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PA-824 is as effective as isoniazid against latent tuberculosis infection in C3HeB/FeJ mice

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Abstract

The bicyclic nitroimidazole-like molecule PA-824 has activity both against replicating and hypoxic non-replicating *Mycobacterium tuberculosis*, raising the possibility that it may have a role in the treatment of latent tuberculosis infection (LTBI). This study aimed to examine the bactericidal and sterilising activities of PA-824 against LTBI in C3HeB/FeJ mice, which develop hypoxic, necrotic granulomas histologically resembling their human counterparts. Female 5–6-week-old C3HeB/FeJ mice were immunised via the aerosol route with a recombinant BCG strain overexpressing the 30-kD major secretory protein (rBCG30) and were aerosol-infected 6 weeks later with virulent *M. tuberculosis* H37Rv. Six weeks after *M. tuberculosis* infection, separate groups of mice were left untreated (negative controls) or were treated with either rifampicin, isoniazid (INH) or PA-824. Culture-positive relapse was assessed in subgroups of mice after 2 months and 4 months of treatment. Human-equivalent doses of PA-824 given five times weekly showed similar bactericidal activity as INH at Months 1, 2 and 4 of treatment, and 15/15 mice treated with either PA-824 or INH showed lung-culture relapse 3 months after completion of treatment. To our knowledge, this is the first report examining the sterilising activity of PA-824 in an animal model of LTBI. This model may be useful for screening the efficacy of novel drugs against LTBI, particularly those with specific activity against bacilli residing within necrotic lung granulomas.

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Ethical approval: All procedures involving animals were performed in compliance with the US Animal Welfare Act regulations and Public Health Service Policy according to protocols approved by the Institutional Animal Care and Use Committee at Johns Hopkins University (Baltimore, MD) [institutional animal welfare no. A3272-01].

Keywords

Latent tuberculosis infection; Animal models; Sterilising; Bactericidal activity; C3HeB/FeJ mice; PA-824

1. Introduction

It is estimated that one-third of the world's population is latently infected with *Mycobacterium tuberculosis*, representing a vast potential reservoir for subsequent reactivation disease, particularly in the setting of the human immunodeficiency virus (HIV) pandemic. Although not proven, latent tuberculosis infection (LTBI) is thought to represent the immunological control of a paucibacillary population of non-replicating and slowly metabolising organisms that have adapted to the unfavourable conditions within lung caseous granulomas, the microenvironment of which may include hypoxia, nutrient limitation and acidic pH [1]. Importantly, these 'dormant' bacilli exhibit antibiotic tolerance as they become, in the absence of genetic mutations, less susceptible to the bactericidal drug isoniazid (INH), which inhibits the mycolic acid synthesis pathway required for cell wall synthesis, but remain susceptible to the transcriptional inhibitor rifampicin (RIF). Thus, clinical studies have shown that LTBI can be eradicated using 9 months of daily treatment with INH or 4 months of daily treatment with RIF [1]. The success of global tuberculosis (TB) eradication efforts is contingent upon the development of new drugs that can accelerate the clearance of LTBI [2].

Recently, the bicyclic nitroimidazole-like molecule PA-824 has been shown to have activity both against replicating and hypoxic non-replicating *M. tuberculosis* [3–5]. PA-824 exhibits bactericidal and sterilising activity against active TB infection in BALB/c mice [6,7] and guinea pigs [6,8,9] as well as substantial early bactericidal activity in human TB disease [10]. PA-824 is a pro-drug that undergoes nitroreduction to one or more active compounds. In addition to inhibiting keto-mycolic acid and protein synthesis, PA-824 also kills *M. tuberculosis* through a novel mechanism involving generation of intracellular nitric oxide [5]. Recently, we developed a novel model of LTBI in C3HeB/FeJ mice [11], which develop hypoxic, necrotic TB lung granulomas histologically resembling their human counterparts. In the current study, the efficacy of PA-824 given alone at human-equivalent doses in clearing paucibacillary infection was evaluated in this model.

2. Methods

2.1. Study design

All animal-related procedures were approved by the Johns Hopkins University School of Medicine Animal Care and Use Committee (Baltimore, MD). Female C3HeB/FeJ mice (5–6-weeks-old) were immunised via the aerosol route with a recombinant BCG strain overexpressing the 30-kD major secretory protein (rBCG30) and were aerosol-infected 6 weeks later with virulent *M. tuberculosis* H37Rv as previously described [11]. In this model, a stable lung count of ca. 10^4 bacilli is established and the mice do not succumb to TB. Six weeks after aerosol *M. tuberculosis* infection, separate groups of mice were left untreated or were randomised to daily (5 days/week) oral treatment with one of the following antibiotic

regimens at human-equivalent doses: 10 mg/kg INH; 10 mg/kg RIF; or 50 mg/kg PA-824. Treatment was discontinued for groups of 15 mice after completion of 2 months or 4 months of antibiotic treatment for assessment of relapse. Animal total body, lung and spleen weights were recorded, and the lungs and spleen were examined grossly for visible lesions and were photographed at the time of sacrifice. Endpoints included the bactericidal activity of each regimen at defined time points and relapse rates. Relapse was defined as positive culture upon plating entire undiluted lung homogenates, with a theoretical detection limit of 1 bacillus/lung. CFU counts reported for *M. tuberculosis* represent colonies growing on thiophene-2-carboxylic acid hydrazide-containing plates during indicated treatment. CFU and relapse data for INH and RIF control groups, obtained in parallel with those for the PA-824 group, have been published previously in graphical form [11].

2.2. Statistical analysis

Pairwise comparisons of group mean values for organ weights and $\log_{10}$-transformed CFU counts were made using Student's *t*-test and one-way analysis of variance (ANOVA) and Bonferroni's post-test with GraphPad InStat v.3.05 (GraphPad Software Inc., San Diego, CA).

3. Results

All untreated and treated mice survived until they were sacrificed according to the protocol (Table 1). After 4 months of treatment, both untreated and treated animals gained weight throughout, without any significant differences in total body weight (Supplementary Table S1) or normalised mean lung and spleen weights (Supplementary Table S2; Supplementary Fig. S1) between groups. All regimens displayed bactericidal activity but differed in their potency (Table 1). RIF was more effective than INH and PA-824 ($P < 0.05$ for both comparisons), rendering all mice culture-negative at Month 4. Although PA-824 showed mildly superior bactericidal activity to INH at Month 1 ($P = 0.04$) and Month 2 ($P = 0.37$), INH showed statistically significantly superior activity to PA-824 at Month 4 ($P = 0.03$). As shown in Table 1, 4 months of treatment with PA-824 was not sufficient to sterilise mouse lungs, as 100% of animals (15/15) relapsed 3 months after treatment completion. Relapse rates were equivalent in PA-824 and INH groups and were inferior to those in RIF-treated mice.

4. Discussion

These data suggest that PA-824 has relatively limited activity, similar to INH, against LTBI in C3HeB/FeJ mice. The apparent discrepancy in the known in vitro activity of the drug against hypoxic cultures of *M. tuberculosis* and the current findings may be explained by reduced bioavailability of the drug within mouse necrotic lung granulomas, which was not assessed in this study. Other drugs, such as moxifloxacin, have been shown to have good distribution in cellular regions of granulomas and uptake by macrophages, but decreased penetration into the cores of necrotic lesions in rabbits [12]. The same bioavailability profile was predicted recently for the standard TB drugs using mathematical modelling and was confirmed through plasma and tissue pharmacokinetic studies [13]. Furthermore, the activity of PA-824 may be overrepresented in animal models in which infection is predominantly

intracellular relative to the mouse strain used in these studies [14], in which the majority of bacilli are located in the extracellular compartment. Consistent with this hypothesis, recent data derived from the same model show that PA-824 has inferior bactericidal activity relative to RIF and is comparable with that of INH [15]. Based on previous data from in the guinea pig model and a phase 2 early bactericidal activity study [8,10], we believe that PA-824 may have a role as a companion drug, in conjunction with pyrazinamide, bedaquiline or sutezolid, for the treatment of LTBI.

Future studies will investigate the basis for the limited activity of PA-824 in this model, including assessment of intralesional pharmacokinetics of the drug and selection of drug-resistant mutants upon relapse. Further studies are also warranted to evaluate novel drugs in this clinically relevant model, with the goal of identifying more potent regimens that can more effectively eradicate drug-susceptible and drug-resistant LTBI. In particular, this model may be a powerful tool to identity new derivatives of bicyclic nitroimidazoles with more potent sterilising activity than PA-824.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Bacillary burden in the lungs of latently infected mice [a] during treatment, and relapse rates

Group	Mean (± S.D.) $\log_{10}$ CFU count at:					Proportion (%) relapse, assessed 3 months after completion of treatment for:	
	Month–1.5	Month 0	Month 1	Month 2	Month 4	2 months	4 months
Untreated	1.15 ± 0.12	4.24 ± 0.13	4.30 ± 0.16	4.01 ± 0.27	4.12 ± 0.25	N/D	N/D
INH (10 mg/kg)			2.80 ± 0.30	1.92 ± 0.32	1.12 ± 0.33	15/15 (100)	15/15 (100)
RIF (10 mg/kg)			1.93 ± 0.31	0.81 ± 0.2	0 ± 0	15/15 (100)	5/15 (33)
PA-824(50 mg/kg)			2.41 ± 0.19	1.75 ± 0.22	1.54 ± 0.13	15/15 (100)	15/15 (100)
Total mice	5	5	20	20		45	45

S.D., standard deviation; N/D, not done; INH, isoniazid; RIF, rifampicin.

[a] Mice were immunised via aerosol with a recombinant BCG strain overexpressing the 30-kD major secretory protein (rBCG30), resulting in mean (± S.D.) implanted lung CFU counts of 3.61 ± 0.07 $\log_{10}$. Six weeks later, the mean lung rBCG30 CFU counts had increased to 5.86 ± 0.18 $\log_{10}$.

Data from control groups (untreated, INH and RIF), conducted in parallel with PA-824 treatment, are derived from our recently published study [11].