AAC Journals.ASM.org

Rifapentine Is Not More Active than Rifampin against Chronic Tuberculosis in Guinea Pigs

Noton K. Dutta,[a] Peter B. Illei,[b] Charles A. Peloquin,[c] Michael L. Pinn,[a] Khisimuzi E. Mdluli,[d] Eric L. Nuermberger,[a,e] Jacques H. Grosset,[a] and Petros C. Karakousis[a,e,f]

Departments of Medicine[a] and Pathology[b] and Center for Tuberculosis Research,[f] Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; College of Pharmacy, University of Florida, Gainesville, Florida, USA[c]; Global Alliance for TB Drug Development, New York, New York, USA[d]; and Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA[e]

Rifamycins are key sterilizing drugs in the current treatment of active tuberculosis (TB). Daily dosing of rifapentine (P), a potent rifamycin with high intracellular accumulation, in place of rifampin (R) in the standard antitubercular regimen significantly shortens the duration of treatment needed to prevent relapse in a murine model of active TB. We undertook the current study to compare directly the activities of human-equivalent doses of P and R in a guinea pig model of chronic TB, in which bacilli are predominantly extracellular within human-like necrotic granulomas. Hartley strain guinea pigs were aerosol infected with ~200 bacilli of *Mycobacterium tuberculosis* H37Rv, and treatment given 5 days/week was initiated 6 weeks later. R at 100 mg/kg of body weight and P at 100 mg/kg were given orally alone or in combination with isoniazid (H) at 60 mg/kg and pyrazinamide (Z) at 300 mg/kg. Culture-positive relapse was assessed in subgroups of guinea pigs after completion of 1 and 2 months of treatment. Human-equivalent doses of R and P showed equivalent bactericidal activity when used alone and in combination therapy. In guinea pigs treated with rifampin, isoniazid, and pyrazinamide (RHZ) or PHZ, microbiological relapse occurred in the lungs of 8/10 animals treated for 1 month and in 0/10 animals treated for 2 months. Substitution of P for R in the standard antitubercular regimen did not shorten the time to cure in this guinea pig model of chronic TB. Data from ongoing clinical trials comparing the activity of these two drugs are awaited to determine the relevance of the guinea pig TB model in preclinical drug screening.

Rifapentine (P), a cyclopentyl rifamycin with a much longer half-life than rifampin (R) (10 to 15 h compared to 2 to 5 h, respectively), was developed with the goal of allowing highly active once-weekly therapy for tuberculosis (TB) (37). However, relative to twice- or thrice-weekly rifampin-isoniazid (RH), once-weekly PH during the continuation phase of treatment was associated with greater drug-susceptible relapse among HIV-negative patients with lung cavitation (8) and a significant incidence of acquired rifamycin monoresistance among HIV-positive patients (35). Recent data in a murine model of active TB showed that increasing rifamycin exposure by daily dosing of P in place of R improved sterilizing activity, suggesting that daily P might permit shortening of treatment duration (28–30). These encouraging results prompted a large-scale phase 2 clinical trial conducted by the Tuberculosis Trials Consortium (TBTC study 29) to evaluate the safety and efficacy of a regimen in which P is substituted for R during the initial 8 weeks of treatment (13).

P is known to have significantly higher intracellular accumulation relative to R, as the MIC and minimal bactericidal concentration (MBC) of P against *Mycobacterium tuberculosis* H37Rv within macrophages are 4-fold lower than the corresponding values against extracellular bacilli (22). On the other hand, the MIC and MBC of R against intracellular *M. tuberculosis* are 2-fold higher than the corresponding values against extracellular bacilli, suggesting that P may be 8-fold more potent than R against intracellular *M. tuberculosis* (22). The impact of higher protein binding of P (97% or higher) relative to R (80 to 85%) on differences in pharmacokinetic and pharmacodynamic properties of the drugs is unknown (9). We hypothesized that the efficacy of P relative to R may be overrepresented in the murine model of TB, in which the bacilli are almost exclusively located in the intracellular compartment (30). In addition, since human TB is characterized histologically by the presence of necrotic granulomas harboring persistent bacilli (34), which are absent in BALB/c mice (7, 17, 31), it is possible that penetration of P into such lesions may be limited due to its relatively high protein binding. In the current study, we directly compared the activity of human-equivalent exposures of P and R, both alone and in combination with the first-line drugs isoniazid (H) and pyrazinamide (Z), in a guinea pig model of chronic TB, which is characterized by a predominantly extracellular population of bacilli residing within necrotic lung granulomas histologically resembling their human counterparts (1–5, 18, 19, 21, 23).

MATERIALS AND METHODS

***Mycobacterium tuberculosis* strains.** The Johns Hopkins Center for Tuberculosis Research laboratory reference strain *M. tuberculosis* H37Rv (17) was used. Aliquots were thawed and grown to logarithmic phase (optical density at 600 nm of 0.6) in supplemented Middlebrook 7H9 broth (Difco) prior to aerosol infection (2).

Animals. Female outbred Hartley guinea pigs (250 to 300 g) with and without jugular vein vascular catheters were purchased from Charles River (Wilmington, MA). All procedures followed protocols approved by the Institutional Animal Care and Use Committee at Johns Hopkins University.

Received 26 March 2012 Returned for modification 5 April 2012
Accepted 23 April 2012
Published ahead of print 30 April 2012
Address correspondence to Petros C. Karakousis, petros@jhmi.edu.
Supplemental material for this article may be found at http://aac.asm.org/.

doi:10.1128/AAC.00500-12

TABLE 1 Basic experimental scheme

Group[a]	No. of guinea pigs at the following time point (mo)[b]: −1.5	0	1 (+3)[c]	2 (+3)	Total no. of guinea pigs (+3)
Untreated group	4	4	4	4	16
Treated groups					
R_{50}			4	4	8
R_{100}			4	4	8
P_{50}			4	4	8
P_{100}			4	4	8
$R_{100}H_{60}Z_{300}$			4 (10)	4 (10)	8 (20)
$P_{100}H_{60}Z_{300}$			4 (10)	4 (10)	8 (20)
All groups					104

[a] The drugs were given alone or in combination and are abbreviated as follows: R, rifampin; P, rifapentine; H, isoniazid; Z, pyrazinamide. The drug doses (in milligrams per kilogram of body weight) are indicated by the subscript numbers. Doses of each drug were determined to be equivalent based on area under the serum drug concentration-time curve (AUC) and were given daily (5 days a week) by gavage.
[b] Time points: month −1.5, day after infection with *M. tuberculosis*; month 0, day of treatment initiation; month 1, 1 month after treatment initiation, and so on.
[c] Some guinea pigs were held for 3 months after treatment completion (+3) before being killed for the relapse assessment. The number of guinea pigs held for 3 months after treatment completion is shown in parentheses.

Pharmacokinetic studies. A single dose of rifapentine (Priftin; Sanofi-aventis) was given to separate groups of catheterized guinea pigs. One dose of 50 mg of rifapentine per kg of body weight (P_{50}) was given to three guinea pigs, and one dose of 100 mg/kg of body weight (P_{100}) was given to six guinea pigs. Doses were prepared in water in a final volume of 0.5 ml and administered orally, as previously described (4). Blood samples (~0.3 ml) were drawn serially from guinea pigs through the intravenous catheter at the following time points after antibiotic dosing: 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h. Serum samples were separated, stored at −80°C, and analyzed using a validated high-performance liquid chromatography (HPLC) assay, whose performance characteristics have been described previously (28, 30).

Aerosol infections. Log-phase cultures of *M. tuberculosis* H37Rv were diluted 500-fold (to ~10^5 bacilli per ml) in 1× phosphate-buffered saline (PBS) for aerosol infection. A total of 104 guinea pigs were aerosol infected with a Madison chamber aerosol generation device (University of Wisconsin, Madison, WI) calibrated to deliver approximately 200 bacilli into guinea pig lungs (1, 4).

Antibiotic treatment. Based on the area under the serum drug concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$), the human-equivalent doses of R, H, and Z in guinea pigs were determined previously to be 100 mg/kg (R_{100}), 60 mg/kg (H_{60}), and 300 mg/kg (Z_{300}), respectively (3, 4). Daily (5 days/week) oral treatment with R_{50} or P_{50} alone and R_{100} or P_{100} alone and in combination with H_{60} and Z_{300} was initiated 6 weeks after infection for a total duration of 8 weeks (Table 1). R and P doses were given before the other drugs by at least 1 h to prevent pharmacokinetic antagonism (14). Animals were treated with a formulation consisting of 40% sucrose (wt/vol), 20% pumpkin (wt/vol) (Libby's 100% pure pumpkin) mixture supplemented with vitamin C (50 mg/kg mean body weight) and commercial *Lactobacillus* (BD Lactinex) (Walmart, Towson, MD) (23). Treatment was discontinued for groups of 10 guinea pigs after completion of 1 month or 2 months of RHZ or PHZ treatment for relapse assessment.

Study endpoints. Four animals were sacrificed on the day after infection and 6 weeks after infection to determine the number of implanted bacilli and the bacillary burden at treatment onset, respectively (Table 1). Four animals from each group were sacrificed after 1 and 2 months of treatment to assess the bactericidal activity of each regimen.

The total animal body, lung, and spleen weights were recorded, and the lungs were examined grossly for visible lesions at necropsy. To account for the physiological increase in organ weights in aging animals, the organ weights were normalized using the following formula: organ weight at sacrifice × (mean body weight on day after infection/body weight at sacrifice). Lungs were sectioned in a standardized fashion along the longitudinal axis (apex to lower lobe), traversing the maximum horizontal dimension (through the hilum). Lung samples were placed into 10% buffered formaldehyde, processed, and embedded in paraffin for histological staining. At least one entire hematoxylin-and-eosin-stained lung cross section per animal (4 animals/group) was assessed, and the surface area occupied by granulomatous inflammation was determined using manual semiquantitative analysis using low and intermediate power magnification (20 to 100×), similar to clinical assessment of Her2/Neu expression in breast cancer. Granulomas were classified as primary (necrotizing) and secondary (nonnecrotizing) and analyzed for the presence of acid-fast bacilli on Kinyoun-stained consecutive sections using high magnification (400×), and the number of organisms were counted per individual granuloma. The overwhelming majority of organisms were seen in necrotizing granulomas. Histological analysis was performed by a board-certified pathologist (P.B.I.), who was unaware (or blinded) of which animals were in which treatment groups.

The remainder of the lung samples was homogenized in 10 to 20 ml of PBS (18). Spleen samples were homogenized in 5 to 15 ml of PBS using glass homogenizers. After mixing, serial 0.5-ml organ tissue aliquots were plated on selective 7H11 agar plates and incubated at 37°C for 6 weeks for CFU determination.

Groups of 10 animals treated with $R_{100}H_{60}Z_{300}$ or $P_{100}H_{60}Z_{300}$ for a total of 1 month or 2 months were sacrificed 3 months after treatment discontinuation to assess the sterilizing activity of each combination regimen. Relapse was defined as positive culture upon plating entire undiluted lung homogenates (15 to 20 plates per animal lung), with a theoretical detection limit of 1 bacillus/lung.

Statistical analysis. For pharmacokinetics studies, data represent median values. Organ CFU values were log transformed prior to calculating the means and standard deviations for each group. All statistical variation values presented in this study refer to standard deviations of the means. Quantitative results are presented using the mean values from four guinea pigs per group and were statistically compared using parametric measures (Student's *t* test and analysis of variance [ANOVA]); a *P* value of <0.05 was considered significant.

RESULTS

Pharmacokinetics of rifapentine in guinea pigs. After oral administration of P in guinea pigs, the median peak serum drug

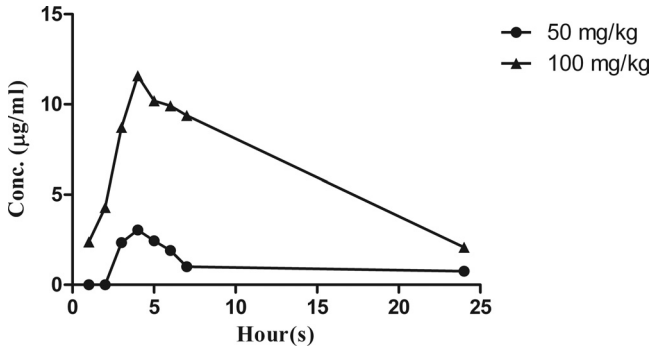

FIG 1 Plasma rifapentine concentration profile in guinea pigs after single doses of 50 mg/kg ($n = 3$) and 100 mg/kg ($n = 6$). Median values are shown in the graph.

TABLE 2 Comparison of rifapentine pharmacokinetics in guinea pigs, mice, and humans

Test species	Drug dosage	Population	Frequency[a]	Food	PK sampling dose	*n*	C_{max} (μg/ml)		AUC_{0-24} (mg · h/liter)		$t_{1/2}$ (h)	
							Median	Range	Median	Range	Median	Range
Guinea pig	50 mg/kg	Healthy	D1	Yes	Single	3	3.16	2.35–3.89	30.25	23.52–45.85	15.61	11.37–21.45
Guinea pig	100 mg/kg	Healthy	D1	Yes	Single	6	11.58	3.55–22.26	187.50	67.32–297.90	21.87	9.05–41.03
Mouse[b]	10 mg/kg	Healthy	D1	Yes	Single	6	19.26	18.05–21.92	321.49	292.46–354.43	20.56	15.24–22.66
Mouse[c]	10 mg/kg	Healthy	D1	Yes	Single	3	28.23	28.14–29.87	282.44	264.09–304.54	25.58	18.98–33.63
Mouse[d]	10 mg/kg	Healthy	D1	Yes	Single	3	14.77	11.41–15.24	237.25	236.14–240.20	21.65	18.98–39.05
Mouse[e]	10 mg/kg	Healthy	SS	Yes	2 wk	3	18.25	17.15–23.68	345.08	267.14–396.40	20.22	19.21–20.46
Human[f]	600 mg	TB	SS	No	Weekly	15	13.36	6.52–25.54	182.14	104.28–434.88	13.86	10.33–20.45
Human[f]	900 mg	TB	SS	No	Weekly	7	15.14	6.57–36.91	229.08	101.81–504.84	16.08	11.99–18.91
Human[f]	1,200 mg	TB	SS	No	Weekly	13	18.95	6.76–54.16	286.96	95.74–706.79	15.30	10.50–19.09

[a] D1, once daily; SS, steady state.
[b] Data from reference 6.
[c] Data from E. L. Nuermberger, unpublished data.
[d] Data from reference 30.
[e] Data from I. M. Rosenthal, R. Tasneen, C. A. Peloquin, M. Zhang, D. Almeida, K. Mdluli, P. C. Karakousis, J. H. Grosset, and E. L. Nuermberger, submitted for publication.
[f] Data from reference 36.

concentrations (C_{max}) were 3.16 and 11.58 μg/ml after 50 (P_{50}) and 100 (P_{100}) mg/kg doses, respectively (Fig. 1 and Table 2). Area under the serum drug concentration-time curve from 0 to 24 h (AUC_{0-24}) values were 30.25 and 187.50 mg · h/liter for P_{50} and P_{100}, respectively. The C_{max} and AUC_{0-24} of P_{100} in guinea pigs closely approximate the corresponding values for weekly P dosing (600 mg) in humans and after a single dose of P (10 mg/kg) in mice (Table 2). The pharmacodynamics of P (36) are not as well-established as for R (24, 25, 38) (Tables 2 and 3). However, it is likely that concentration-dependent activity, which is profound for R (Table 3) is a class effect (15, 16). Therefore, C_{max}/MIC and AUC/MIC likely are the most important parameters for matching P exposures (6, 30).

Morbidity, mortality, and organ pathology during treatment. No deaths were observed in either the treated or untreated groups of guinea pigs during the course of the experiment. After 2 months of infection, untreated animals began to lose weight (see Table S1 in the supplemental material), ultimately requiring euthanasia due to TB-related morbidity (data not shown), whereas treated animals gained weight throughout. The normalized mean lung and spleen weights of treated guinea pigs showed decreasing trends over time (Table S2). At the initiation of antibiotic treatment, all guinea pig lungs showed well-demarcated tubercle lesions (see Fig. S1 in the supplemental material) and granulomatous inflammation (Fig. S2). The lungs of animals that received 4 weeks of drug therapy had less gross inflammation (Fig. S1) and a reduction in the surface area involved by granulomas compared to animal lungs at the start of treatment (Table S3). R and P treatment reduced the number and size of lung lesions and prevented the development of necrosis in granulomas when used alone and in combination therapy during the first 8 weeks of treatment (Table S3). Each of the treatment groups showed a similar reduction in the number of visible acid-fast bacilli (AFB) within necrotic granulomas during therapy (Table S3). However, upon treatment

TABLE 3 Comparison of rifampin pharmacokinetics in guinea pigs, mice, and humans

Test species	Drug dosage	Population	Frequency[a]	Food	PK sampling dose	*n*	C_{max} (μg/ml)		AUC_{0-24} (mg · h/liter)		$t_{1/2}$ (h)	
							Median	Range	Median	Range	Median	Range
Guinea pig[b]	100 mg/kg	Healthy	D1	Yes	Single	6	14.80	6.27–16.86	71.06	32.66–124.24	2.30	1.77–4.44
Mouse[c]	10 mg/kg	Healthy	D1	Yes	Single	3	17.11	10.99–18.92	122.07	118.88–135.80	5.29	5.21–5.55
Mouse[d]	10 mg/kg	Healthy	D1	Yes	Single	3	10.57	9.70–10.62	105.06	90.64–110.94	4.68	4.12–5.24
Mouse[e]	10 mg/kg	Healthy	SS	Yes	2 weeks	3	14.06	12.96–15.66	128.13	114.67–138.61	1.97	1.73–2.32
Human[f]	600 mg	Healthy	D1	No	Single	24	11.80	9.65–24.99	70.46	49.30–139.73	3.10	2.10–6.22
Human[g]	600 mg	Healthy	D1	No	Single	14	11.06	6.20–17.36	52.63	29.02–98.96	3.03	1.85–4.88
Human[h]	600 mg	Healthy	D10	No	10	16	10.31	3.48–17.76	39.56	20.39–96.17	1.81	1.40–2.64
Human[h]	600 mg	TB	D9 to 40	No	3 to 5	70	6.37	0.87–25.23	38.56	8.97–128.18	2.08	0.88–9.08

[a] D10, every 10 days; SS, steady state.
[b] Data from reference 4.
[c] Data from reference 6.
[d] Data from reference 30.
[e] Data from reference 35.
[f] Data from reference 24.
[g] Data from reference 25.
[h] Data from reference 38.

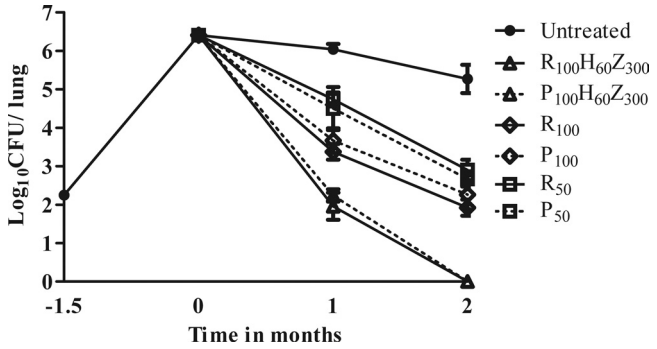

FIG 2 Antituberculosis activity of indicated drugs in infected guinea pigs. Animals were infected via aerosol with ~10^2 CFU of *M. tuberculosis* H37Rv strain and were either left untreated or treated with drugs beginning 6 weeks after infection. Values are the means of log-transformed lung CFU ± standard deviations of the means (error bars) for four animals.

completion and at the relapse assessment time points, few AFB could still be detected in some animals within the necrotic centers of remaining granulomas (Table S3), which were present in the periphery of the lungs (Fig. S3).

Bactericidal activity of R or P alone and in combination with HZ against chronic TB infection in guinea pigs. On the day after aerosol infection (month −1.5 of treatment), 2.2 ± 0.1 $\log_{10}$ CFU were recovered from the lungs of guinea pigs. The bacilli grew exponentially to a peak lung burden of 6.4 ± 0.1 $\log_{10}$ CFU at the time of treatment initiation (month 0). R and P given alone each showed statistically significant dose-dependent killing, which was very similar in magnitude for equivalent rifamycin doses. Lung CFU declined by an average of 1.9 and 3.0 $\log_{10}$ CFU ($P < 0.01$) following daily monotherapy with R_{50} and R_{100}, respectively, and by an average of 1.7 and 2.7 $\log_{10}$ CFU ($P < 0.01$) following monotherapy with P_{50} and P_{100}, respectively. On completion of 2 months of treatment, the lungs of guinea pigs receiving daily monotherapy with R_{50} and R_{100} harbored 2.6 ± 0.1 and 1.90 ± 0.2 $\log_{10}$ CFU, respectively, while those of guinea pigs receiving P_{50} and P_{100} contained 2.8 ± 0.2 and 2.2 ± 0.3 $\log_{10}$ CFU, respectively. Although in each monotherapy regimen mean $\log_{10}$ CFU counts were significantly reduced ($P < 0.01$) compared to lung CFU counts at treatment initiation (Fig. 2), similar doses of each rifamycin given individually did not yield statistically different bacillary killing at any time point (*P* values of 0.42 and 0.1 for R_{50} versus P_{50} at month 1 and month 2, respectively; *P* values of 0.18 and 0.08 for R_{100} versus P_{100} at month 1 and month 2, respectively).

The daily combination regimens RHZ and PHZ each showed potent bactericidal activity, as lung CFU declined in each group by approximately 4.0 $\log_{10}$ CFU ($P < 0.01$) after the first month of treatment, and all lungs were culture negative in each group after completion of 2 months of treatment (Fig. 2). Substitution of R_{100} with P_{100} did not result in a significant difference in bactericidal activity of the combination regimen ($P = 0.16$ at month 1).

Sterilizing activity of RHZ/PHZ in guinea pigs. Groups of 10 guinea pigs were held without treatment after completion of 1 and 2 months of RHZ or PHZ to assess relapse rates. Microbiological relapse occurred in the lungs of 8/10 animals completing 1 month of RHZ (mean $\log_{10}$ CFU = 0.4 ± 0.4) or PHZ ($\log_{10}$ CFU = 0.4 ± 0.5) and in 0/10 animals completing 2 months of either combination regimen. Interestingly, CFU were detectable in the spleens of relapse group animals that had completed 2 months of treatment with RHZ (mean $\log_{10}$ CFU = 0.7 ± 0.3) or PHZ (mean $\log_{10}$ CFU = 0.6 ± 0.3) ($P = 0.58$).

DISCUSSION

Consistent with our initial hypothesis, our data show that substitution of P for R does not enhance the bactericidal or sterilizing activity of daily combination treatment with the companion drugs HZ in chronically infected guinea pigs.

Our findings appear to conflict with those of murine studies showing that daily dosing of P in place of R in the standard antituberculous regimen significantly shortens the duration of treatment needed to prevent relapse (29, 30). More recently, daily dosing of PHZ in *M. tuberculosis*-infected nude mice led to lung sterilization, whereas treatment failure associated with H resistance was observed with daily dosing of RHZ (39). In these studies, the superior activity of PHZ relative to RHZ was attributed largely to the greater rifamycin exposure obtained by substituting P for R because of the longer elimination half-life of P. In the current study, the P and R exposures used in the combination regimens appear equivalent to those used in the mouse studies based on AUC_{0-24} and C_{max}. Drug exposures were matched on the basis of AUC_{0-24} rather than $AUC_{0-\infty}$ (4), since the former does not require extrapolation to infinity. A precise estimate of $AUC_{0-\infty}$ for P in guinea pigs could not be obtained, since the estimated half-life of the drug approached the end of the sampling interval (24 h). Thus, relatively small errors in the calculation of the half-life could have significant effects on the magnitude of the $AUC_{0-\infty}$, but not on the AUC_{0-24}. However, since P and R doses in the current study were based on single-dose pharmacokinetic (PK) experiments, it is possible that these may not accurately reflect steady-state values of each drug in guinea pigs. Differences in R autoinduction have been observed across different animal species (33), although these properties have not been characterized for P. If P induces its own metabolism to a greater extent in guinea pigs than in mice, its antituberculous activity potentially could be underrepresented in the former species. However, based on the significantly longer elimination half-life for P relative to R in guinea pigs, steady-state AUC values would be expected to be disproportionately greater for P relative to R, as has been shown in mice (30), which would not explain the lack of superiority of P against *M. tuberculosis* in guinea pigs relative to R. On the other hand, serum AUC values may not accurately reflect rifamycin exposures encountered by *M. tuberculosis* at sites of infection. Thus, although the calculated serum AUC for P_{100} was more than 2-fold greater than that of R_{100}, free drug exposures within necrotic granulomas, where persistent bacilli are believed to reside (39), may have favored R, which has lower protein binding than P (9, 16). In addition, the greater intracellular accumulation of P relative to R (22), which may have rendered P-containing regimens more potent against predominantly intracellular TB infection in BALB/c mice relative to R-containing regimens (29, 30), may not have provided an advantage in chronically infected guinea pigs, which harbor primarily extracellular bacilli within necrotic lung granulomas. A direct comparison of the antitubercular activities of RHZ and PHZ in C3HeB/FeJ mice (11, 12), which, like guinea pigs, develop necrotic lung granulomas following *M. tuberculosis* infection but share common drug metabolism properties with BALB/c mice (26), could help determine the role of lung pathology on the potency of each combination regimen. Ultimately, protein-binding

studies of each drug in guinea pigs and microdialysis studies of infected guinea pig lungs, or those of larger species, will be required to elucidate the protein-binding properties and penetration of each drug in necrotic lung lesions harboring persistent bacilli.

In contrast to our findings in guinea pigs, treatment with RHZ requires 5 to 6 months to render their lungs culture negative in the standard mouse model and a similar length of time is needed to prevent relapse in clinical cases of drug-susceptible TB. This may reflect more effective immune responses to *M. tuberculosis* infection in guinea pigs relative to mice and humans, leading to enhanced bacillary killing in the face of TB treatment (4). Consistent with this hypothesis, *katG*-deficient isoniazid-resistant mutants could not be isolated following isoniazid monotherapy in guinea pigs (3), whereas such mutants are readily recoverable from mouse lungs and sputum samples from TB patients. Interestingly, microscopic examination of culture-negative lungs of guinea pigs completing 2 months of RHZ or PHZ treatment and held for an additional 3 months revealed the presence of acid-fast bacilli within the necrotic core of granulomas. We have made similar observations following 4 months of treatment with RHZ in guinea pigs (2), consistent with either delayed clearance of antibiotic-killed bacilli, which continue to stain positively (19, 27), or the presence of noncultivable persisters. In the current study, mycobacteria were still detectable in the spleens of guinea pigs assessed at relapse after 2 months of RHZ or PHZ, indicating that TB infection was not eradicated in these animals. Difficulty in sterilizing *M. tuberculosis*-infected guinea pig tissues was also observed by Shang et al., who showed that although a combination regimen containing the novel diarylquinolone TMC207 with R and Z rapidly reduced the bacillary load in the lungs to undetectable levels by 8 weeks of treatment, more than one fifth of animals developed clinical relapse as late as 11 months after the treatment was discontinued (32). The reason for the inferior activity of the two rifamycin-containing regimens against *M. tuberculosis* in the spleens relative to the lungs observed in the current study requires further investigation.

Although the precise role of the guinea pig (21) in TB chemotherapy has yet to be defined, it has been postulated that the model is useful in discriminating between purely bactericidal and sterilizing drugs. Thus, while the bactericidal drugs isoniazid and streptomycin showed early potent activity, their activity was dramatically reduced against persistent bacilli (3, 5). In contrast, the uniquely sterilizing drug Z was shown to have dose-dependent activity against chronic TB infection in guinea pigs and to exhibit synergy with R, as in humans (1). Corroborating previous reports (2), we found that increasing the rifamycin exposure in guinea pigs receiving R or P monotherapy resulted in greater bactericidal activity. These findings are consistent with those of clinical studies showing that RHZ is less active when administered intermittently rather than daily (10, 20). In the current study, nutritional supplementation (23) was sufficient to avert severe gastrointestinal toxicity associated with larger daily doses of rifamycins in *M. tuberculosis*-infected guinea pigs (4). Thus, this appears to be a promising model to study the sterilizing activity of escalating doses of rifamycins, as well as the contribution of novel antituberculous drugs to rifamycin-containing regimens against persistent, extracellular bacilli within necrotic lung granulomas.

In conclusion, our findings indicate that daily dosing of P, alone or in combination with the first-line drugs H and Z, does not yield greater bactericidal or sterilizing activity against chronic TB infection in guinea pigs when P is substituted for R. The results of ongoing clinical studies evaluating the efficacy of substitution of P for R in treating active TB are awaited to determine the clinical relevance of our findings in the guinea pig model.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (grant AI083125 to P.C.K.) and FDA (grant U18FD004004 to P.C.K. and E.L.N.). We have no conflicts of interest.

REFERENCES

1. **Ahmad Z, et al.** 2011. Dose-dependent activity of pyrazinamide in animal models of intracellular and extracellular tuberculosis infections. Antimicrob. Agents Chemother. **55**:1527–1532.
2. **Ahmad Z, et al.** 2011. Effectiveness of tuberculosis chemotherapy correlates with resistance to *Mycobacterium tuberculosis* infection in animal models. J. Antimicrob. Chemother. **66**:1560–1566.
3. **Ahmad Z, et al.** 2009. Biphasic kill curve of isoniazid reveals the presence of drug-tolerant, not drug-resistant, *Mycobacterium tuberculosis* in the guinea pig. J. Infect. Dis. **200**:1136–1143.
4. **Ahmad Z, et al.** 2010. Comparison of the 'Denver regimen' against acute tuberculosis in the mouse and guinea pig. J. Antimicrob. Chemother. **65**:729–734.
5. **Ahmad Z, et al.** 2010. The potent bactericidal activity of streptomycin in the guinea pig model of tuberculosis ceases due to the presence of persisters. J. Antimicrob. Chemother. **65**:2172–2175.
6. **Almeida D, et al.** 2011. Activities of rifampin, rifapentine and clarithromycin alone and in combination against *Mycobacterium ulcerans* disease in mice. PLoS Negl. Trop. Dis. **5**:e933. doi:10.1371/journal.pntd.0000933.
7. **Aly S, et al.** 2006. Oxygen status of lung granulomas in Mycobacterium tuberculosis-infected mice. J. Pathol. **210**:298–305.
8. **Benator D, et al.** 2002. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. Lancet **360**:528–534.
9. **Burman WJ, Gallicano K, Peloquin C.** 2001. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin. Pharmacokinet. **40**:327–341.
10. **Chang KC, Leung CC, Yew WW, Ho SC, Tam CM.** 2004. A nested case-control study on treatment-related risk factors for early relapse of tuberculosis. Am. J. Respir. Crit. Care Med. **170**:1124–1130.
11. **Davis SL, et al.** 2009. Bacterial thymidine kinase as a non-invasive imaging reporter for *Mycobacterium tuberculosis* in live animals. PLoS One **4**:e6297. doi:10.1371/journal.pone.0006297.
12. **Davis SL, et al.** 2009. Noninvasive pulmonary [18F]-2-fluoro-deoxy-D-glucose positron emission tomography correlates with bactericidal activity of tuberculosis drug treatment. Antimicrob. Agents Chemother. **53**:4879–4884.
13. **Dorman S, et al.** 2011. A phase II study of a rifapentine-containing regimen for intensive phase treatment of pulmonary tuberculosis: preliminary results for tuberculosis trials consortium study 29. Am. J. Respir. Crit. Care Med. **183**:A6413.
14. **Grosset J, Truffot-Pernot C, Lacroix C, Ji B.** 1992. Antagonism between isoniazid and the combination pyrazinamide-rifampin against tuberculosis infection in mice. Antimicrob. Agents Chemother. **36**:548–551.
15. **Gumbo T, et al.** 2007. Concentration-dependent *Mycobacterium tuberculosis* killing and prevention of resistance by rifampin. Antimicrob. Agents Chemother. **51**:3781–3788.
16. **Jayaram R, et al.** 2003. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. Antimicrob. Agents Chemother. **47**:2118–2124.
17. **Karakousis PC, Williams EP, Bishai WR.** 2008. Altered expression of isoniazid-regulated genes in drug-treated dormant *Mycobacterium tuberculosis*. J. Antimicrob. Chemother. **61**:323–331.
18. **Klinkenberg LG, Sutherland LA, Bishai WR, Karakousis PC.** 2008. Metronidazole lacks activity against *Mycobacterium tuberculosis* in an in vivo hypoxic granuloma model of latency. J. Infect. Dis. **198**:275–283.
19. **Lenaerts AJ, et al.** 2007. Location of persisting mycobacteria in a guinea pig model of tuberculosis revealed by r207910. Antimicrob. Agents Chemother. **51**:3338–3345.

20. **Li J, Munsiff SS, Driver CR, Sackoff J.** 2005. Relapse and acquired rifampin resistance in HIV-infected patients with tuberculosis treated with rifampin- or rifabutin-based regimens in New York City, 1997–2000. Clin. Infect. Dis. **41**:83–91.
21. **McMurray DN.** 2001. Disease model: pulmonary tuberculosis. Trends Mol. Med. **7**:135–137.
22. **Mor N, Simon B, Mezo N, Heifets L.** 1995. Comparison of activities of rifapentine and rifampin against *Mycobacterium tuberculosis* residing in human macrophages. Antimicrob. Agents Chemother. **39**:2073–2077.
23. **Ordway DJ, et al.** 2010. Evaluation of standard chemotherapy in the guinea pig model of tuberculosis. Antimicrob. Agents Chemother. **54**: 1820–1833.
24. **Peloquin CA, et al.** 1997. Population pharmacokinetic modeling of isoniazid, rifampin, and pyrazinamide. Antimicrob. Agents Chemother. **41**: 2670–2679.
25. **Peloquin CA, Namdar R, Singleton MD, Nix DE.** 1999. Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. Chest **115**:12–18.
26. **Pichugin AV, Yan BS, Sloutsky A, Kobzik L, Kramnik I.** 2009. Dominant role of the sst1 locus in pathogenesis of necrotizing lung granulomas during chronic tuberculosis infection and reactivation in genetically resistant hosts. Am. J. Pathol. **174**:2190–2201.
27. **Rees RJ, Hart PD.** 1961. Analysis of the host-parasite equilibrium in chronic murine tuberculosis by total and viable bacillary counts. Br. J. Exp. Pathol. **42**:83–88.
28. **Rosenthal IM, et al.** 2006. Potent twice-weekly rifapentine-containing regimens in murine tuberculosis. Am. J. Respir. Crit. Care Med. **174**:94–101.
29. **Rosenthal IM, Zhang M, Almeida D, Grosset JH, Nuermberger EL.** 2008. Isoniazid or moxifloxacin in rifapentine-based regimens for experimental tuberculosis? Am. J. Respir. Crit. Care Med. **178**:989–993.
30. **Rosenthal IM, et al.** 2007. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. PLoS Med. **4**:e344. doi: 10.1371/journal.pmed.0040344.
31. **Roy CJ, et al.** 2012. Aerosolized gentamicin reduces the burden of tuberculosis in a murine model. Antimicrob. Agents Chemother. **56**:883–886.
32. **Shang S, et al.** 2011. Activities of TMC207, rifampin, and pyrazinamide against *Mycobacterium tuberculosis* infection in guinea pigs. Antimicrob. Agents Chemother. **55**:124–131.
33. **Strolin BM, Dostert P.** 1994. Induction and autoinduction properties of rifamycin derivatives: a review of animal and human studies. Environ. Health Perspect. **102**:101–105.
34. **Vandiviere HM, Loring WE, Melvin I, Willis S.** 1956. The treated pulmonary lesion and its tubercle bacillus. II. The death and resurrection. Am. J. Med. Sci. **232**:30–37.
35. **Vernon A, Burman W, Benator D, Khan A, Bozeman L.** 1999. Acquired rifamycin monoresistance in patients with HIV-related tuberculosis treated with once-weekly rifapentine and isoniazid. Tuberculosis Trials Consortium. Lancet **353**:1843–1847.
36. **Weiner M, et al.** 2004. Pharmacokinetics of rifapentine at 600, 900, and 1,200 mg during once-weekly tuberculosis therapy. Am. J. Respir. Crit. Care Med. **169**:1191–1197.
37. **Weiner M, et al.** 2003. Low isoniazid concentrations and outcome of tuberculosis treatment with once-weekly isoniazid and rifapentine. Am. J. Respir. Crit. Care. Med. **167**:1341–1347.
38. **Weiner M, et al.** 2010. Effects of tuberculosis, race, and human gene SLCO1B1 polymorphisms on rifampin concentrations. Antimicrob. Agents Chemother. **54**:4192–4200.
39. **Zhang M, et al.** 2011. Treatment of tuberculosis with rifamycin-containing regimens in immune-deficient mice. Am. J. Respir. Crit. Care Med. **183**:1254–1261.