Evaluation of Rifalazil in a Combination Treatment Regimen as an Alternative to Isoniazid-Rifampin Therapy in a Mouse Tuberculosis Model

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The newer rifamycin, rifalazil (RLZ) (previously known as KRM-1648), has been shown in prior experiments to be a highly potent drug against *Mycobacterium tuberculosis*. In this report, we studied the efficacy of RLZ in combination with pyrazinamide (PZA) and ethambutol (EMB) in a long-term in vivo experiment and compared their activity with the isoniazid (INH)-rifampin (RIF) combination which is presently used in the clinic. Combinations of RLZ with PZA alone or with both PZA and EMB were both found to have sterilizing activities comparable to that of the INH-RIF combination but significantly better activity with respect to relapse of infection. These results suggest that RLZ, or other agents with similar activity, could be combined with available agents to act as a potential alternative drug regimen to the currently used INH-RIF combination.

Therapy for tuberculosis is arduous due to the long duration of therapy and multidrug regimens. The current standard regimen of isoniazid (INH), rifampin (RIF), and pyrazinamide (PZA) requires 6 to 9 months of daily treatment. Approaches to improve patient compliance include instituting an intermittent treatment regimen or shortening the duration of therapy. With the discovery of the newer rifamycins, rifapentine and rifalazil (RLZ) (previously known as KRM-1648), the potential was created for shortening existing treatment regimens.

In the last decade, several groups extensively compared the efficacy of the newer rifamycins in *Mycobacterium tuberculosis* in vitro (6, 14, 15) and in vivo mouse models (1, 2, 5, 7, 8, 9, 16). RLZ is the most active single drug available against *M. tuberculosis* today. In previous experiments in our laboratory, we compared the activity of RLZ to that of RIF when given as a single drug or in a combination regimen with INH. RLZ was significantly more active than RIF as a single drug (11). The combination of RLZ and INH was more effective than that of RIF and INH, reducing the treatment period to apparent sterilization by half (6 versus 12 weeks). Six months following cessation of a 12-week RLZ-INH treatment regimen, the organs still appeared to be sterilized, whereas the RIF-INH regimen yields regrowth as early as 4 weeks after completion of therapy (10).

In this report, we studied the efficacy of RLZ in combination with PZA and ethambutol (EMB) in long-term in vivo experiments in order to test their ability in potential alternative regimens to the currently used INH, RIF, and PZA combination.

Six-week-old outbred female Swiss mice (Charles River, Wilmington, Mass.) were infected intravenously through a caudal vein with *M. tuberculosis* ATCC 35801 (strain Erdman) as described previously (13). Each mouse received 3.6×10^7 viable organisms suspended in 0.2 ml of modified 7H10 broth. The inoculum size was verified by plating serial dilutions of the bacterial suspension in triplicate on 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment. Plates were incubated at 37°C in ambient air for 4 weeks prior to counting viable *M. tuberculosis* colonies (CFU). Every group consisted of eight mice at each time point unless stated otherwise. Treatment was started 1 week postinfection. A control group of infected mice was sacrificed at the start of treatment (early control group). A second group of infected but untreated mice was sacrificed 4 weeks after therapy was initiated (late control group). Drugs were administered orally by gavage, 5 days per week for 12 weeks. RLZ (20 mg/kg) was given alone or in combination with PZA (150 mg/kg) and/or EMB (150 mg/kg) and was compared to RIF (20 mg/kg) combined with INH (25 mg/kg). RLZ was provided by Kaneka Corporation, Osaka, Japan. PZA, RIF, EMB, and INH were purchased from Sigma Chemical Co., St. Louis, Mo. RLZ and RIF were dissolved in dimethyl sulfoxide (DMSO), with subsequent dilution in distilled water prior to administration. The final concentration of DMSO in the drug preparations was 5%. INH, EMB, and PZA were dissolved in water. The water-soluble drugs were administered in the morning; the DMSO-soluble drugs were given in the afternoon. The MICs of RLZ, RIF, and INH for ATCC strain 35801 are 0.00047, 0.06, and 0.03 μg/ml, respectively. MICs were determined in modified 7H10 broth (pH 6.6) as described previously (3). Mice were sacrificed after completion of the 12-week treatment period, and viable cell counts were determined from the homogenates of spleens and lungs. Parallel treatment groups were sacrificed after a 12-week observation period. Sacrifice was performed by CO_2 inhalation. Spleens and right lungs were aseptically removed and ground in a tissue homogenizer (IdeaWorks! Laboratory Devices, Syracuse, N.Y.). The number of viable organisms was determined as described above. The entire volume of organ homogenates was plated to determine the number of culturable mycobacteria per organ. Statistical analysis to evaluate the number of mice showing regrowth of infection after cessation of treatment was evaluated by the Fisher exact test for a 2-by-2 contingency (4).

The results of the study are displayed in Tables 1 and 2. After 12 weeks of treatment, in every treatment group a significant reduction in the number of *M. tuberculosis* organisms was achieved, with an apparent clearance of the organs in the

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TABLE 1. *M. tuberculosis* CFU counts in spleens and
lungs after 12 weeks of therapy

Treatment group	Spleen		Lung	
	Growth[c]	Mean CFU	Growth[c]	Mean CFU
Early control	8/ 8	2.2×10^8	8/8	2.5×10^7
Late control[a]	1/1	2.8×10^7	1/1	1.5×10^8
INH plus RIF	0/8	0	0/8	0
RLZ	1/8	1	1/8	2
RLZ plus EMB[b]	2/7	30	1/7	14
RLZ plus PZA	1/8	7	1/8	1
RLZ plus PZA plus EMB	0/8	0	0/8	0

[a] Only one mouse survived.
[b] One mouse died during the first week of treatment.
[c] Growth, the number of mice with mycobacterial growth/the total number of mice in the group.

INH-RIF and RLZ-PZA-EMB groups (Table 1). The numbers of culture-positive mice between the treatment groups were not significantly different. At 12 weeks after the cessation of therapy, regrowth was detected in all groups. Of note, however, only one mouse in the RLZ-PZA-EMB group demonstrated regrowth in the lungs (one colony). Also, in the spleens of mice from this group, regrowth was observed in only half of the mice compared to almost all mice in the other groups (Table 2). In spleens, no significant difference was observed in the number of mice showing relapse of infection between all treatment groups. In contrast, in the lungs of the RLZ-PZA and RLZ-PZA-EMB groups the number of culture-positive mice was significantly lower than in the RLZ and RLZ-EMB groups. The addition of EMB to RLZ-PZA did not significantly improve the treatment outcome.

We conclude from this long-term drug study that the combinations RLZ-PZA and RLZ-PZA-EMB were the most active regimens evaluated. The addition of EMB did not significantly improve the treatment outcome in our experimental setting. However, it is possible the three-drug therapy might be

TABLE 2. *M. tuberculosis* CFU counts in spleens and lungs after
12 weeks of therapy and 12 weeks of observation

Treatment group	Spleen		Lung	
	Regrowth[f]	Mean CFU $\pm$ SD[g]	Regrowth[f]	Mean CFU $\pm$ SD[g]
INH plus RIF	7/8	339 ± 402	7/8	479 ± 670
RLZ	8/8	2,009 ± 1,956	6/8[b]	983 ± 1,233
RLZ plus EMB[e]	6/6[a]	1,720 ± 1,026	5/6[b]	1,146 ± 885
RLZ plus PZA	7/8[b,c]	1,013 ± 907	3/8[b]	1,299 ± 852
RLZ plus PZA plus EMB	4/8[d]	1,157 ± 1,524	1/8[c]	0

[a] Three of six spleens had too-many-to-count (TMTC) colonies; data represent those animals with countable colonies.
[b] One mouse had TMTC colonies.
[c] One mouse had one colony.
[d] one mouse had two colonies.
[e] Two mice died in the first week of treatment.
[f] Regrowth, the number of mice with mycobacterial regrowth/the total number of mice in the group.
[g] The results reflect the mean ± the standard deviation where there was regrowth.

superior to the two-drug regimen with regard to decreasing the emergence of drug resistance. Future experiments studying the frequency of drug resistance in long-term experiments might reveal a beneficial effect of adding a third drug. In conclusion, a sufficiently strong agent such as RLZ in combination with agents with modest activities, e.g., PZA and EMB, might be a valuable alternative to the presently used INH combination with RIF and PZA and may allow for a significantly shorter course regimen.

REFERENCES

1. **Brooks, J. A., and I. M. Orme.** 1998. Evaluation of once-weekly therapy for tuberculosis using isoniazid plus rifamycins in the mouse aerosol infection model. Antimicrob. Agents Chemother. **42:**3047–3048.
2. **Chapuis, L., B. Ji, C. Truffot-Pernot, R. J. O'Brien, M. C. Raviglione, and J. H. Grosset.** 1994. Preventive therapy of tuberculosis with rifapentine in immunocompetent and nude mice. Am. J. Respir. Crit. Care Med. **150:**1355–1362.
3. **Cynamon, M. H.** 1985. Comparative in vitro activities of MDL-473, rifamycin, and ansamycin against *Mycobacterium intracellulare.* Antimicrob. Agents Chemother. **28:**440–441.
4. **Daniel, W. W.** 1978. The Fisher exact test, p. 110–114. *In* Applied nonparametric statistics. Houghton Mifflin Co., Boston, Mass.
5. **Dhillon, J., J. M. Dickinson, J. A. Guy, T. K. Ng, and D. A. Mitchison.** 1992. Activity of two long-acting rifamycins, rifapentine and FCE 22807, in experimental murine tuberculosis. Tubercle Lung Dis. **73:**116–123.
6. **Dhillon, J., and D. A. Mitchison.** 1992. Activity in vitro of rifabutin, FCE 22807, rifapentine, and rifampin against *Mycobacterium microti* and *M. tuberculosis* and their penetration into mouse peritoneal macrophages. Am. Rev. Respir. Dis. **145:**212–214.
7. **Grosset, J., N. Lounis, C. Truffot-Pernot, R. J. O'Brien, M. C. Raviglione and B. Ji.** 1998. Once-weekly rifapentine-containing regimens for treatment of tuberculosis in mice. Am. J. Respir. Crit. Care Med. **157:**1436–1440.
8. **Ji, B., C. Truffot-Pernot, C. Lacroix, M. C. Raviglione, R. J. O'Brien, P. Olliaro, G. Roscigno, and J. Grosset.** 1993. Effectiveness of rifampin, rifabutin, and rifapentine for preventive therapy of tuberculosis in mice. Am. Rev. Respir. Dis. **148:**1541–1546.
9. **Kelly, B. P., S. K. Furney, M. T. Jessen, and I. M. Orme.** 1996. Low-dose aerosol infection model for testing drugs for efficacy against *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **40:**2809–2812.
10. **Klemens, S. P., and M. H. Cynamon.** 1996. Activity of KRM-1648 in combination with isoniazid against *Mycobacterium tuberculosis* in a murine model. Antimicrob. Agents Chemother. **40:**298–301.
11. **Klemens, S. P., M. A. Grossi, and M. H. Cynamon.** 1994. Activity of KRM-1648, a new benzoxazinorifamycin, against *Mycobacterium tuberculosis* in a murine model. Antimicrob. Agents Chemother. **38:**2245–2248.
12. **Klemens, S. P., C. A. Sharpe, and M. H. Cynamon.** 1994. Activity of levofloxacin in a murine model of tuberculosis. Antimicrob. Agents Chemother. **38:**1476–1479.
13. **Lenaerts, A. M. J. A., S. E. Chase, A. J. Chmielewski, and M. H. Cynamon.** 1999. Evaluation of rifapentine in long-term treatment regimens for tuberculosis in mice. Antimicrob. Agents Chemother. **43:**2356–2360.
14. **Moghazeh, S. L., X. Pan, T. Arain, C. K. Stover, J. M. Musser, and B. N. Kreiswirth.** 1996. Comparative antimicrobial activities of rifampin, rifapentine and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known rpoB mutations. Antimicrob. Agents Chemother. **40:**2655–2657.
15. **Mor, N., B. Simon, N. Mezo, and L. Heifets.** 1995. Comparison of activities of rifapentine and rifampin against *Mycobacterium tuberculosis* residing in human macrophages. Antimicrob. Agents Chemother. **39:**2073–2077.
16. **Shoen, C. M., S. E. Chase, M. S. DeStefano, T. S. Harpster, A. J. Chmielewski, and M. H. Cynamon.** 2000. Evaluation of rifalazil in long-term treatment regimens for tuberculosis in mice. Antimicrob. Agents Chemother. **44:**1458–1462.