Fully weekly antituberculosis regimen: a proof-of-concept study

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ABSTRACT

Background: The World Health Organization recommends supervising the treatment of tuberculosis. Intermittent regimens have the potential to simplify the supervision and improve compliance. Our objective was to analyse the sterilising activity of once-weekly regimens based on drugs with a long half-life, bedaquiline and rifapentine, in a murine model of tuberculosis.

Methods: 300 Swiss mice were infected intravenously infected with $\times 10^{-6}$ CFU *Mycobacterium tuberculosis* H37Rv. Mice were treated once weekly with regimens containing: 1) bedaquiline, rifapentine and pyrazinamide (BPZ); 2) BPZ plus moxifloxacin (BPZM); 3) BPZM plus clofazimine (BPZMC); 4) the standard daily regimen of tuberculosis. All regimens were given for 4 or 6 months. Bactericidal and sterilising activity were assessed.

Results: After 2 months of treatment, the mean count in lungs was $0.76 \pm 0.60 \log_{10}$ CFU in mice treated with the daily control regimen and negative in all mice treated with once-weekly regimens ($p < 0.05$ compared to the daily control). All mice had negative lung cultures on completion of either 4 or 6 months of treatment, whereas 3 months after 4 and 6 months of treatment, respectively, the relapse rate was 64% and 13% in the standard daily regimen, 5% and 0% in BPZ, 0% and 0% in BPMZ and 0% and 5% in BPMZC ($p < 0.05$ for all once-weekly regimens *versus* 4-month daily control; $p > 0.05$ for all once-weekly regimens *versus* 6-month daily control).

Conclusions: BPZ-based once-weekly regimens have higher sterilising activity than the standard daily regimen and could greatly simplify treatment administration and possibly shorten the duration of tuberculosis treatment.

Introduction

The treatment of drug-susceptible tuberculosis is based on a four-drug combination given for 6 months which has not changed for the past 40 years, despite evidence that it was not optimally designed [1]. The complexity and length of this regimen limits treatment compliance, and this can be the cause of treatment failure and emergence of drug resistance. Indeed, a study has shown that, in a daily regimen, ⩽90% adherence significantly increases the rate of unfavorable outcome [2, 3].

In recent years, multiple clinical trials have been conducted in order to reduce the duration of tuberculosis treatment. These efforts have not been successful with fluoroquinolones [4–6] and are currently ongoing with daily rifapentine and high-dose rifampin [7–9], as well as with new drugs such as bedaquiline and pretomanid [10, 11]. However, reducing the duration of treatment is not the only way to simplify the treatment and its supervision. Another approach is to space out drug administration. Current treatment regimens are given either daily or twice- or thrice-weekly. These intermittent regimens are based on old studies and may not be as active as the daily one. Efforts have been made to develop once-weekly regimens based on drugs with a long half-life [12–14]. Some of these studies have been designed and conducted based on the use of a long half-life rifamycin, rifapentine. The first studies have not been successful because rifapentine was combined with isoniazid, a drug with short half-life. Thus, the once-weekly combination of isoniazid and rifapentine was in fact a rifapentine monotherapy for 6 days out of seven, and has led to treatment failure with selection of rifamycin-resistant mutants [15]. In order to avoid this risk, rifapentine should be combined with at least one companion drug with a long half-life, or be used only in cases with low bacillary burden to reduce the risk of treatment failure and selection of drug-resistant mutants. Two different studies have demonstrated the efficacy of such strategies. In the first one, a randomised controlled clinical trial, an experimental arm containing rifapentine combined with moxifloxacin once weekly during the 4 months of treatment continuation achieved non-inferiority compared to the daily control regimen [6]. In a second clinical trial, a rifapentine–isoniazid weekly combination was used successfully for the treatment of latent tuberculosis infection [16–18]. Bedaquiline is a new anti-tuberculosis drug which has been mainly evaluated for the treatment of drug-resistant tuberculosis [19–22]. However, it has a very long half-life and could thus be a good candidate for a once-weekly regimen in combination with rifapentine, despite the known drug–drug interactions between bedaquiline and rifamycins. Since for both bedaquiline and rifapentine the main parameter predicting efficacy is the area under curve (AUC), a weekly regimen should be as active as daily one, given that the weekly AUC is the same [23–25].

Previously, we demonstrated in a murine model of tuberculosis that once-weekly regimens based on the rifapentine–bedaquiline combination have higher bactericidal activity than the standard daily regimen after 1 and 2 months [26]. The objective of the present study was to evaluate the sterilising activity of such regimens over the whole duration of treatment.

Methods

Antimicrobial agents

Bedaquiline was provided by Janssen (Beerse, Belgium). The other compounds were purchased from the following manufacturers: rifampin from Sandoz (Holzkirchen, Germany), isoniazid from Galien (Nevers, France), pyrazinamide from Sanofi (Paris, France), moxifloxacin from Eurogenerics (Manila, the Phillipines), clofazimine from Sigma (St Louis, MO, USA) and rifapentine from Carbosynth (Compton, UK).

Drugs were diluted in water containing 0.05% agar, except for bedaquiline, which was diluted in aqueous 20% 2-hydroxypropyl-β-cyclodextrin.

Drug suspensions were prepared weekly, except for rifampin (once every 2 weeks) and bedaquiline (once for the whole experiment), and were stored at 4°C.

Ethics

The study was reviewed by ethical committee Charles Darwin and conducted under approval of Ministère de l'Enseignement Supérieur de la Recherche et de l'Innovation N° 123802017112809414820v3.

Mycobacterium tuberculosis strain

The H37Rv strain of *M. tuberculosis* was grown on Lowenstein–Jensen medium. This strain was obtained from a lung of an untreated mouse from a previous experiment stored at −80°C [27]. Minimal inhibitory concentrations of drugs used in this experiment are presented in supplementary table S1.

Colonies were subcultured in Dubos broth plus 10% oleic acid–albumin–dextrose–catalase for 7 days at 37°C. The turbidity of the resulting suspension was adjusted with normal saline to match that of standard

1 mg·mL^{-1} suspension of *Mycobacterium bovis* bacille Calmette–Guérin and was further diluted with normal saline to obtain a suspension for mouse inoculation containing 2.06×10^6 CFU·mL^{-1}.

Intravenous infection

300 6-week-old Swiss female mice were purchased from the Janvier Breeding Centre (Le Genest Saint Isle, France). They were intravenously infected in the tail vein with 0.5 mL of bacterial suspension containing 1.03×10^6 CFU *Mycobacterium tuberculosis* H37Rv. Intravenous infection has been used historically in the laboratory and has shown a better correlation with relapses in clinical trials than aerosol infection [28, 29].

Chemotherapy

After infection, mice were randomised into eight groups (table 1). The negative control group contained 40 untreated mice, of which 10 were sacrificed the day after infection (D−27), 20 were sacrificed at the beginning of the treatment (D0) and 10 were sacrificed at 2 months of treatment. In the positive control group, mice received the daily standard regimen of drug-susceptible tuberculosis: 2 months of rifampin, isoniazid and pyrazinamide followed by 2 or 4 months of rifampin and isoniazid. In the experimental groups, mice were treated once weekly with 1) bedaquiline, rifapentine and pyrazinamide (BPZ); b) BPZ plus moxifloxacin (BPZM); c) BPZM plus clofazimine (BPZMC). Each experimental regimen was tested for two treatment durations: 4 and 6 months.

Treatment began 4 weeks after infection (D0). Drugs were administrated by gavage, either 5 days per week or once weekly, at the following dosing: rifampin 10 mg·kg^{-1}, isoniazid 25 mg·kg^{-1}, pyrazinamide 150 mg·kg^{-1} once daily (daily control) or 300 mg·kg^{-1} once weekly (experimental regimens); bedaquiline 62.5 mg·kg^{-1}; rifapentine 20 mg·kg^{-1}; moxifloxacin 200 mg·kg^{-1}; clofazimine 20 mg·kg^{-1}. Rifampin was administered 1 h before the other drugs to avoid drug–drug interactions. Drug dosings were selected to provide AUC values in mice comparable with those achievable in humans (supplementary table S2). For bedaquiline, since the AUC is the main parameter predictive of efficacy, weekly dosing generates an efficacy equivalent to that of daily dosing given that the total weekly amount of drug is the same. As a consequence, and as demonstrated previously, a 125 mg·kg^{-1} dosing once weekly generates the same bactericidal activity as a 25 mg·kg^{-1} dosing five times a week [23, 26]. The 25 mg·kg^{-1} dose in the mouse generates an AUC of 19 µg·h^{-1}·mL^{-1}; thus a 125 mg·kg^{-1} dosing should generate an AUC ~100 µg·h^{-1}·mL^{-1}. Since rifapentine reduces by 50% the AUC of bedaquiline in humans, and since this interaction does not exist in the mouse, we used a half-weekly dosing of bedaquiline in order to mimic the interaction seen in humans [30, 31]. Such dosing should generate an AUC close to 50 µg·h^{-1}·mL^{-1} after a single dose of bedaquiline, which corresponds to 50% of the AUC of bedaquiline after a single dose of 700 mg of bedaquiline in humans [32]. Bedaquiline undergoes accumulation both in the murine model and in the human, leading to a doubling of the AUC after multiple doses (supplementary tables S3 and S4).

Assessment of treatment efficacy

Treated mice were sacrificed through cervical elongation at 2 months and at the end of the treatment (4 or 6 months) to assess bactericidal activity. Survival rate, mean spleen weights, gross lung lesions and mean lung CFU were analysed. Lung CFU were determined by plating three serial 10-fold dilutions of

TABLE 1 Experimental design

	Mice sacrificed at:						
	Day −27	Day 0	2 months	4 months	6 months	4+3 months	6+3 months
Untreated	10	20	10				
5/7 days							
2 months RHZ/4 months RH			10		10		30
1/7 days							
4 months BPZ			10	10		20	
6 months BPZ					10		20
4 months BPMZ			10	10		20	
6 months BPMZ					10		20
4 months BPMZC			10	10		20	
6 months BPMZC					10		20
Total	10	20	50	30	40	60	90

Data are presented as n. R: rifampin; H: isoniazid; Z: pyrazinamide; B: bedaquiline; P: rifapentine; M: moxifloxacin; C: clofazimine.

homogenised suspensions onto triplicate Lowenstein–Jensen slants that were incubated at 37°C for 6 weeks.

Treated mice were sacrificed 3 months after treatment completion to assess sterilising activity. Mean spleen weights, gross lung lesions and proportion of relapsing mice with positive lung culture (relapsing mice) were analysed.

Statistical analysis

Mean spleen weights and lung CFU were compared using the Mann–Whitney test. Proportions of mice with a positive *M. tuberculosis* culture from lung tissue 3 months after the completion of treatment were compared using Fisher's test. Mice with contaminated lung cultures were excluded from analysis.

Statistical tests were performed using BioStaTGV (https://biostatgv.sentiweb.fr/).

Gene sequencing

Among mice relapsing 3 months after treatment completion in the experimental groups, gene sequencing was performed on colonies grown on Lowenstein–Jensen slants for *Rv0678*, *atpE*, *rpoB*, *pncA*, *gyrA* and *gyrB* genes, as described previously (supplementary table S5).

Results

Survival rates

Among untreated mice, one out of 10 died on day 56; the others were sacrificed at 2 months.

Among treated mice, some died of gavage accidents: seven out of 70 in the BPZ group; five out of 70 in the BPZM group; and two out of 70 in the BPZMC group. The proportion of gavage accidents was not different between daily and weekly groups (p>0.05 Fisher's exact test).

Mean spleen weights

The day after infection (D−27), the mean spleen weight was 152±20 mg and increased to 413±250 mg 4 weeks later (D0). After 2 months of treatment, the mean spleen weight decreased, although not significantly so, across all treated groups.

The mean spleen weight did not differ significantly between treated mice 2 months and mice at the end of treatment (4 or 6 months), except between treated mice at 2 months and treated mice at 4 months in the BPZMC group (259±76 mg *versus* 164±37 mg, p=0.006). The mean spleen weight was similar between mice sacrificed at 7 months (3 months after the 4-month treatment completion) and at 4 months. The mean spleen weight of mice sacrificed at 9 months (3 months after the 6-month treatment completion) was significantly lower than at 6 months in once-weekly regimen groups, but not in daily standard regimen group.

Bactericidal activity

The mean CFU count in the lungs was 2.30±0.66 $\log_{10}$ the day after infection (D−27) and increased significantly to 4.60±1.26 $\log_{10}$ at the beginning of the treatment (D0) (p=0.007) (table 2). After 2 months

TABLE 2 Bacterial counts in the lungs of mice and proportion of mice with positive lung culture after 2, 4 and 6 months of treatment in murine tuberculosis[#]

| | Proportion of mice with positive culture Bacterial counts $\log_{10}$ CFU | | | | | |
	Day 0	2 months	4 months	6 months	4+3 months	6+3 months
Untreated	4.60±1.26 (20/20)	6.03±1.70 (9/9)				
RHZ/RH		0.76±0.60 (6/9)	0 (0/5)	0 (0/5)	2.19±0.75 (9/14)	0.93±1.31 (2/15)
BPZ		0* (0/8)	0 (0/7)	0 (0/8)	1.52 (1/19)*	0 (0/17)
BPZM		0* (0/9)	0 (0/7)	0 (0/10)	0 (0/20)*	0 (0/17)
BPZMC		0* (0/10)	0 (0/9)	0 (0/9)	0 (0/20)*	2.44 (1/19)

Data are presented as mean±SD (n/N). R: rifampin; H: isoniazid; Z: pyrazinamide; B: bedaquiline; P: rifapentine; M: moxifloxacin; C: clofazimine. [#]: mice with contaminated lung culture were excluded from the analysis: RHZ n=2 (n=1 at 2 months and n=1 at 4+3 months), BPZ n=4 (n=1 at 2 months, n=1 at 4 months and n=2 at 6+3 months), BPZM n=2 (n=1 at 2 months, n=1 at 6+3 months) and BPZMC n=1 (at 4 months). *: p<0.05 *versus* control arm RHZ.

of treatment, all infected and untreated mice were sacrificed and their mean lung CFU count was 6.03 ±1.70 $\log_{10}$.

The bactericidal activity was significantly greater in the once-weekly regimen groups than in the daily standard regimen group after 2 months of treatment: the mean lung CFU count was 0.76±0.60 $\log_{10}$ in the daily standard regimen group (with six out of nine positive mice), and negative in all mice in the BPZ, BPZM and BPZMC groups (p=0.008, p=0.005 and p=0.003, respectively as compared to daily control regimen).

On completion of the 4-month and 6-month treatments, all treated mice, either with the daily standard regimen or with the once-weekly regimens, had a negative lung culture.

Sterilising activity

After 4 months of treatment and 3 months of follow-up, nine (64%) out of 14 mice relapsed in the daily standard regimen group *versus* only one (5%) out of 19 mice in the BPZ group and none out of 20 mice in both the BPZM and BPZMC groups (figure 1). The mean lung CFU counts in the relapsing mice were 2.19±0.75 $\log_{10}$ in the standard regimen group and 1.52 $\log_{10}$ in the positive mouse of the BPZ group. The relapse rate 3 months after 4 months of once-weekly treatment with BPZ, BPZM and BPZMC was significantly lower than after 4 months of daily standard treatment (p<0.001) (table 2).

After 6 months of treatment and 3 months of follow-up, two (13%) out of 15 mice relapsed in the daily standard regimen group *versus* one (5%) out of 19 in the BPZMC group, with 0.93±1.31 $\log_{10}$ and 2.44 $\log_{10}$ lung CFU counts, respectively, and no relapse in the BPZ and BPZM groups. The difference in the relapse rate between 6 months of daily standard regimen and 6 months of weekly regimen with BPZ, BPZM or BPZMC (p=0.32, p=0.32 and p=0.93, respectively), or between 6 months of daily standard regimen and 4 months of weekly regimen with BPZ, or BPZM or BPZMC (p=0.57, p=0.18 and p=0.18, respectively), did not achieve statistical significance.

In the mouse that relapsed at 6 months of treatment and 3 months of follow-up in the BPZMC group, *Rv0678*, *atpE*, *rpoB*, *pncA*, *gyrA* and *gyrB* gene sequencing did not show any mutation.

Discussion

This study demonstrates, for the first time to our knowledge, that once-weekly 4- or 6-month regimens based on the BPZ combination are at least as active as the 6-month daily standard regimen of tuberculosis in a murine model.

In a previous experiment in this model, we showed that the bactericidal activity of bedaquiline is the same whatever the frequency of administration (once weekly, twice weekly or five times per week), provided that
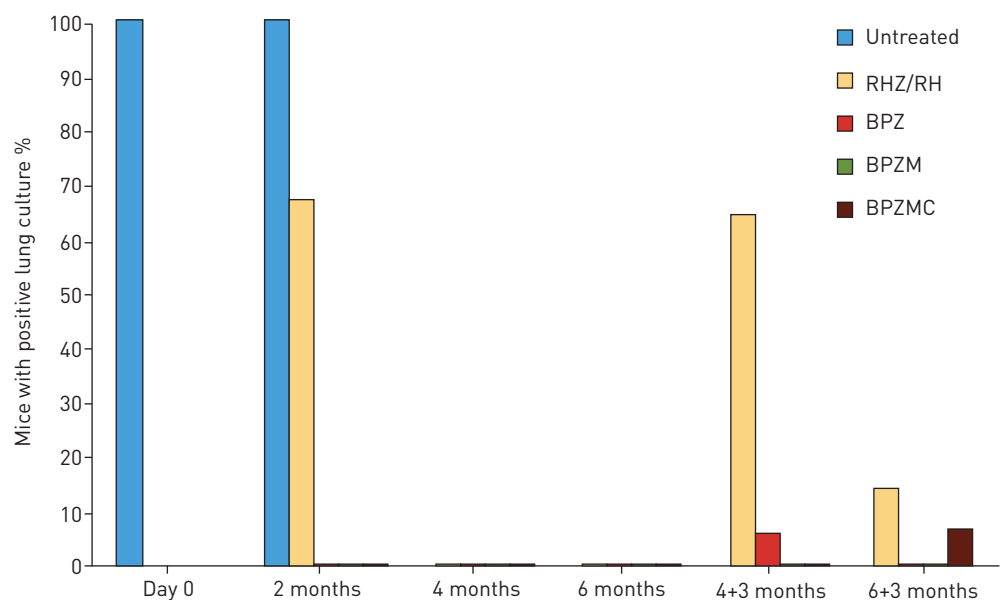

FIGURE 1 Proportion of mice with positive lung culture after 2, 4 and 6 months of treatment and 3 months after treatment competion in murine tuberculosis. R: rifampin; H: isoniazid; Z: pyrazinamide; B: bedaquiline; P: rifapentine; M: moxifloxacin; C: clofazimine.

the total weekly dose is the same [26]. However, in this previous study, we did not take into account the pharmacokinetic interaction between rifapentine and bedaquiline. Indeed, it was shown later that rifapentine reduces bedaquiline AUC by 50% [33]. As a consequence, in the current study we reduced the bedaquiline weekly dose by half, from 125 to 62.5 mg·kg^{-1}, in order to mimic the AUC reduction seen in humans in presence of rifapentine and which does not exist in mouse [34].

Despite this drug–drug interaction, we believe that there is a clear benefit in combining these two drugs. We showed previously that in a daily regimen, the most active combinations with bedaquiline included rifampin and pyrazinamide [28]. A daily regimen of bedaquiline, pyrazinamide and rifapentine given for just 3 months resulted in a similar proportion of relapses as the standard regimen after 6 months [35]. As these drugs all have potent sterilising activities, it is not surprising that their combination is very active.

In the current study, all three BPZ once-weekly groups showed superiority compared to standard daily regimen in terms of bactericidal activity at 2 months and of sterilising activity at 4 months of treatment and 3 months of follow-up. At 6 months of treatment and 3 months of follow-up, the difference in bactericidal and sterilising activity was no longer significant, but at this point the relapse rate of the control regimen was low (13%). A higher number of individuals per arm might be required to show a statistically significant difference. In addition, it must be underlined that the relapse rate of the once-weekly test regimens after 4 months was not different from that of the control regimen after 6 months. Therefore, it is possible that a BPZ regimen may reduce both the frequency of drug administration and the total duration of treatment.

Based on previous studies, we hypothesised that the addition of clofazimine and moxifloxacin could increase the sterilising activity of the BPZ combination [34, 36, 37]. In our study, no difference was shown in the groups treated with BPZ plus moxifloxacin and clofazimine–moxifloxacin. This might be partially explained by the high sterilising activity of the BPZ regimen. ⩾80 mice per group and per time-point sacrifice would be required in order to show a 20% statistical difference. Similarly, in another study testing daily treatment with BPZ, the addition of moxifloxacin did not increase the sterilising activity of the regimen [35].

Based on our knowledge of antituberculosis chemotherapy, an initial "intensive phase" with daily treatment could be warranted before switching to once-weekly dosing. Such daily intensive phase could rapidly reduce the bacillary load. This is unlikely to occur with a once-weekly bedaquiline-based regimen, given the delayed early bactericidal activity exerted by bedaquiline [38, 39]. In our opinion, moxifloxacin could be included in such a clinical trial regimen, despite the lack of additive effect shown in our study. Indeed, moxifloxacin has demonstrated early bactericidal activity [40]; in addition, the long half-life of this drug makes it a good companion drug for rifapentine [6]. Regarding clofazimine, at present, there has been no clinical proof of its activity in the context of drug-susceptible tuberculosis and skin pigmentation can be a limit to its use, even if it may be less pronounced if given weekly. Conversely, data from murine studies support the use of clofazimine against drug-susceptible tuberculosis [36, 41]; in addition, clofazimine has been shown to act synergistically with bedaquiline in a murine model [36] and to have a long half-life [42, 43], making it an interesting choice for weekly regimens. However, bedaquiline, clofazimine and moxifloxacin are all known to prolong the QT interval and should be used carefully in patients either in clinical trials or in programmatic use.

When discussing findings from preclinical models, a crucial question is to what extent are they predictive for treatment outcomes in humans. The model used here (*i.v.*-infected Swiss mice) can be considered as a very conservative one and therefore not expected to generate results which are too "optimistic", compared to what eventually is seen in humans [44]. This was illustrated in the evaluation of the ability of fluoroquinolones to shorten treatment. While in a model of aerosol-infected BALB/c mice the 4-month moxifloxacin-based regimen was as active as the standard 6-month daily regimen, this was not the case in our model [28, 29]. Ultimately, the clinical studies confirmed the inferiority of the 4-month moxifloxacin (REMOX study) and gatifloxacin (OFLOTUB study) regimen [4–6].

Another limit is the number of animals used in the study, which, although high for a murine study (300 mice), remains low compared to the expected sample size of a clinical trial (~500 patients per arm). Thus, like others conducted in murine models, this study must be seen as a proof-of-concept that needs to be confirmed in a clinical trial.

Additionally, toxicity in relation to high peak serum levels the day of drug intake may be a limiting factor. Finally, the reduction of the bedaquiline AUC in presence of rifapentine may be seen as a limitation, since this reduction may vary from patient to patient and generate heterogeneous exposures to bedaquiline, requiring therapeutic drug monitoring [45]. A possible solution would be to replace rifapentine with another drug with a long half-life: moxifloxacin, which has shown interesting activity combined with

bedaquiline without rifapentine, or other newly approved drugs or those under development [34, 46–48]. Whatever the choice, the potential candidate should have sterilising properties at least as strong as those of the rifamycins; a preliminary pharmacokinetic study assessing the different drug interactions in human would be required before starting a therapeutic trial.

Until now, bedaquiline development has focused on drug-resistant tuberculosis, similarly to two other recently approved drugs, delamanid and pretomanid. We believe that these new drugs could also play a role in improving treatment of drug-susceptible tuberculosis. Overall, the development of intermittent regimens has been somewhat neglected in favour of shortened regimens. It should be recalled that to date, whereas all shorter fluoroquinolone-based regimens failed to show non-inferiority to the standard daily 6-month regimen, a regimen including once-weekly rifapentine–moxifloxacin during the continuation phase managed to show non-inferiority [6]. If confirmed in a clinical trial, such a weekly regimen would reduce the number of days with drug intakes from 182 to 26. A daily regimen would have to be reduced to <1 month in order to reach a similar low number of drug intakes. Once-weekly regimens should be explored to increase compliance.

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