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Activity of KRM-1648, a New Benzoxazinorifamycin, against *Mycobacterium tuberculosis* in a Murine Model

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The activity of KRM-1648 was evaluated in a murine model of tuberculosis. Approximately 10^7 viable *Mycobacterium tuberculosis* ATCC 35801 organisms were given intravenously to 4-week-old female outbred mice. Treatment was started 1 week postinfection and given by gavage for 4 weeks. Viable-cell counts were determined from homogenates of spleen and lung tissues. The activity of KRM-1648 was compared with those of rifampin and rifabutin at 20 mg/kg of body weight. KRM-1648 was more active than either rifampin or rifabutin against organisms in spleens and lungs. KRM-1648 alone and in combination with either isoniazid, ethambutol, pyrazinamide, or levofloxacin was evaluated. Other treatment groups received isoniazid, ethambutol, pyrazinamide, or levofloxacin as single agents. KRM-1648 was the most active single agent evaluated. KRM-1648–pyrazinamide and KRM-1648–isoniazid were the most active combinations. These combinations were more active than KRM-1648 alone. The promising activity of KRM-1648 in *M. tuberculosis*-infected mice suggests that it is a good candidate for clinical development as a new antituberculosis agent.

KRM-1648 (KRM) is a rifamycin analog with the chemical structure 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl)-benzoxazinorifamycin (14). This new rifamycin is considerably more active than rifampin in vitro against a number of mycobacterial species, including *Mycobacterium tuberculosis*, *M. avium* complex, *M. kansasii*, and *M. marinum* (7, 11, 13). This promising in vitro activity is borne out in animal models of infection with *M. tuberculosis*, *M. avium* complex, and *M. leprae* (3, 4, 10, 12).

The purpose of the present study was to evaluate the activity of KRM in a murine model of tuberculosis. KRM was administered at 20 mg/kg of body weight because of the availability of pharmacokinetic data for mice given this dose (12). The activity of KRM was compared with those of the first-line antituberculosis agents and with those of rifabutin and levofloxacin. The activity of KRM in combination with isoniazid, ethambutol, pyrazinamide, and levofloxacin was also evaluated.

MATERIALS AND METHODS

KRM was provided by Kaneka Corporation, Osaka, Japan. Rifabutin (RBT) was provided by Adria Pharmaceuticals, Columbus, Ohio. Levofloxacin (LEV) was provided by R.W. Johnson Pharmaceutical Research Institute, Raritan, N.J. Isoniazid (INH), rifampin (RIF), ethambutol (EMB), and pyrazinamide (PZA) were obtained from Sigma Chemical Co., St. Louis, Mo. KRM, RBT, and RIF were dissolved in dimethyl sulfoxide with subsequent dilution in distilled water prior to administration. LEV was dissolved in absolute ethanol with subsequent dilution in water. Two drops of 0.1 N NaOH were required for complete solubilization. INH, EMB, and PZA were dissolved in water. Drugs were freshly prepared each morning prior to administration.

Isolate. *M. tuberculosis* ATCC 35801 (strain Erdman) was obtained from the American Type Culture Collection, Rockville, Md. The MICs of all antimicrobial agents except PZA were determined in modified Middlebrook 7H10 broth (7H10 agar formulation with agar and malachite green omitted), pH 6.6, supplemented with 10% Middlebrook OADC (oleic acid-albumin-dextrose-catalase) enrichment (Difco Laboratories, Detroit, Mich.) and 0.05% Tween 80 (1). The MIC of PZA was determined in 7H10 agar, pH 5.8, supplemented with 10% Middlebrook OADC enrichment (5, 6). The MICs (in micrograms per milliliter) of KRM, RBT, RIF, LEV, INH, EMB, and PZA are 0.00047, 0.015, 0.06, 2, 0.03, 1, and 32, respectively.

Medium. The organism was grown in modified 7H10 broth with 10% OADC enrichment and 0.05% Tween 80 on a rotary shaker for 5 days. The culture suspension was diluted in modified 7H10 broth to yield 100 Klett units/ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.), or approximately 5×10^7 CFU/ml. The size of the inoculum was determined by titration and counting from triplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% OADC enrichment. The plates were incubated at 37°C for 4 weeks prior to counting.

Infection studies. The infection studies were performed in two separate experiments. Four-week-old female outbred CD-1 mice (Charles River, Wilmington, Mass.) were infected intravenously through a caudal vein. Each mouse received approximately 10^7 viable organisms suspended in 0.2 ml of modified 7H10 broth. There were 8 to 10 mice per group.

Treatment was started 1 week after infection. A control group of infected mice (designated early controls) was sacrificed at the start of treatment. Treatment was given 5 days/week for 4 weeks. Remaining animals in a control group of infected but untreated mice (late controls) were sacrificed at the end of the treatment period. Animals were sacrificed by cervical dislocation 3 to 5 days after the last dose of drug. Spleens and lungs were aseptically removed and ground in a tissue homogenizer. The number of viable organisms was determined by titration on 7H10 agar plates.

Statistical evaluation. The viable-cell counts were converted to logarithms, which were then evaluated with one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by the Tukey honestly significant difference test (8) to make pairwise

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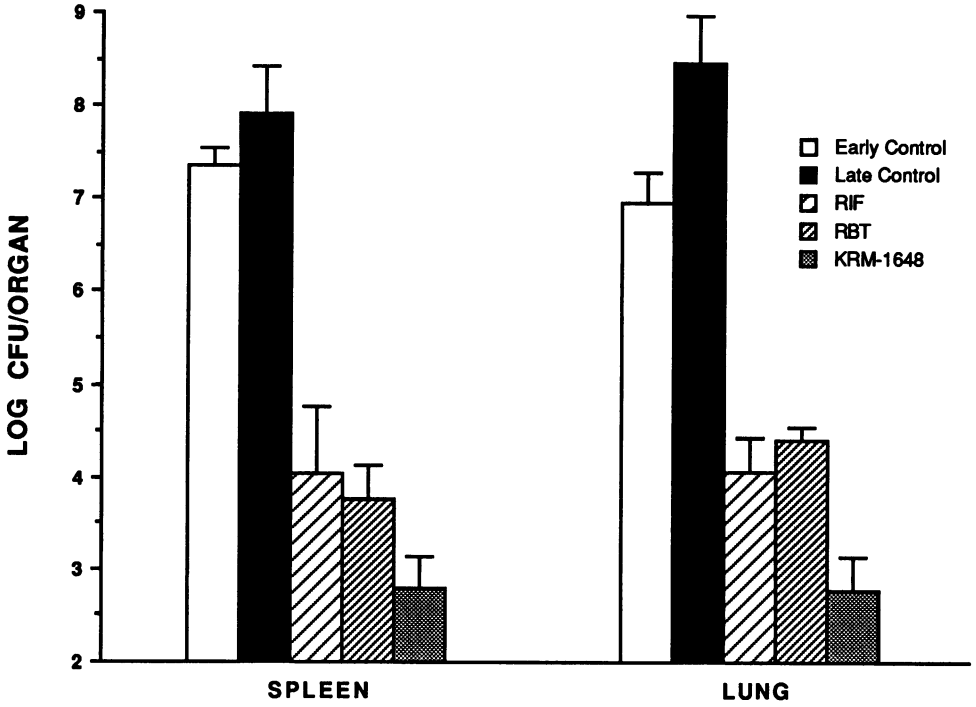

FIG. 1. Comparative activities of KRM, RIF, and RBT. Results are means for 8 to 10 mice per group; error bars reflect standard deviations.

comparisons among means. The results of the statistical evaluations are summarized in the following section.

RESULTS

Comparative activities of KRM, RIF, and RBT. KRM, RIF, and RBT (20 mg/kg of body weight) were each given 5 days/week for 4 weeks to female mice which had been infected with 2.2×10^7 viable *M. tuberculosis* organisms.

The increase in cell counts between the early and late control groups was significant for organisms in lungs ($P < 0.01$) but not for organisms in spleens ($P > 0.05$). Mortality in the late control group was 40%, with deaths occurring on days 15, 21, 25, and 28 postinfection. There were no deaths in any group receiving treatment.

Treatment with KRM, RIF, and RBT reduced cell counts in spleens and lungs compared with both early controls ($P < 0.01$ for all agents) and late controls ($P < 0.01$ for all agents). KRM was more active than RIF or RBT against organisms in spleens and lungs ($P < 0.01$). The differences in organ cell counts between groups receiving RIF and RBT were not significant ($P > 0.05$) (Fig. 1).

Comparison of KRM with INH, EMB, PZA, and LEV alone and in combination. KRM alone (20 mg/kg) and in combination with INH (25 mg/kg), PZA (150 mg/kg), EMB (125 mg/kg), or LEV (200 mg/kg) was given 5 days/week for 4 weeks to female mice which had been infected with 2.2×10^7 viable *M. tuberculosis* organisms. Other treatment groups received INH, PZA, EMB, and LEV as single agents.

The difference in cell counts between early and late control groups was not significant for organisms in spleens ($P > 0.05$). The increase in cell counts between early and late control groups was significant for organisms in lungs ($P < 0.01$). Mortality in the late control group was 50%, with deaths occurring on days 14 (one mouse), 22 (two mice), and 23 (two mice) postinfection. There were two deaths in the LEV treatment group (days 14 and 28 postinfection).

Treatment with KRM, INH, PZA, EMB, and LEV reduced cell counts in spleens compared with early controls ($P < 0.01$ for KRM, INH, EMB, and LEV; $P < 0.05$ for PZA). Treatment with KRM, INH, EMB, and LEV reduced cell counts in spleens compared with late controls ($P < 0.01$ for each). Treatment with PZA did not reduce cell counts in spleens compared with late controls ($P > 0.05$). KRM was the most active single agent against organisms in spleens. The differences in splenic cell counts between the group receiving KRM and groups receiving INH, PZA, EMB, or LEV were significant ($P < 0.01$ for each) (Fig. 2; Table 1).

Only the combination of KRM plus PZA was more active against organisms in spleens than treatment with KRM alone ($P < 0.01$). Differences in splenic cell counts between all other combinations and treatment with KRM alone were not significant ($P > 0.05$). The difference in cell counts between groups receiving KRM plus INH and KRM plus PZA did not reach statistical significance ($P > 0.05$).

Treatment with KRM and INH as single agents reduced cell counts in lungs compared with early controls ($P < 0.01$). Treatment with PZA, EMB, or LEV did not reduce lung cell counts compared with early controls ($P > 0.05$). Treatment with KRM, INH, EMB, and LEV reduced cell counts in lungs compared with late controls ($P < 0.01$). PZA did not reduce cell counts in lungs compared with late controls ($P > 0.05$). KRM was the most active single agent against organisms in lungs. The differences in lung cell counts between the group receiving KRM and groups receiving INH, PZA, EMB, or LEV were significant ($P < 0.01$ for each).

The combinations of KRM plus INH and KRM plus PZA were more active against organisms in lungs than treatment with KRM alone ($P < 0.05$ for KRM plus INH; $P < 0.01$ for KRM plus PZA). There was no difference between KRM plus

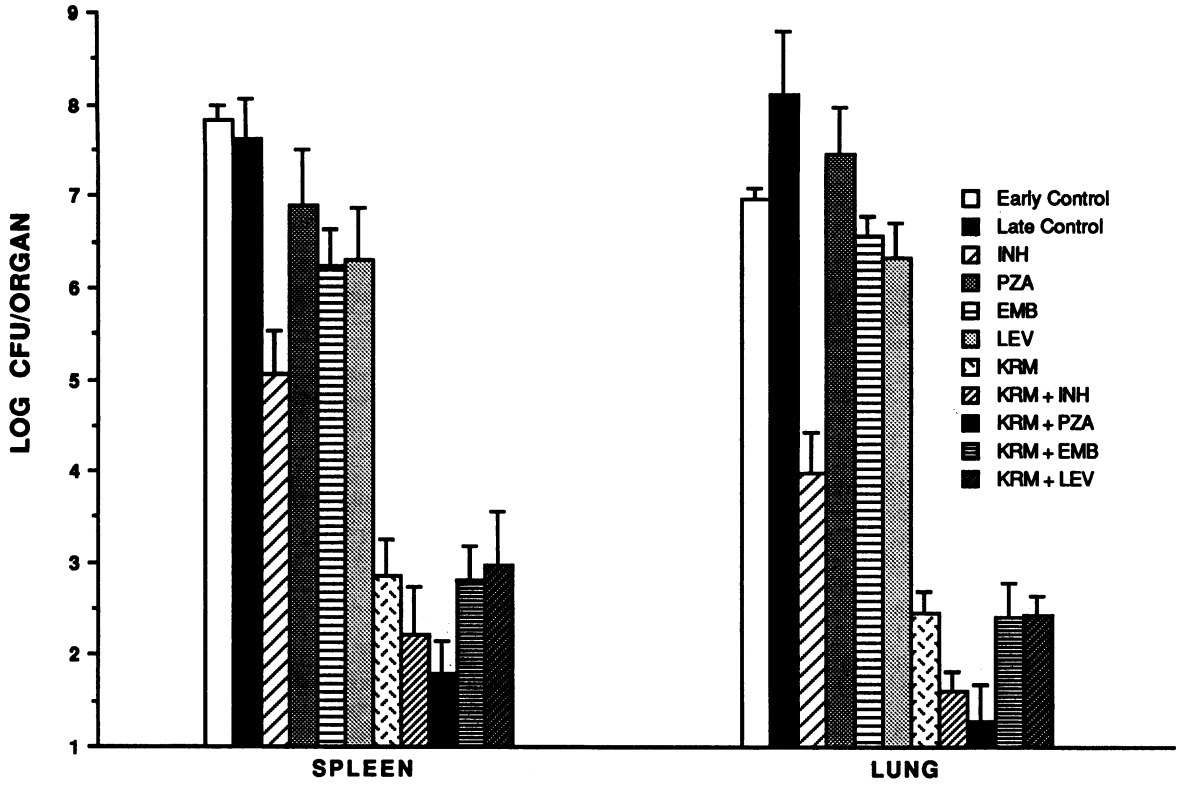

FIG. 2. Comparison of KRM with INH, EMB, PZA, and LEV alone and in combination.

INH and KRM plus PZA against organisms in lungs ($P > 0.05$). Differences in lung cell counts between groups receiving KRM plus EMB or KRM plus LEV and treatment with KRM alone were not significant ($P > 0.05$).

DISCUSSION

The MIC of KRM against *M. tuberculosis* ATCC 35801 is 0.00047, considerably lower than the broth-determined MICs of RIF and RBT. The MICs of KRM and RIF for 90% of isolates, as determined by agar dilution on 7H11 agar against a group of *M. tuberculosis* strains ($n = 16$), are ≤0.0125 and 0.8, respectively (11). The superior in vitro activity of KRM is paralleled by enhanced in vivo activity against *M. tuberculosis* in the murine model. Kuze et al. previously reported promising activity of KRM against *M. tuberculosis* H37Rv in ddY mice as measured by a reduction in mortality compared with that in untreated control mice (10). KRM resulted in at least a 1-log-greater reduction in organ cell counts than treatment with either RIF or RBT in the current short-term treatment study.

TABLE 1. Reduction of *M. tuberculosis* cell counts by KRM alone and in combination

Group	Mean log CFU in:	
	Spleens	Lungs
Early control	7.83 ± 0.17	6.95 ± 0.13
Late control	7.61 ± 0.46	8.12 ± 0.66
INH	5.07 ± 0.45	3.99 ± 0.44
PZA	6.89 ± 0.62	7.46 ± 0.50
EMB	6.24 ± 0.43	6.55 ± 0.24
LEV	6.30 ± 0.57	6.33 ± 0.38
KRM	2.85 ± 0.40	2.45 ± 0.23
KRM + INH	2.23 ± 0.50	1.61 ± 0.21
KRM + PZA	1.80 ± 0.34	1.29 ± 0.40
KRM + EMB	2.81 ± 0.38	2.40 ± 0.38
KRM + LEV	2.98 ± 0.57	2.43 ± 0.22

Tomioka et al. reported the comparative pharmacokinetics of KRM, RIF, and RBT determined in male ddY mice following administration of a single 20-mg/kg dose by gavage (12). The peak concentration of KRM in plasma was 0.60 μg/ml 8 h after administration. KRM appears to be concentrated within tissues, as peak levels achieved in lungs and spleens were 5.27 and 9.14 μg/g, respectively (12).

KRM was more active than INH, RIF, RBT, PZA, EMB, or LEV in this short-term treatment study. Treatment with PZA, EMB, and LEV tended to inhibit growth of organisms, while treatment with KRM, RIF, RBT, and INH reduced cell counts in both organs compared with early controls. LEV at higher doses (300 and 400 mg/kg) is more active in the murine system; however, the dose in mice that corresponds to the maximum tolerated daily dose in humans has not yet been established (9).

The combinations of KRM plus INH and KRM plus PZA were more active than KRM alone. Additional studies of KRM in combination with INH or PZA in longer-term experiments would be of interest. Evaluation for regrowth following cessation of therapy would help estimate the potential sterilizing activity of these agents in combination. The promising activity of KRM in *M. tuberculosis*-infected mice suggests that it is a good candidate for clinical development as a new antituberculosis agent. It is hoped that the enhanced activity of KRM compared with that of RIF will allow for ultra-short-course therapy, i.e., treatment regimens of 4 months or less (2).

Studies in murine tuberculosis test systems are in progress to evaluate this possibility.

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REFERENCES

1. **Cynamon, M. H.** 1985. Comparative in vitro activities of MDL 473, rifampin, and ansamycin against *Mycobacterium intracellulare*. Antimicrob. Agents Chemother. **28:**440–441.
2. **Cynamon, M. H., and S. P. Klemens.** 1994. Chemotherapeutic agents for mycobacterial infections, p. 237–257. *In* L. N. Friedman (ed.), Tuberculosis: current concepts and treatment. CRC Press, Inc., Boca Raton, Fla.
3. **Emori, M., H. Saito, K. Sato, H. Tomioka, T. Setogawa, and T. Hidaka.** 1993. Therapeutic efficacy of the benzoxazinorifamycin KRM-1648 against experimental *Mycobacterium avium* infection induced in rabbits. Antimicrob. Agents Chemother. **37:**722–728.
4. **Gidoh, M., S. Tsutsumi, T. Yamane, K. Yamashita, K. Hosoe, and T. Hidaka.** 1992. Bactericidal action at low doses of a new rifamycin derivative, 3′-hydroxy-5′-(4-isobutyl-1-piperazinyl) benzoxazinorifamycin (KRM-1648) on *Mycobacterium leprae* inoculated into footpads of nude mice. Lepr. Rev. **63:**319–328.
5. **Hawkins, J. E.** 1984. Drug susceptibility testing, p. 179–180. *In* G. P. Kubica and L. G. Wayne (ed.), The mycobacteria: a sourcebook. M. Dekker, Inc., New York, N.Y.
6. **Heifets, L. B.** 1991. Drug susceptibility tests in the management of chemotherapy of tuberculosis, p. 109–111. *In* L. B. Heifets (ed.), Drug susceptibility in the chemotherapy of mycobacterial infections. CRC Press, Inc., Boca Raton, Fla.
7. **Inderlied, C. B., L. E. Bermudez, L. Barbara-Burnham, M. Wu, and L. S. Young.** 1993. In vitro and macrophage activity of KRM1648 against the *Mycobacterium avium* complex (MAC), abstr. 69. Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother.
8. **Kirk, R. E.** 1986. Experimental design: procedures for the behavioral sciences. Brooks-Cole Publishing Co., Belmont, Calif.
9. **Klemens, S. P., C. A. Sharpe, M. C. Rogge, and M. H. Cynamon.** 1994. Activity of levofloxacin in a murine model of tuberculosis. Antimicrob. Agents Chemother. **38:**1476–1479.
10. **Kuze, F., T. Yamamoto, R. Amitani, and K. Suzuki.** 1990. *In vivo* activities of new rifamycin derivatives against mycobacteria. Kekkaku **66:**7–12.
11. **Saito, H., H. Tomioka, K. Sato, M. Emori, T. Yamane, K. Yamashita, K. Hosoe, and T. Hidaka.** 1991. In vitro antimycobacterial activities of newly synthesized benzoxazinorifamycins. Antimicrob. Agents Chemother. **35:**542–547.
12. **Tomioka, H., H. Saito, K. Sato, T. Yamane, K. Yamashita, K. Hosoe, K. Fujii, and T. Hidaka.** 1992. Chemotherapeutic efficacy of a newly synthesized benzoxazinorifamycin, KRM-1648, against *Mycobacterium avium* complex infection induced in mice. Antimicrob. Agents Chemother. **36:**387–393.
13. **Yamamoto, T., R. Amitani, F. Kuze, and K. Suzuki.** 1990. *In vitro* activities of new rifamycin derivatives against *Mycobacterium tuberculosis* and *M. avium* complex. Kekkaku **65:**31–36.
14. **Yamane, T., T. Hashizume, K. Yamashita, E. Konishi, K. Hosoe, T. Hidaka, K. Watanabe, H. Kawaharada, T. Yamamoto, and F. Kuze.** 1993. Synthesis and biological activity of 3′-hydroxy-5′-aminobenzoxazinorifamycin derivatives. Chem. Pharm. Bull. **41:** 148–155.