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Potent Rifamycin-Sparing Regimen Cures Guinea Pig Tuberculosis as Rapidly as the Standard Regimen

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Strategies involving new drug combinations, as well as new uses of existing drugs, are urgently needed to reduce the time required to cure patients with drug-sensitive or multidrug-resistant (MDR) tuberculosis (TB). We compared the sterilizing activity of the standard first-line antitubercular regimen, rifampin-isoniazid-pyrazinamide (RHZ), with that of the novel regimen PA-824–moxifloxacin–pyrazinamide (PaMZ), which is currently being studied in clinical trials (NCT01498419), in the guinea pig model of chronic TB infection, in which animals develop necrotic granulomas histologically resembling their human counterparts. Guinea pigs were aerosol infected with ~2 $\log_{10}$ bacilli of wild-type *Mycobacterium tuberculosis* H37Rv, and antibiotic treatment was initiated 6 weeks after infection. Separate groups of animals received RHZ, PaMZ, or single or two-drug components of the latter regimen administered at human-equivalent doses 5 days/week for a total of 8 weeks. Relapse rates were assessed 3 months after discontinuation of treatment to determine the sterilizing activity of each combination regimen. PaMZ given at human-equivalent doses was safe and well tolerated for the entire treatment period and rendered guinea pig lungs culture negative more rapidly than RHZ did. After 1 month of treatment, 80% and 50% of animals in the RHZ and PaMZ groups, respectively, had lung culture-positive relapse. Both combination regimens prevented microbiological relapse when administered for a total of 2 months. Our data support the use of PaMZ as a novel isoniazid- and rifamycin-sparing regimen suitable for treatment of both drug-sensitive TB and MDR-TB.

The increasing global burden of drug-resistant tuberculosis (TB) underscores the need for new drugs but also for shorter, better-tolerated regimens to reduce treatment durations from the current 18 to 24 months for multidrug-resistant TB (MDR-TB) (1, 2). Shorter regimens are expected to improve adherence to treatment, thereby reducing transmission and the emergence of new drug resistance, as well as facilitating coadministration with antiretroviral regimens and curbing costs. As a result of continuing efforts to evaluate novel drug combinations with treatment-shortening potential, several new drugs have been shown to contribute to reducing the time required to achieve a relapse-free state in animal models (3–6).

A paradigm shift from "drug development" to "regimen development," wherein the regimen, not an individual drug, is the unit of development, seems a more feasible approach to deliver sufficiently novel combinations more rapidly (1). The novel regimen PaMZ, comprising PA-824 (Pa), moxifloxacin (M), and pyrazinamide (Z), a combination containing neither rifampin (R) nor isoniazid (H), was found to have a sterilizing activity superior to that of the current first-line regimen, RHZ, in mice (3, 7). In addition, studies in the mouse model have revealed synergism between the agents in the novel combination (3, 7). Recently, a phase II clinical study confirmed the potent initial activity of PaMZ observed in mice, demonstrating that the early bactericidal activity (EBA) of this novel regimen is not inferior to that of the standard antitubercular regimen (8). However, it remains unclear whether the potent sterilizing activity and treatment-shortening potential of PaMZ in mice will also be observed in humans.

Although the mouse model has been used historically in TB chemotherapy studies, the guinea pig model, in which animals develop pathology more closely resembling human TB lesions, may be advantageous for testing the sterilizing activity of novel anti-TB drugs (9–14). Because of the limited data available in the literature (15–17), we first characterized the pharmacokinetic (PK) parameters of Pa and M in guinea pigs to establish human-equivalent doses. We then studied the bactericidal and sterilizing activities of PaMZ against chronic tuberculosis in guinea pigs to validate results from earlier mouse studies and to evaluate the potential of PaMZ as a novel regimen for the treatment of drug-susceptible TB and MDR-TB.

MATERIALS AND METHODS

***Mycobacterium tuberculosis* strain.** *M. tuberculosis* strain H37Rv-JHU (18) was used for these studies. Prior to aerosol infection, cultures were grown in Middlebrook 7H9 broth (Difco Laboratories, Detroit, MI) supplemented with 10% OADC (oleic acid-albumin-dextrose-catalase; Becton, Dickinson), 0.05% Tween, and 0.1% glycerol to mid-log phase (optical density at 600 nm [OD_{600}] of ~0.6).

Animals. All animals were maintained under specific-pathogen-free conditions and provided water and standard feed *ad libitum*. All procedures followed protocols approved by the Institutional Animal Care and Use Committee at Johns Hopkins University. Female outbred Hartley

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guinea pigs (250 to 300 g) with and without jugular vein vascular catheters were purchased from Charles River Labs (Wilmington, MA).

Pharmacokinetic experiments. Separate groups of three catheterized guinea pigs each were given (i) a single dose of Pa at 12.5 or 50 mg/kg of body weight; (ii) two doses of Pa at 50 mg/kg, separated by 8 h (q8/16h); or (iii) a single dose of M at 100, 150, 175, or 200 mg/kg. The primary and secondary goals of the dosing studies were to match the human area under the concentration-time curve (AUC) and to approximate human C_{max} (maximum concentration of drug in serum) measurements, respectively. To obtain steady-state data (19), four guinea pigs were treated twice daily (q8/16h) for 7 days with Pa at 25 mg/kg or M at 90 mg/kg. Doses were prepared in 40% sucrose water in a final volume of 0.5 ml and delivered to the posterior oropharynx by use of an automatic pipette with a disposable tip. Blood (~0.3 ml) was drawn serially from guinea pigs through an intravenous catheter at the following time points after antibiotic dosing: 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 24 h in the single-dose study and before drug administration on day 0 and day 8 and 0.5, 1, 2, 4, 6, 8, and 24 h after the last dosing in the steady-state study, which is a robust sampling scheme. Briefly, serum was separated, stored at −80°C, and later analyzed for the Pa (20, 21) or M (22–24) concentration by use of validated assays on a Thermo-Finnigan P4000 high-performance liquid chromatography (HPLC) pump (Thermo-Finnigan, San Jose, CA) with a model AS1000 fixed-volume autosampler, a model UV2000 UV detector (Pa) (both from Thermo Electron Corporation, Waltham, MA) or McPherson fluorescence detector (M) (McPherson, Chelmsford, MA), a Gateway E-series computer (Gateway, Poway, CA), and the Chromquest HPLC data management system (Thermo Electron Corporation). The plasma standard concentration curve for Pa ranged from 0.20 to 50 μg/ml (20). The absolute recovery of Pa from plasma was 88.2%. The overall precision of the validation assay across all standards was 0.67 to 5.38%. The six-point standard curve for M ranged from 0.2 to 15 μg/ml, with linearity extending well above this range (23). The recovery of M from plasma was approximately 90%. The overall validation precision for M was 4.19 to 6.62% (23). All of our results represent total drug concentrations. Population values for typical protein binding can be applied to the C_{max} and AUC. We performed a noncompartmental analysis using Phoenix WinNonlin 6.3 (Pharsight, Mountain View, CA). This included the C_{max}, time to obtain C_{max} (T_{max}), AUC, and half-life ($t_{1/2}$).

A one-compartment oral absorption model was used to simulate total and free Pa (assuming 5, 7, 10, or 15% unbound drug) and M (assuming 60% unbound drug) concentrations at steady state for different dosing regimens. We used the steady-state median values obtained from the noncompartmental analysis to determine the volume of distribution and elimination rate constant, while the absorption rate constant was adjusted to match the median serum concentrations measured at steady state. The resultant free, unbound (f) concentrations were used to calculate pharmacodynamic (PD) indices (fAUC/MIC ratio, fC_{max}/MIC ratio, and fT_{MIC} [portion of a 24-h period that the free, unbound fraction of drug exceeded the MIC under steady-state pharmacokinetic conditions]) over 168 h (7 days), using an Excel sheet. For Pa, we performed the PD analysis over MIC values ranging from 0.03125 to 0.25 μg/ml. For M, we performed the PD analysis over MIC values ranging from 0.25 to 1 μg/ml. Simulations were deterministic, with the objective of comparing data between dosing regimens, not assessing the variability in exposure within each dosing regimen.

Aerosol infections. The basic experimental scheme is shown in Table 1. A total of 132 guinea pigs were aerosol infected with H37Rv-JHU by use of a Madison chamber aerosol generation device (University of Wisconsin, Madison, WI) calibrated to deliver ~2 $\log_{10}$ CFU to the lungs (10–14, 25).

Antibiotic therapy. Oral therapy daily (5 days/week) with human-equivalent doses of PaMZ (Pa at 25 mg/kg twice daily, M at 25 mg/kg twice daily, and Z at 300 mg/kg once daily), RHZ (R at 100 mg/kg, H at 60 mg/kg, and Z at 300 mg/kg, all once daily), or single or two-drug (PaM, PaZ, and MZ) components of the PaMZ regimen was initiated 6 weeks after infection and continued for a total of 2 months (Table 1). For each drug given twice daily, the morning dose preceded the afternoon dose by 8 h and the latter preceded the following dose by 16 h (q8/16h). For the RHZ-treated group, the R dose preceded that of the other drugs (H and Z) by at least 1 h to prevent PK antagonism (26, 27). Animals were treated with a formulation consisting of 40% (wt/vol) sucrose, 20% (wt/vol) pumpkin (Libby's 100% pure pumpkin) mixture supplemented with vitamin C (50 mg/kg of mean body weight), and commercial *Lactobacillus* (BD Lactinex) (all purchased from Walmart, Towson, MD) to help stabilize the enteric flora and prevent gastrointestinal dysbacteriosis or antibiotic-associated enteritis (10).

TABLE 1 Basic experimental scheme

Group[a]	No. of animals sacrificed at mo[b]:					
	−1.5	0	0.5	1 (+3)	2 (+3)	Total
Untreated	4	4	4	4	4	20
$R_{100}H_{60}Z_{300}$			4	4 (10)	4 (10)	12 (20)
$Pa_{25\ BID}$			4	4		8
$M_{90\ BID}$			4	4		8
Z_{300}			4	4		8
$Pa_{25\ BID}M_{90\ BID}$			4	4		8
$Pa_{25\ BID}Z_{300}$			4	4		8
$M_{90\ BID}Z_{300}$			4	4		8
$Pa_{25\ BID}M_{90\ BID}Z_{300}$			4	4 (10)	4 (10)	12 (20)
Total	4	4	32	32 (20)	8 (20)	132

[a] Drug doses (in mg/kg; single or twice-daily dose) are indicated by subscripts. BID, twice daily, every 16 and 8 h. Doses of each drug were determined to be human equivalent based on the AUC and were given orally daily (5 days/week).
[b] Month −1.5, day after infection with *M. tuberculosis*; month 0, day of treatment initiation; month 0.5, 0.5 month after treatment initiation; and so on. "(+3)" indicates that the number of guinea pigs in parentheses was held for 3 months after treatment completion before sacrifice for relapse assessment.

Study endpoints. Four animals were sacrificed at month −1.5 and month 0 relative to treatment initiation in order to determine the number of bacilli implanted in each lung on the day after infection and the lung bacillary load at the start of treatment, respectively. In order to determine the bactericidal activity of each regimen, 4 animals from each group were sacrificed at months 0.5 and 1 and, for the three-drug combinations, also at month 2.

Animal body weights and lung and spleen weights were recorded and organs photographed at the time of sacrifice. In order to account for the physiological increase in organ weights in aging animals, organ weights were normalized using the following formula: sacrificed animal organ weight on day of sacrifice × (mean body weight on day after infection/sacrificed animal body weight on day of sacrifice). The lungs and spleen of each animal were examined at necropsy for grossly visible lesions, and small sections from the left lung were dissected, placed into 10% buffered formaldehyde, and paraffin embedded for histopathological staining with hematoxylin and eosin (H&E) and Kinyoun stain for acid-fast bacillus (AFB) detection. At least one entire H&E-stained cross section per animal lung (4 animals/group) was analyzed for degree of inflammation. Histological analysis was performed by two board-certified pathologists (T. J. Gniadek and D. A. Belchis), who were blinded to the identity of each sample. The remainder of each tissue was homogenized and plated as previously described (9–12, 14). Plates were incubated at 37°C for 4 weeks before final CFU counts were determined.

After the completion of 1 month and 2 months of treatment with PaMZ and RHZ, groups of 10 animals were held for an additional 3 months without treatment to determine the sterilizing activity of each combination regimen. Relapse was defined as the presence of mycobacterial colonies upon plating of entire undiluted lung homogenates.

Statistical analysis. For pharmacokinetic studies, data represent means ± standard deviations (SD). Organ CFU values were log trans-

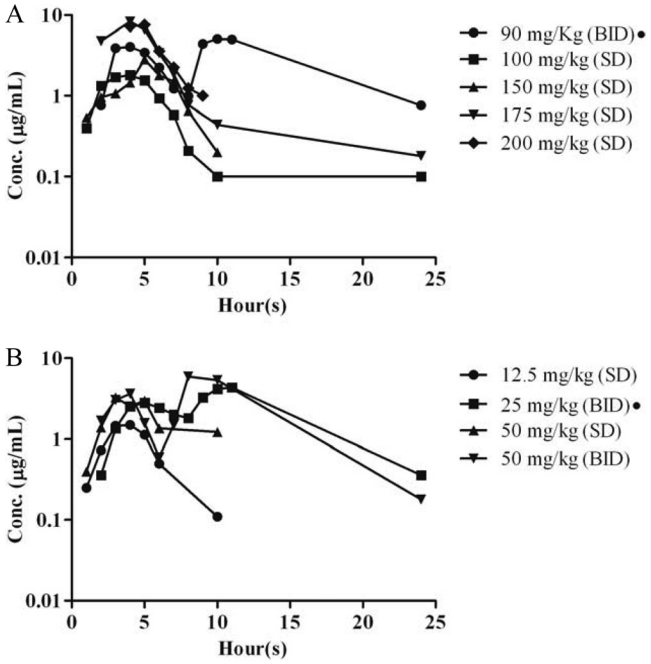

FIG 1 Plasma concentration profiles of moxifloxacin (A) and PA-824 (B) in guinea pigs. The graphs present median values ($n = 3$ or 4 per time point). SD, single dose; BID, twice a day (q8/16h). A black circle next to the drug dose indicates repeated doses.

formed prior to calculating the means and standard deviations for each group. Comparisons of data among experimental groups were performed by Student's *t* test and analysis of variance followed by Bonferroni multiple-comparison tests, using GraphPad Prism software. *P* values of <0.05 were considered statistically significant.

RESULTS

Identification of human-equivalent doses of PaMZ in guinea pigs. Based on the area under the serum 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 (9, 10). In the current study, we determined the human-equivalent doses of Pa and M (Fig. 1; Table 2). In a preliminary single-dose study for M, the $t_{1/2}$ was found to be 3-fold longer in humans. Although M at 150 mg/kg yielded a C_{max} in guinea pigs matching that following standard dosing in humans, this dose yielded a relatively low AUC (17.2 mg-h/liter for guinea pigs versus 55 mg-h/liter for humans). We found that M at 175 mg/kg in guinea pigs produced a more human-like AUC (39.70 mg-h/liter), but the resulting C_{max} was significantly higher (9.83 μg/ml). On the other hand, splitting this dose into 90 mg/kg twice daily (q8/16h) yielded a C_{max} and AUC_{0-24} closely approximating the corresponding steady-state values following daily dosing with M at 400 mg in humans (Table 2).

The $AUC_{0-\alpha}$ in guinea pigs receiving a single dose of Pa at 12.5 mg/kg (11.19 mg-h/liter) was somewhat lower than that in humans, though the C_{max} was similar to steady-state values in humans receiving 200 mg. At a 50-mg/kg single dose, the C_{max} was higher, with a variable AUC. The 50-mg/kg twice-daily (q8/16h) dose yielded an AUC approximately twice that of the target human AUC. The half-life of Pa is markedly longer in humans than in guinea pigs, resulting in significant accumulation of the drug in the former. However, the median AUC following repeated dosing of Pa at 25 mg/kg q8/16h closely matches human exposures at steady state (28).

Pharmacodynamic simulations. Pa at the dose used in this study (25 mg/kg q8/16h) showed favorable PD parameters (fT_{MIC} and *f*AUC/MIC ratio) at the low MICs (0.03125 and 0.0625 μg/ml). At the higher MICs, its activity was dependent on the un-

TABLE 2 Moxifloxacin and PA-824 pharmacokinetics in guinea pigs, mice, and humans[a]

Regimen	Test species	Drug dosage (frequency)	Sampling dose	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg-h/liter)
Moxifloxacin	Guinea pig	90 mg/kg (BID)	Weekly	4.55 ± 2.04	1.4 ± 1.47	3.14 ± 0.95	41.47 ± 12.04
	Guinea pig	100 mg/kg (QD)	Single	1.91 ± 0.36	1.68 ± 0.59	2.35 ± 0.39	11.1 ± 2.3
	Guinea pig	150 mg/kg (QD)	Single	5.08 ± 1.97	1.25 ± 0.64	2.37 ± 0.45	17.2 ± 2.4
	Guinea pig	175 mg/kg (QD)	Single	9.83 ± 3.02	1.0 ± 0	2.23 ± 0.76	39.70 ± 6.13
	Guinea pig	200 mg/kg* (QD)	Single	6.67 ± 2.34	2.47 ± 0.33	2.63 ± 0.26	29.4 ± 6.7
	Mouse[b]	100 mg/kg (QD)	Single	14.2 ± 3.9	0.25	2.32 ± 0.14	23.6
	Human[c]	400mg (QD)	SS	6.11 ± 1.41	1.22 ± 0.44	7.55 ± 1.88	55.84 ± 13.19
	Human[d]	400mg (QD)	5th	6.1 (4.5–9.0)	1 (1.0–2.0)	8.1 (5.1–9.9)	55.3 (36.0–79.1)
PA-824	Guinea pig	12.5 mg/kg (D1)	Single	1.68 ± 0.34	2.65 ± 1.17	1.94 ± 0.38	11.19 ± 2.04
	Guinea pig	25 mg/kg (BID)	Weekly	2.99 ± 0.70	2.25 ± 1.25	4.7 ± 2.27	42.19 ± 21.04
	Guinea pig	50 mg/kg (D1)	Single	5.84 ± 4.08	2.66 ± 1.15	3.16 ± 1.11	39.79 ± 27.88
	Guinea pig	50 mg/kg (BID)	Single	5.79 ± 1.46	7.0 ± 4.14	2.16 ± 1.41	70.95 ± 9.61
	Mouse[e]	100 mg/kg (D1)	Single	21.4 ± 5.7	4.7 ± 3.1	12.8 ± 1.0	327.6 ± 77.1
	Human[f]	200 mg/kg (QD)	SS	1.7 ± 0.3	4.5 (2–8)	16.0 ± 1.6	30.2 ± 3.7

[a] C_{max}, maximum concentration; T_{max}, time to obtain C_{max}; $AUC_{0-\infty}$, area under the concentration-time curve from 0 h to infinity; $t_{1/2}$, half-life; QD, once daily; BID, twice a day (the morning dose was separated from the afternoon dose by 8 h); SS, steady state. *, dose given as part of an RMZ combination. In each case, R was given 60 min prior to administration of the other 2 companion drugs. Data represent means ± SD for 3 or 4 animals. Data with parentheses are medians (ranges).

[b] Data are from reference 24.

[c] Data are from reference 23.

[d] Data are from reference 22. For this group, the AUC_{0-24} was determined.

[e] Data are from reference 21. For this group, the AUC_{0-24} was determined.

[f] Data are from reference 28.

TABLE 3 Pharmacodynamic parameters of PA-824 and moxifloxacin in guinea pigs[a]

Drug	Dose (mg/kg; given BID)	MIC (μg/ml)	% free, unbound drug	$\%fT_{MIC}$	*f*AUC/MIC	fC_{max}/MIC
PA-824	25	0.03	15	100.00	223.60	14.13
	25	0.03	10	100.00	149.07	9.42
	25	0.03	7.50	100.00	111.80	7.07
	25	0.03	5	94.64	74.53	4.71
	25	0.06	15	100.00	111.80	7.07
	25	0.06	10	94.64	74.53	4.71
	25	0.06	7.50	85.71	55.90	3.54
	25	0.06	5	73.21	37.27	2.36
	25	0.13	15	85.71	55.90	3.53
	25	0.13	10	73.21	37.27	2.36
	25	0.13	7.50	56.55	27.95	1.77
	25	0.13	5	24.40	18.63	1.18
	25	0.25	15	56.55	27.95	1.77
	25	0.25	10	24.40	18.63	1.18
	25	0.25	7.50	2.98	13.98	0.88
	25	0.25	5	0	9.32	0.59
Moxifloxacin	90	0.25	60	99.40	117.76	11.20
	90	0.5	60	74.40	58.88	5.60
	90	1	60	49.40	29.44	2.80

[a] AUC, area under the serum concentration-time curve; $\%fT_{MIC}$, cumulative percentage of a 24-h period that the free, unbound fraction of drug exceeded the MIC under steady-state pharmacokinetic conditions; BID, twice daily (the morning dose was separated from the afternoon dose by 8 h). Data were obtained by simulation of the dosing regimens on the basis of the pharmacokinetic profiles for PA-824 and moxifloxacin in guinea pigs.

bound fraction. Also, the twice-daily dosing was more consistent in maintaining concentrations above the MIC. For M, the dose used in this study showed good PD activity (*f*AUC/MIC and fC_{max}/MIC) at the low MIC (0.25 μg/ml), but less so at the high MIC (1.0 μg/ml) (Table 3).

Morbidity and mortality during treatment. No deaths were observed in either the treated or untreated groups of guinea pigs during the course of the experiment. After 2 weeks of treatment, treated guinea pigs were found to have lower mean body weights than those of the untreated control group, possibly due to gavage-induced stress (see Table S1 in the supplemental material). The combination groups had greater weight loss than the monotherapy groups. However, mean body weights of treated guinea pigs started to increase thereafter and continued to increase even after completion of treatment in animals kept for relapse studies.

Organ weights, gross pathology, and histology during treatment. On the day after infection, the normalized mean guinea pig lung weight was 2.2 ± 0.1 g, increasing to 2.4 ± 0.4 g at the onset of treatment (see Table S2 in the supplemental material). The normalized mean lung weight of untreated guinea pigs continued to increase, reaching 2.9 ± 0.3 g at month 1 of treatment. In contrast, normalized mean lung weights of treated guinea pigs showed decreasing trends over time, ranging from 1.83 ± 0.28 to 2.8 ± 0.6 g at month 0.5 of treatment, with further declines at month 1 (ranging from 1.65 ± 0.1 to 2.7 ± 0.6 g). However, lung weights increased in animals kept for assessment of relapse rates (see Fig. S1). Similar trends were observed for normalized mean spleen weights of treated and untreated guinea pigs (see Table S2).

PaMZ and RHZ showed equivalent reductions in the number and size of grossly visible lung tubercles, as rapidly as within 2 weeks of treatment initiation (see Fig. S1 in the supplemental material). Histological evaluation revealed diffuse inflammation, nodular aggregates, and multiple necrotic and nonnecrotizing granulomas, which were present at treatment onset and increased in number over the subsequent month in untreated animals. Treatment with PaMZ and RHZ reduced the number and size of granulomas, as well as the number of extracellular AFB within granulomas, to similar degrees 2, 4 (see Fig. S2), and 8 weeks after initiation of therapy.

Bactericidal activity of PaMZ and RHZ for chronic TB infection in guinea pigs. On the day after aerosol infection (month −1.5 of treatment), 2.2 ± 0.08 $\log_{10}$ CFU were recovered from guinea pig lungs. The bacilli grew exponentially, to a peak lung burden of 6.1 ± 0.1 $\log_{10}$ CFU at the time of treatment initiation (month 0). At month 0.5, all combination (two- or three-drug) regimens displayed bactericidal activity, but the three-drug regimens (PaMZ and RHZ) were more potent than the two-drug regimens (PaM, PaZ, and MZ) (Fig. 2). However, the difference was not statistically significant for any of the comparisons (see Fig. S3 in the supplemental material). Although both PaMZ and RHZ regimens demonstrated bactericidal activity, treatment with PaMZ resulted in a mean lung CFU count that was 0.6 $\log_{10}$ lower than that observed after treatment with RHZ ($P = 0.11$). Each of the single-drug regimens showed bactericidal activity in the following rank order: Pa = M > Z. One month of RHZ treatment reduced the CFU count by 4.5 $\log_{10}$. For comparison, 1 month of treatment with PaMZ reduced the CFU count by 5.7 $\log_{10}$ ($P < 0.001$) (Fig. 2; see Fig. S3). The two-drug combinations showed equivalent activities, and the order of activity of the single-drug regimens was unchanged. After completion of 2 months of treatment with PaMZ or RHZ, all lungs were culture negative.

Sterilizing activity of RHZ and PaMZ in guinea pigs. Groups of 10 guinea pigs were held without treatment for 3 months following completion of 1 and 2 months of RHZ or PaMZ treatment in order to assess lung culture-positive relapse rates. After 1 month of treatment, relapse rates in guinea pig lungs were 80% (8/10 animals) and 50% (5/10 animals) for those treated with RHZ (mean $\log_{10}$ CFU = 0.4 ± 0.2) and PaMZ ($\log_{10}$ CFU = 0.24 ±

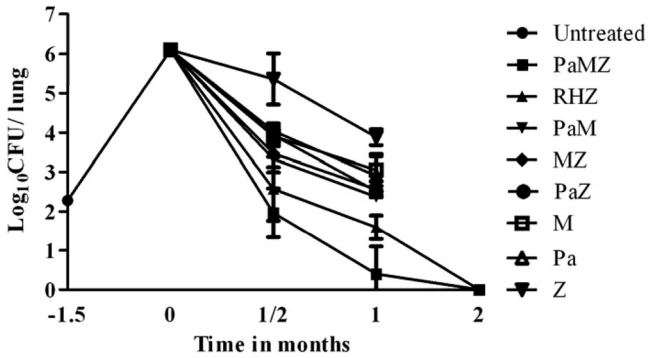

FIG 2 Antitubercular activities of the indicated drugs in infected guinea pigs. Animals were infected via aerosol with ~10^2 CFU of *M. tuberculosis* H37Rv and were either left untreated or treated with drugs daily (5 days/week) beginning 6 weeks after infection. R, rifampin at 100 mg/kg once daily; H, isoniazid at 60 mg/kg once daily; Z, pyrazinamide at 300 mg/kg once daily; Pa, PA-824 at 25 mg/kg twice daily (q8/16h); M, moxifloxacin at 90 mg/kg twice daily (q8/16h).

0.3), respectively. The relapse rate in guinea pig lungs was 0% (0/10 animals) after 2 months of treatment with RHZ or PaMZ. One of the limitations of this study is that we assessed bacillary burdens in the lungs only, not in any extrapulmonary organs such as the spleen or liver, which is why our data do not allow us to conclude that guinea pigs were truly "cured" of TB infection.

DISCUSSION

In this study, we compared the bactericidal and sterilizing activities of the standard antitubercular regimen RHZ against chronic TB infection in guinea pigs with those of the novel regimen PaMZ and investigated the contribution of each single and two-drug component to the activity of each combination regimen. We found that PaMZ was well tolerated and that animals receiving this novel regimen had a more rapid reduction in lung CFU count and achieved a stable cure at least as rapidly as those receiving RHZ.

A key component of PaMZ is Pa, a nitroimidazole effective against drug-sensitive and drug-resistant TB (5, 6, 29–31). In addition to inhibiting ketomycolic acid and protein synthesis, Pa also kills *M. tuberculosis* through a novel mechanism involving generation of intracellular nitric oxide (32). Pa is a prodrug that undergoes nitroreduction to one or more active compounds (17, 20). Because it may kill *M. tuberculosis* by different mechanisms under aerobic and anaerobic conditions (33) and may be activated more efficiently under hypoxic conditions, it warrants evaluation in models that differ in their degree of tissue hypoxia. In an experimental mouse model of TB infection, which lacks hypoxic lesions (34, 35), Pa given as monotherapy at 100 mg/kg exhibited bactericidal activity similar to that of the human-equivalent dose of H during the intensive phase of treatment (36, 37). Subsequently, the PaMZ combination was shown to achieve a more rapid cure than that by the standard RHZ regimen in the murine model of acute TB infection (3). Given its unique activity under hypoxic conditions, Pa has also been studied in the guinea pig model of TB infection, in which animals develop hypoxic necrotic granulomas histologically resembling their human counterparts (38). After 30 days of treatment, Pa given orally at 40 mg/kg exhibited antitubercular activity equivalent to that of H at 25 mg/kg in the lungs and spleens of chronically infected guinea pigs (33). More recently, Pa given as a dry powder via aerosol showed more modest killing in the guinea pig model of TB infection (17). However, in the latter study, guinea pig plasma drug concentrations were significantly lower than corresponding human steady-state values following dosing with Pa at 200 mg (29). Regarding the activity of PA-824 in the murine model (21, 30), the fT_{MIC} provided the best fit of the data, while the *f*AUC/MIC ratio was not far behind (fT_{MIC} was 5% better over 12 to 72 h, based on the R^2 value, and AUC was 1% better over 12 to 48 h). Within each dosing frequency, higher values for all three parameters (fT_{MIC}, *f*AUC/MIC, and fC_{max}/MIC) were associated with a greater kill capacity. All regimens dosed every 144 h resulted in an increase in CFU counts or, in one case, bacteriostasis. Since the best PD parameter clearly depended on the conditions of the analysis, we matched Pa exposures in guinea pigs and humans based on AUC values for the drug. Such doses were recently shown to have early bactericidal activity in clinical trials (29). Based on our simulations, the 25-mg/kg q8/16h dosing strategy produced an fT_{MIC} somewhat similar to that for the 200-mg dose in humans (29, 30).

Previous studies have shown that each drug contributes substantially to the activity of PaMZ in a murine model of acute TB (3). The addition of the third drug to any regimen comprising the other two drugs led to a >100-fold increase in bacillary killing after 1 month and 2 months of treatment. Similarly, in the current study, we found that the addition of each drug significantly increased the bactericidal activity of the companion two-drug regimens after 2 weeks and 1 month of treatment in the guinea pig model of chronic TB. These data are consistent with human EBA studies, in which PaMZ was more effective than PaZ (8), which likewise was more effective than Pa alone (3, 31). Historically, mouse models have represented the activity of existing TB drugs well (3). However, pathological differences between *M. tuberculosis*-infected mice and humans have raised concerns about the "predictiveness" of mouse models with respect to novel drugs or drug regimens, which require more human-like pathology for optimal activity. Human TB is characterized histologically by the presence of necrotic granulomas, which are absent in BALB/c mice (2, 39). Like rabbits and nonhuman primates, guinea pigs infected with *M. tuberculosis* develop necrotic granulomas characterized by tissue hypoxia (9–14, 25, 38), which is not a feature of mouse granulomas (9–14, 25, 34, 35). Evidence in humans (40) and guinea pigs suggests that persistent bacilli reside in the extracellular compartment of such necrotic granulomas (41). In particular, the hypoxia-dependent sterilizing activity of Pa against *M. tuberculosis* persisters may be represented better in the guinea pig model than in the standard mouse model. Although upon first consideration the guinea pig model of TB appears overly "optimistic," in so far as guinea pigs are cured with the standard anti-TB regimen within 2 months, this model has proven useful in discriminating between purely tuberculocidal drugs and those with more potent sterilizing activity (9–13, 42). Thus, the bactericidal drugs isoniazid and streptomycin showed early potent activity in acutely infected guinea pigs that was dramatically reduced against persistent bacilli (13, 14). The uniquely sterilizing drug Z was found to have dose-dependent activity against chronic TB infection in guinea pigs and to exhibit synergy with R, as in humans (12). Furthermore, substitution of rifapentine for R in the standard antitubercular regimen did not shorten the time to cure in the guinea pig model of chronic TB (10), matching results from

human studies (43) but unlike those derived from the mouse model (44). Interestingly, the PaMZ combination appeared less effective than RHZ in preliminary studies in C3HeB/FeJ mice, which develop human-like necrotic lung granulomas characterized by tissue hypoxia (45), raising concern that the relative sterilizing activity of a particular regimen may be dependent on species-specific pathology. However, the selection of Pa-resistant mutants in the latter study may have confounded results. A direct comparison of the sterilizing activities of PaMZ in BALB/c and C3HeB/FeJ mice is ongoing. The experimental design of our study did not allow us to find monotherapy regimens that lead to emergence of drug resistance, as occurs when M (46) or Z (47) is given as monotherapy in humans. It is important that the lung bacillary burden in guinea pigs at the start of treatment in our study was 10^6 CFU, whereas in most cases of human cavitary TB, the number of bacilli exceeds 10^9 (48). We reported earlier that H-resistant mutants may have reduced virulence in this model (14).

Our results indicate that the potent sterilizing activity of PaMZ is sufficient to cure guinea pigs at least as quickly as RHZ does, thus marking for the first time a regimen without R and H that has prevented relapse at least as effectively as the first-line regimen in this model. An oral regimen that cures TB in 6 months or less may open the door to improved treatments for MDR-TB, which currently consist of complex regimens of 18 to 24 months that include the use of injectable agents. Because Pa, which does not induce hepatic metabolism, was able to effectively substitute for R, a potent metabolic inducer of many drugs, including antiretroviral agents and the newly approved TB drug bedaquiline, Pa-containing regimens may provide attractive alternatives to R-containing combinations in evaluations of new drug candidates.

While the PaMZ regimen is expected to be just as effective against the majority of MDR-TB isolates as it is against fully drug-susceptible isolates, Z and M are unlikely to be active against extensively drug-resistant TB (XDR-TB). Moreover, both Z resistance and fluoroquinolone resistance are on the rise in MDR-TB patients (49–52), indicating that use of PaMZ in this population must be accompanied by careful evaluation of drug susceptibility. The findings of this study are timely, as the dosing mirrors that in a clinical trial investigating the same regimen given during the intensive phase of therapy, the results of which are expected soon.

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