Powerful Bactericidal and Sterilizing Activity of a Regimen Containing PA-824, Moxifloxacin, and Pyrazinamide in a Murine Model of Tuberculosis[▽]

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PA-824 is a nitroimidazo-oxazine in clinical testing for the treatment of tuberculosis. We report that the novel combination of PA-824, moxifloxacin, and pyrazinamide cured mice more rapidly than the first-line regimen of rifampin, isoniazid, and pyrazinamide. If applicable to humans, regimens containing this combination may radically shorten the treatment of multidrug-resistant tuberculosis.

The creation of new drug regimens capable of shortening the duration of tuberculosis (TB) treatment is a major priority for drug development (5). PA-824 (Pa) is a nitroimidazo-oxazine in phase II clinical testing. It has an MIC of 0.125 µg/ml for *Mycobacterium tuberculosis*, a unique mechanism of action, and no problems of cross-resistance in relation to other existing TB drugs (6). In a murine model, Pa has bactericidal activity during the initial and continuation phases of treatment (7). In the continuation phase, it is more active than isoniazid (INH) or moxifloxacin (MXF) and nearly as active as rifampin (RIF) plus INH, suggesting bactericidal activity against persisting bacilli. Therefore, we investigated whether the incorporation of Pa could improve the efficacy of existing short-course regimens.

In a mouse model, the combination of RIF-MXF-pyrazinamide (PZA) is more active than the standard regimen of

RIF-INH-PZA and capable of shortening the treatment duration by up to 2 months (i.e., from 6 to 4 months) (3, 4). We hypothesized that the incorporation of Pa would permit a further reduction in the treatment duration from 4 to 3 months. Six-week-old female BALB/c mice were infected via aerosol spray with $3.50 \pm 0.19 \log_{10}$ CFU of *M. tuberculosis* H37Rv, as described previously (2), and began treatment 19 days later when mean CFU counts in lungs and spleens were 7.77 ± 0.09 and $5.29 \pm 0.18 \log_{10}$, respectively. The treatment regimens are described in Table 1. Drug preparation and dosing were described previously (3, 7). All procedures were approved by the institutional Animal Care and Use Committee. Organ CFU counts (for 6 mice/group/time point) were performed as described previously (2).

When Pa was added to RIF-MXF-PZA or substituted for RIF, lung CFU counts fell more than 6 $\log_{10}$ during the first 2

TABLE 1. Treatment regimens and results of the first experiment

Regimen	Treatment ^a	Mean log ₁₀ CFU count (± SD) in lungs		Mean log ₁₀ CFU count (± SD) in spleens		Proportion (%) of mice cured after treatment for 3 mos
		Before treatment	After 2 mos of treatment regimen	Before treatment	After 2 mos of treatment regimen	
No treatment	None	7.77 ± 0.09	ND ^b	5.29 ± 0.18	ND	
1 (control)	2 mos of RIF-MXF-PZA plus 1 mo of RIF-MXF		1.63 ± 0.48		0.80 ± 0.27	13 of 24 (54.2)
2	2 mos of RIF-MXF-PZA-Pa plus 1 mo of RIF-MXF-Pa		1.18 ± 0.54		0.23 ± 0.19 ^c	5 of 24 (20.8)
3	2 mos of Pa-MXF-PZA plus 1 mo of Pa-MXF		1.46 ± 0.38		0.13 ± 0.21 ^d	7 of 27 (25.9)
4	2 mos of RIF-Pa-PZA plus 1 mo of RIF-Pa		3.26 ± 0.41 ^d		1.12 ± 0.52	0 of 24 (0)
5	3 mos of RIF-MXF-Pa		4.32 ± 0.24 ^d		1.91 ± 0.36 ^d	0 of 24 (0)

^a Drug doses, in milligrams per kilogram of body weight: RIF, 10; MXF, 100; PZA, 150; and Pa, 100. All drugs were given orally 5 days a week.

^b ND, not determined because untreated mice died before the 2-month time point.

^c *P* < 0.05 versus regimen 1.

^d *P* < 0.01 versus regimen 1.

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TABLE 2. Treatment regimens and CFU counts in lungs in the second experiment

Regimen	Treatment ^a	Mean log ₁₀ CFU count (± SD) in lungs at:					
		Day 0	Wk 2	Mo 1	Mo 2	Mo 3	Mo 4
No treatment	None	7.13 ± 0.22	8.34 ± 0.45	7.90 ± 0.25	ND ^b	ND	ND
1 (control)	2 mos of RIF-INH-PZA plus 4 mos of RIF-INH		5.87 ± 0.19	5.01 ± 0.17	2.63 ± 0.17	0.34 ± 0.23 ^c	0
2	2 mos of RIF-MXF-PZA plus 3 mos of RIF-MXF		5.91 ± 0.14	3.70 ± 0.09 ^e	1.22 ± 0.24 ^e	0	0
3	2 mos of Pa-MXF-PZA plus 4 mos of Pa-MXF		5.36 ± 0.13 ^f	2.61 ± 0.14 ^f	0.12 ± 0.16 ^{d,f}	0	0
4	2 mos of Pa-MXF		6.24 ± 0.16	5.11 ± 0.20	2.30 ± 0.24	ND	ND
5	2 mos of Pa-PZA		6.09 ± 0.32	4.85 ± 0.19	2.50 ± 0.32	ND	ND
6	2 mos of MXF-PZA		6.85 ± 0.11	5.44 ± 0.23	3.63 ± 0.45	ND	ND

^a Drug doses, in milligrams per kilogram: RIF, 10; INH, 25; PZA, 150; MXF, 100; and Pa, 100. All drugs were given orally 5 days a week.

^b ND, not determined because untreated mice died after the 1-month time point.

^c One of five mice was culture negative.

^d Three of five mice were culture negative.

^e $P < 0.01$ versus regimen 1.

^f $P < 0.01$ versus regimens 1 and 2.

months of therapy, similar to results obtained with RIF-MXF-PZA (Table 1). Spleen CFU counts fell by 4.5 to 5 logs and were modestly lower after treatment with RIF-MXF-PZA-Pa ($P < 0.05$) or Pa-MXF-PZA ($P < 0.01$) than after treatment with RIF-MXF-PZA (one-way analysis of variance with Dunnett's posttest). When Pa was substituted for MXF or PZA, the reductions in CFU counts in lungs and spleens were not as great as those observed after treatment with RIF-MXF-PZA ($P < 0.01$). Because we aimed to determine whether the incorporation of Pa would significantly shorten the 4-month treatment duration possible with RIF-MXF-PZA, treatment stopped after 3 months for all groups. Mice were held for an additional 3 months without treatment and then sacrificed to determine the proportion with negative lung and spleen cultures indicating cure. Treatment with the control regimen—2 months of RIF-MXF-PZA plus 1 month of RIF-MXF—was associated with the highest cure rate (54.2%) (Table 1). Interestingly, the addition of Pa to the control regimen and the substitution of Pa for RIF resulted in lower cure rates of 20.8 and 25.9%, respectively. The cure rates for these two regimens were significantly lower ($P = 0.049$ and 0.036 , respectively) than that for the control regimen before, but not after, alpha was adjusted for multiple comparisons (Fisher's exact test), a result that may reflect the limited sensitivity of proportion-based outcome measures.

Although our hypothesis that the incorporation of Pa would improve the RIF-MXF-PZA combination was not confirmed, the surprising results obtained with Pa-MXF-PZA, a regimen containing neither RIF nor INH, prompted a second experiment comparing Pa-MXF-PZA directly with the standard regi-

men, RIF-INH-PZA, with RIF-MXF-PZA again, and with two-drug combinations of the components of Pa-MXF-PZA: Pa-MXF, Pa-PZA, and MXF-PZA (Table 2). Mice were infected with 4.01 ± 0.18 log₁₀ CFU. Treatment began 13 days later, when the mean lung CFU count was 7.13 ± 0.22 log₁₀. Treatment with RIF-INH-PZA produced a 4.5 log₁₀ reduction after 2 months. Compared to treatment with RIF-INH-PZA, treatment with RIF-MXF-PZA resulted in lower CFU counts at 1 and 2 months ($P < 0.001$). Pa-MXF-PZA was more effective than either comparator at each time point over the first 2 months ($P < 0.001$; one-way analysis of variance with Bonferroni's posttest). Each two-drug combination of the components of Pa-MXF-PZA was less active than the three-drug combination at each time point ($P < 0.01$), confirming that each drug contributes to the activity of Pa-MXF-PZA. However, both Pa-MXF and Pa-PZA were more active than MXF-PZA ($P < 0.001$ at each time point, except $P < 0.05$ for Pa-MXF at 1 month) and had activity similar to that of RIF-INH-PZA.

When treatment was stopped after 4 months and mice were held for three additional months, 10 (50%) of 20 mice treated with RIF-INH-PZA were cured, whereas 19 (95%) and 20 (100%) of the 20 mice treated with RIF-MXF-PZA and Pa-MXF-PZA, respectively, were cured ($P < 0.01$ for each regimen versus RIF-INH-PZA). All mice were cured after 5 or 6 months of treatment with any regimen (Table 3).

Based on the results of these experiments, we conclude that the incorporation of Pa into the experimental 4-month RIF-MXF-PZA regimen did not permit the shortening of the treatment duration to 3 months. In fact, the substitution of Pa for

TABLE 3. Outcomes of test-of-cure assessments in the second experiment

Regimen	Treatment ^a	Proportion (%) of mice cured after treatment for:		
		4 mos	5 mos	6 mos
1	2 mos of RIF-INH-PZA plus 4 mos of RIF-INH	10 of 20 (50)	20 of 20 (100)	20 of 20 (100)
2	2 mos of RIF-MXF-PZA plus 3 mos of RIF-MXF	19 of 20 (95) ^b	20 of 20 (100)	20 of 20 (100)
3	2 mos of Pa-MXF-PZA plus 4 mos of Pa-MXF	20 of 20 (100) ^b	20 of 20 (100)	20 of 20 (100)

^a Drug doses were as given in Table 2, footnote a.

^b $P < 0.01$ versus regimen 1.

MXF or PZA was detrimental, underscoring the important contribution of these drugs to the regimen's activity. Remarkably, however, Pa substituted quite well for RIF. The resultant Pa-MXF-PZA regimen was at least as effective as RIF-MXF-PZA in reducing organ CFU counts but may be somewhat less effective in producing a durable culture-negative state after treatment. Still, the sterilizing activity of Pa-MXF-PZA was enough to cure mice more quickly than RIF-INH-PZA, marking the first time a regimen without RIF and INH has prevented relapse more effectively than the first-line regimen. An oral regimen that cures TB in 6 months or less may open the door to improved treatments for multidrug-resistant TB, which currently consist of complex regimens including injectable agents and lasting 18 to 24 months. Because Pa substituted effectively for RIF, a potent metabolic inducer of many compounds, and does not induce hepatic metabolism itself, Pa-containing regimens may provide attractive alternatives to RIF-containing combinations in evaluations of new drug candidates metabolized by P450 enzymes, such as TMC207.

The Pa exposure levels produced in mice in this experiment may or may not be reproduced by the ultimate human dose. Because Pa has dose-dependent activity (7), lower-level exposures may not yield the same powerful additive activity with MXF-PZA. Even so, our results provide compelling support for optimizing Pa exposure levels during development and developing other nitroimidazole derivatives, including the nitroimidazo-oxazole OPC-67683 (1).

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