapse, developed more than 60 years ago by McCune *et al.*[22, 23] It has been used to assess the pharmacodynamics of TB drug regimens and pave the way for drugs from critical preclinical evaluation to clinical application.[29] Our previous results demonstrated that RPF-dependent bacilli constituted a major pool for disease relapse in the Cornell model.[13] It has been repeatedly shown that the standard rifampicin dose (10 mg/kg) regimen was unable to eliminate the undetectable persistent bacteria leading to a high disease relapse.[8, 13, 24] With high dose rifampicin (50 mg/kg) regimen, treatment duration was shortened from 14 to 6 weeks and free of relapse. [13] This was attributed to the eradication of CF-responsive persistent bacilli from the infected organs. In this study, we showed that double the standard dose size of rifampicin failed to remove CF-dependent persisters at both 11 weeks and 14 weeks of treatment with a relapse rate of 33% (Table 5). When the drug reached 30 mg/kg, cfu count zero was achieved at 8 weeks with low number of CF-dependent persisters and a further treatment period (up to 11 weeks) was needed to sterilize the organs (Table 4). The regimens with rifampicin at 40 and 50 mg/kg rendered true tubercle bacilli-sterility (negativity for both cfu count and CF-resuscitatable bacteria) in lungs and spleens at 8 weeks of treatment.

We present clear evidence that we were able to predict disease relapse by assessing CF-dependent persisters. For the first time, we demonstrated that in mice, rifampicin dose size of 30 mg/kg (a minimum threshold) or higher was able to eradicate persistent bacilli leading to about 21 to 43% shortened treatment period with no disease relapse. Based on this observation, it may be argued that patients treated with higher than 30 mg/kg of rifampicin

are likely to achieve cfu count negative sputum faster with low number or no persistent bacteria leading to shortened treatment duration. This is evidenced in humans, that rifampicin at 35 mg/kg was able to improve time to stable culture conversion in liquid media [20] although treatment outcome is unknown in term of treatment duration and relapse. Our data offered a potential prediction of high dose rifampicin at 30 to 50 mg/kg to improve current clinical treatment, namely shortening the treatment duration and reducing relapse. This highly promising proof-of-principle work has pioneered a novel clinical method to identify and quantify persistent bacteria by RPF resuscitation to assess the clinical effectiveness of higher dose rifampicin in humans (A. Jindani, St George's University of London, personal communications).

In addition, we demonstrated that rifampicin in combination with isoniazid and pryzinimide showed a linear relationship between its dose level and plasma exposure (Cmax and AUC) in both uninfected and infected mice. We also showed that the plasma exposures of rifampicin were similar in both infected and uninfected animals (Fig. 2). The drug exposures were about two fold higher than those in our previous and other group's reports.[13, 14, 17, 19] Importantly, the rifampicin dose linearity of plasma exposure coincided with the linear trend in cfu count elimination (Fig. 1). There was a clear linearity of the bacterial elimination rate constant as a measure of efficacy with increasing dose of rifampicin within the range of doses examined (Fig 1). Similarly, the dose-dependent drug exposure of rifampicin is closely associated with the persistent bacterial elimination at the time points when cfu counts were negative. The linear trend in elimination rate constant was in agreement with the deduction of persister counts and relapse rate, namely faster elimination rates at higher doses concurred with lower persister counts and lower relapse rates. The same may be true in humans because interestingly, the linearity of rifampicin plasma exposure with dose shown in this study is consistent with the linearity of rifampicin PK over the range of 10 to 35 mg/kg in humans.[14]

It has been shown that in the standard dose of rifampicin (10 mg/kg), 90% of the drug was bound to human plasma proteins [30] and 97% was bound to mouse proteins,[19] therefore, only a very low amount of free drug was able to diffuse into tuberculous lesions. Here we showed that increasing dose of rifampicin exhibited an accelerated dose-dependent eradication of persistent bacteria (Table 4). When rifampicin concentration was increased to 30 mg/kg and above, high blood Cmax and AUC were achieved, leading to higher levels of biologically available rifampicin which were able to kill persistent bacteria.[13]

The drug exposure and the unbound drug for the same dose size between mice and humans are different for rifampicin. In mice, AUCs and Cmax of rifampicin are at least threefold higher than those in humans. In contrast, the free fraction of the drug is almost threefold greater in humans than that in mice. This suggests that the levels of the active and free drug in mice leading to the greater efficacy shown in this study can be effectively reached in humans at the dose levels which were currently studied in human clinical trials.

The implication of our mouse data to patient's benefits must be taken with caution. Tuberculosis in humans and in mice differs in the histopathology of the disease. In humans, TB rarely kills the host in the initial infection. Active disease is associated with a wide range of granuloma lesions, including bacterial bearing, necrotic granulomas undergoing central liquefaction and large open cavities, as well as closed granulomas with central caseum, fibrotic and calcified lesions. In contrast, in the standard Cornell model, infection is initiated by a high dose of *M. tuberculosis* (10^5 cfu/mouse) and treatment is commenced 2 to 3 weeks after infection when adaptive immunity is just established. There are no granuloma-like structures in the lungs.

In conclusion, the current recommended dosage of rifampicin at 10 mg/kg is insufficient to kill persistent bacilli in the Cornell mouse model. Rifampicin at 30 mg/kg or higher in combination with isoniazid and pyrazinamide significantly shortened the treatment and

prevented disease relapse by removing persistent bacteria. PK exposure of rifampicin and the observed cfu elimination rate constants were both linear in the range of rifampicin doses from 10 to 50 mg/kg in the combination therapy. Optimizing rifampicin to its maximal therapeutic efficacy with acceptable side-effect profiles will provide valuable information in human studies and can potentially revolutionize current tuberculosis chemotherapy.

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Transparency declarations

None to declare

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403
404

405 Figure legends

406 Figure 1. Treatment profiles of *M. tuberculosis* H37Rv with different dose size of rifampicin
407 (R) in combination with isoniazid (H) and pyrazinamide (Z) in the Cornell mouse model. a.
408 Elimination of cfu counts in lungs. b. Elimination of cfu counts in spleens. The solid arrow
409 indicates the treatment starting at 3 weeks of post infection. The empty arrow indicates
410 starting steroid treatment after the termination of 14 week therapy. c. Elimination rate
411 constant against rifampicin doses in lungs. d. Elimination rate constant against rifampicin
412 doses in spleens.

413 Figure 2. Rifampicin pharmacokinetic relationship between dose sizes and drug exposure in
414 infected and uninfected mice. a. Linear relationship between rifampicin dose and Cmax. b.
415 Linear relationship between rifampicin dose and AUC. c. Clearance of rifampicin with
416 different dose sizes of the drug.

417

Table 1. Cornell model experimental design

Treatment groups[a]	No. of mice[b]	D0	D21	2W	4W	6W	8W	11W	14W	22W[c]
Control	8	4	4							
R10HZ	71			8	8	8	8	8	8	23
R20HZ	71			8	8	8	8	8	8	23
R30HZ	71			8	8	8	8	8	8	23
R40HZ	71			8	8	8	8	8	8	23
R50HZ	71			8	8	8	8	8	8	23

a Mice were intravenously infected at day 0. Treatment commenced at 21 days after infection. Dosages for each drug were as follows: rifampicin (R) 10, 20, 30, 40 or 50 mg/kg, isoniazid (H) 25 mg/kg and pryzinimide (Z) 150 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. Bactericidal and sterilizing activities of the experimental regimens against *M. tuberculosis* in mouse lungs and spleens

Organs	Time	Control	R10HZ	R20HZ	R30HZ	R40HZ	R50HZ
Lung	D0[a]	4.36 ± 0.26					
	D21[b]	6.93 ± 0.07					
	2 week[c]		5.58 ± 0.43	5.12 ± 0.14	4.68 ± 0.27	4.35 ± 0.29	4.00 ± 0.23
	4 week		4.58 ± 0.33	4.12 ± 0.06	3.35 ± 0.46	2.80 ± 0.41	1.99 ± 0.02
	6 week		3.71 ± 0.05	3.08 ± 0.52	1.88 ± 0.70	1.14 ± 0.62	0
	8 week		2.58 ± 0.27	1.95 ± 0.43	0	0	0
	11 week		1.01 ± 0.43	0	0	0	0
	14 week		0	0	0	0	0
Spleen	D0[a]	5.30 ± 0.16					
	D21[b]	7.43 ± 0.21					
	2 week[c]		6.36 ± 0.29	5.73 ± 0.96	5.07 ± 0.52	4.61 ± 0.56	3.94 ± 0.46
	4 week		5.20 ± 0.23	4.17 ± 0.48	3.24 ± 0.13	2.00 ± 0.48	1.40 ± 0.42
	6 week		3.65 ± 0.45	2.54 ± 0.49	1.69 ± 0.46	0	0
	8 week		2.34 ± 0.36	1.49 ± 0.53	0	0	0
	11 week		0.92 ± 0.46	0	0	0	0
	14 week		0	0	0	0	0

a. 2 hours post-infection. b. 21 days post-infection. c. week 2 post-treatment.
Zero cfu count from each drug regimen was derived from one third of tissue homogenate and limit detection was 3 cfu/organ.
The data presented as mean of 4 mice for the control and 8 mice for the treatment groups with standard deviation.

Table 3. Estimates of exponential rate constants during pre-treatment (knet_no_drug) and treatment (knet_with_drug) in mouse lungs and spleens

Treatment group	Elimination rate constant (wk-1)			
	Lungs		Spleens	
	est.[a]	%RSE[b]	est.[a]	%RSE[b]
R10HZ	-0.52	2.0	-0.56	3.4
R20HZ	-0.58	4.9	-0.69	6.3
R30HZ	-0.75	8.2	-0.88	5.4
R40HZ	-0.84	6.2	-1.32	6.7
R50HZ	-1.07	5.2	-1.40	6.9

[a] estimate. [b] percentage relative standard error.

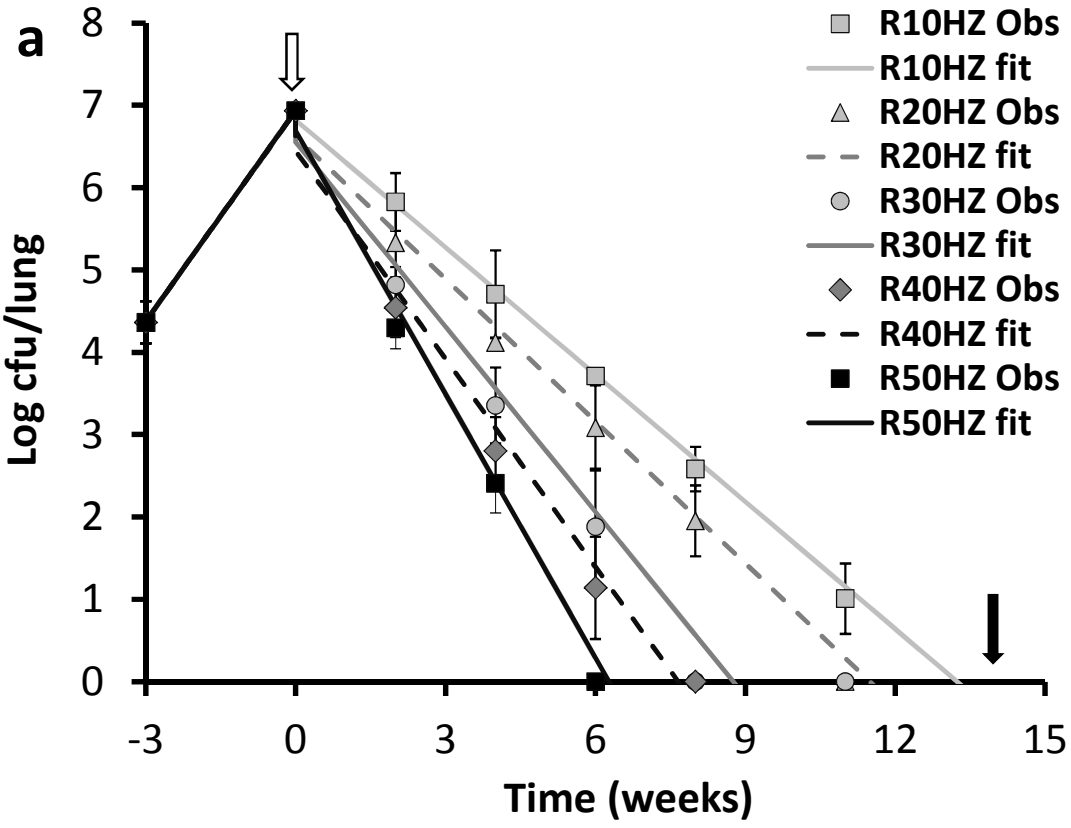
Table 4. Resuscitation of *M. tuberculosis* H37Rv in mouse lungs and spleens in the Cornell mouse model after treatment with regimens containing different doses of rifampicin

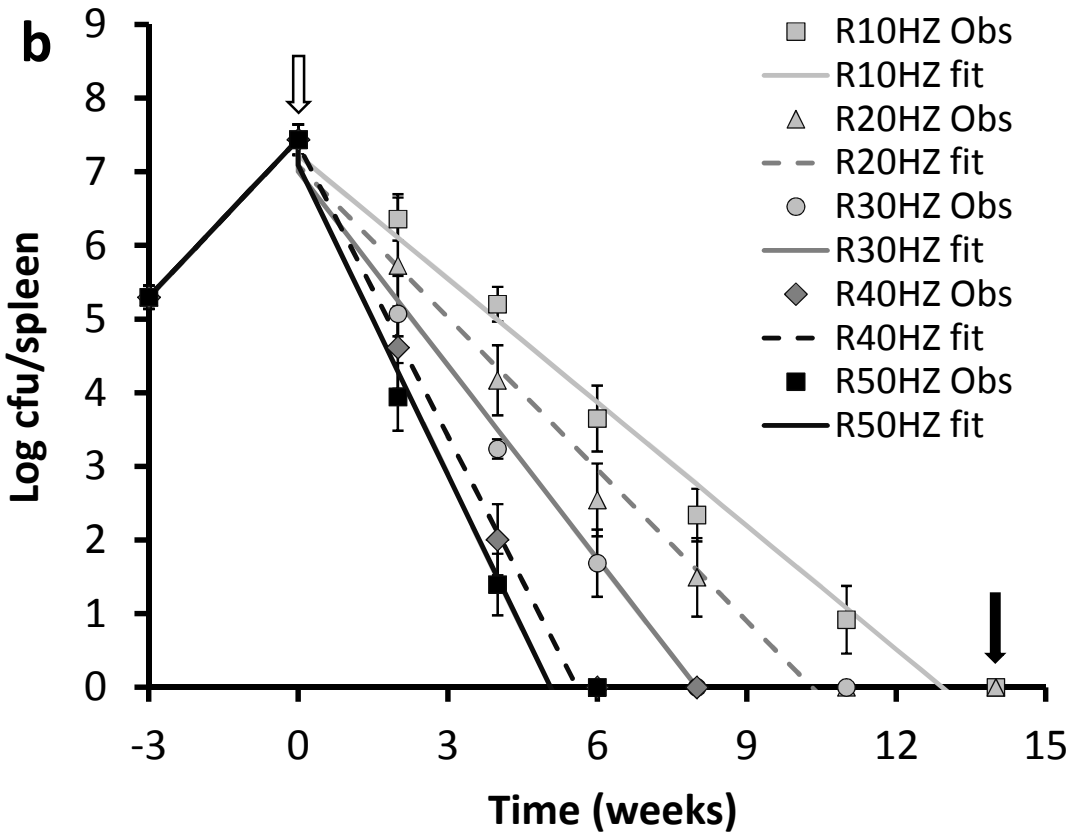
		MPN counts (CF)[a]				
Organs	Weeks of treatment	R10HZ	R20HZ	R30HZ	R40HZ	R50HZ
Lung	6	-	-	-	-	1 ± 4
	8	-	-	9 ± 9	0	0
	11	-	90 ± 30	0	0	0
	14	245 ± 28	20 ± 23	0	0	0
Spleen	6	-	-	-	-	3 ± 5
	8	-	-	18 ± 10	0	0
	11	-	122 ± 83	0	0	0
	14	308 ± 440	58 ± 21	0	0	0

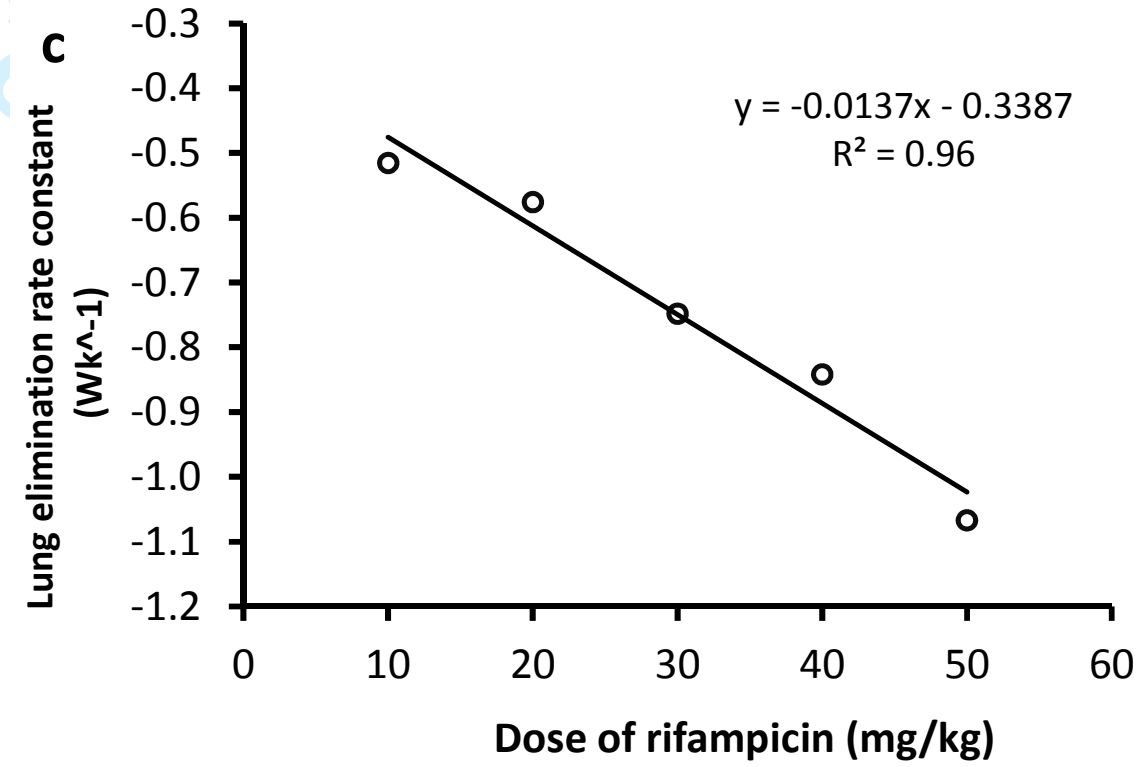
[a]determined by MPN of the diluted organ homogenies (n=8) with the culture filtrates, mean MPN ± standard deviations. Broth counts were derived from one third of tissue homogenate and calculated to represent the MPN of entire organ. The limit of detection was 1 MPN/organ. -, Colony count positive and MPN counts not performed organs.

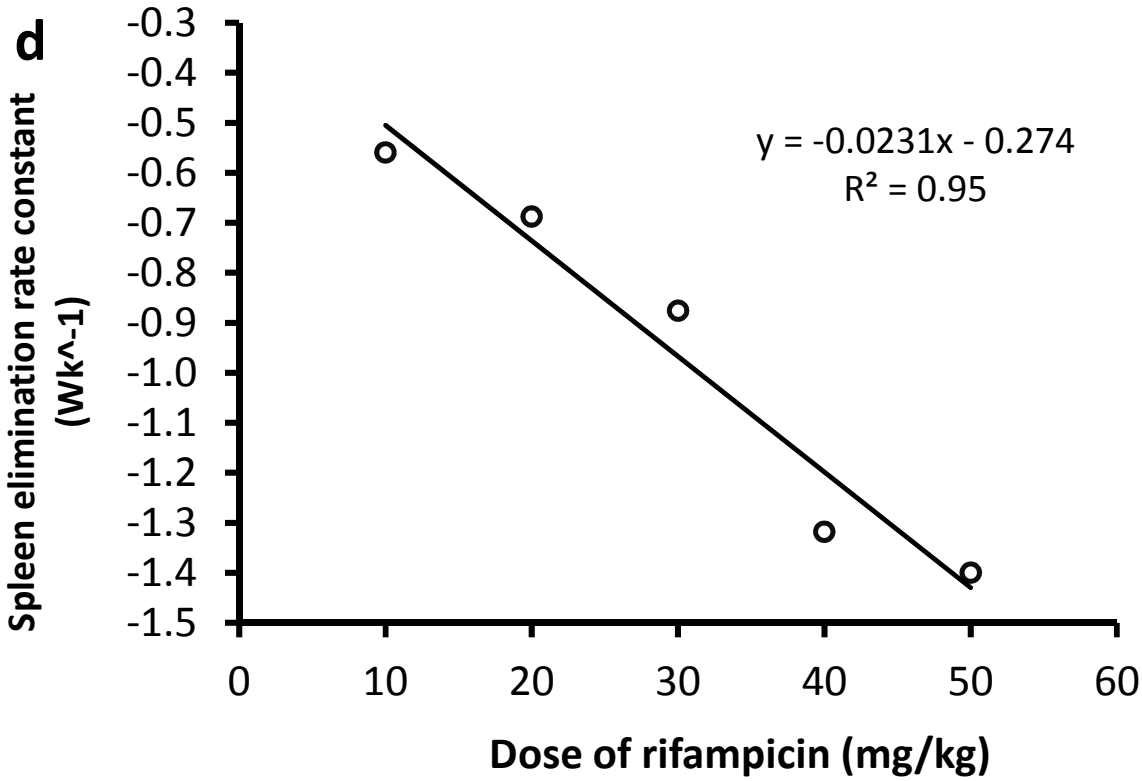
Table 5. Relapse rates of mice after treatment with regimens containing different doses of rifampicin for 14 weeks

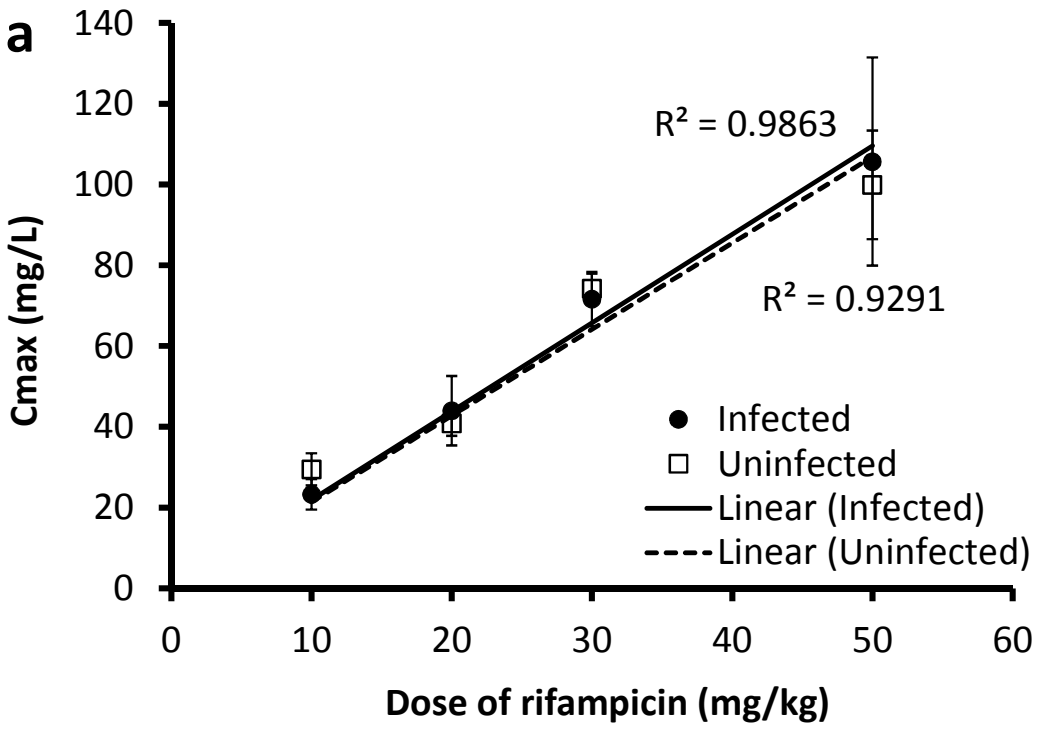
CFU counts detected from	R10HZ	R20HZ	R30HZ	R40HZ	R50HZ
Both organs	6	1	0	0	0
Lungs	7	5	0	0	0
Spleens	6	2	0	0	0
Negative organs	3	16	22	23	22
Total mice	22	24	22	23	22
% relapse	86.36	33	0	0	0

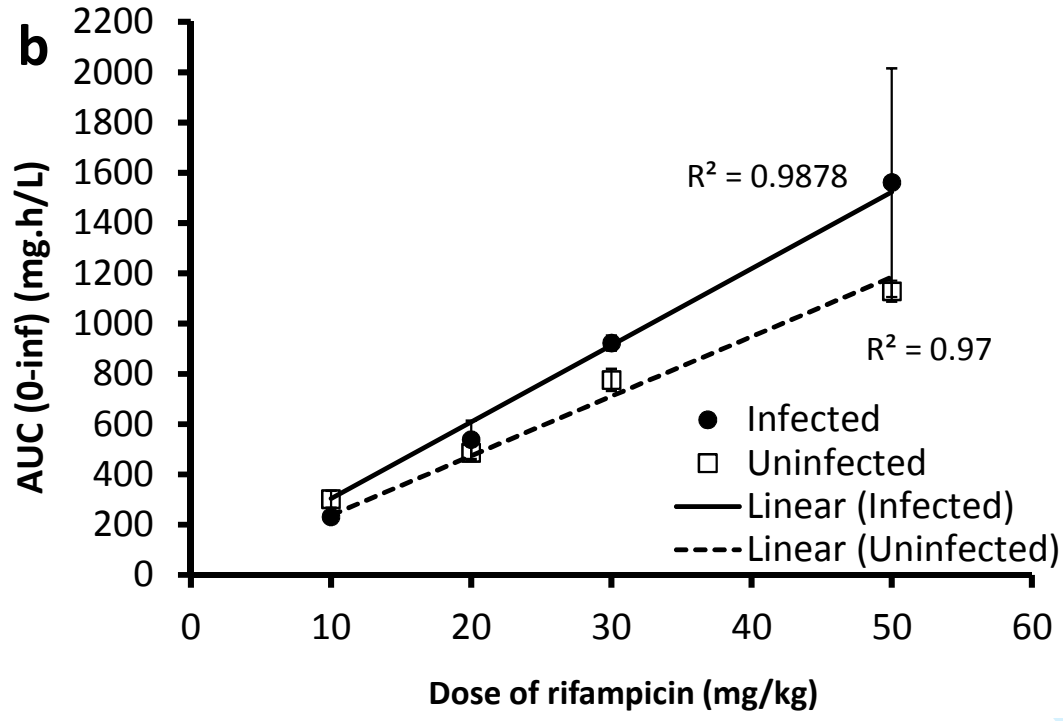

a
Log cfu/lung
Time (weeks)
R10HZ Obs
R10HZ fit
R20HZ Obs
R20HZ fit
R30HZ Obs
R30HZ fit
R40HZ Obs
R40HZ fit
R50HZ Obs
R50HZ fit


b
Log cfu/spleen
Time (weeks)
R10HZ Obs
R10HZ fit
R20HZ Obs
R20HZ fit
R30HZ Obs
R30HZ fit
R40HZ Obs
R40HZ fit
R50HZ Obs
R50HZ fit


c
Lung elimination rate constant (Wk^-1)
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
-1.2
y = -0.0137x - 0.3387
R² = 0.96
0
10
20
30
40
50
60
Dose of rifampicin (mg/kg)


d
Spleen elimination rate constant (Wk^-1)
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
-1.2
-1.3
-1.4
-1.5
y = -0.0231x - 0.274
R² = 0.95
0
10
20
30
40
50
60
Dose of rifampicin (mg/kg)


a
Cmax (mg/L)
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
Dose of rifampicin (mg/kg)
R² = 0.9863
R² = 0.9291
Infected
Uninfected
Linear (Infected)
Linear (Uninfected)


b
AUC (0-inf) (mg.h/L)
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Dose of rifampicin (mg/kg)
R² = 0.9878
R² = 0.97
Infected
Uninfected
Linear (Infected)
Linear (Uninfected)