

## Evaluation of Once-Weekly Therapy for Tuberculosis Using Isoniazid plus Rifamycins in the Mouse Aerosol Infection Model

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**Once-weekly therapy with combinations of isoniazid plus a rifamycin was tested in the mouse low-dose aerosol infection model against two strains of *Mycobacterium tuberculosis*. Combinations of isoniazid and rifalizil and isoniazid and rifapentine were both highly effective. These animal model data thus support the evaluation of these regimens under clinical conditions.**

The therapy of disease caused by *Mycobacterium tuberculosis* is now further complicated by the emergence of increasing drug resistance. One of the factors contributing to this specific problem, and indeed to the therapy of tuberculosis as a whole, is patient compliance (1). To address this issue, the World Health Organization has instigated a program of directly observed therapy (2, 3, 5, 6, 14, 20).

Among approaches to encourage patient compliance are shortening the therapy period (some regimens in the past have required a year or more of daily treatment) and lengthening the period between doses, e.g., instituting once-weekly therapy. This latter approach is particularly attractive in areas of the world, such as Africa, in which patients may be widely dispersed in rural areas away from the nearest clinic (8, 10, 17, 24, 25).

In this study, we used the mouse aerosol infection model (18) to evaluate the combination of isoniazid and the newly available rifamycins (4, 7, 9, 11-13, 15, 16, 19, 21, 22) given once weekly. Combinations of isoniazid with rifapentine (RPT) or rifalizil (RLZ) were the most effective in this model. These data suggest that such regimens are indeed worthy of evaluation in clinical trials.

Female specific-pathogen-free C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine) were aerogenically infected with *M. tuberculosis* Erdman (TMC107) or the highly virulent (drug-sensitive) strain CSU93 (23) with a Middlebrook aerosol generation device (Glas-Col Inc., Terre Haute, Ind.). Mice were exposed to a standardized inoculum that reproducibly deposits 50 to 100 bacilli into the lung tissues. The course of the infection was then monitored against time by plating serial dilutions of individual whole-organ homogenates on nutrient 7H11 agar and counting bacterial colony formation 14 to 21 days later after incubation at 37°C in humidified air. RLZ, previously designated KRM-1648, was provided by PathoGenesis Corp., Seattle, Wash.; rifampin (RIF) was purchased from Sigma Chemical Co., St. Louis, Mo. RPT was provided by Hoechst Marion Roussel, Inc., Cincinnati, Ohio; rifabutin (RBT) was provided by Pharmacia Upjohn, Columbus, Ohio. The MICs (of RIF, 0.125 µg; of RPT, 0.03 µg; of RBT, 0.03 µg; and of RLZ, 0.008 µg), determined by plating inocula onto agar con-

taining dilutions of drug, for the two strains were similar. For use in vivo, each drug was initially dissolved in a small volume of ethanol and then further dissolved in 0.05% methylcellulose and 0.04% Tween 80 in distilled water prior to oral gavage. Therapy, started at week 3, was given once weekly for 5 weeks. All drugs were given at a dose of 25 mg/kg of body weight/day.

The results of the study are shown in Fig. 1. One week after the 5-week therapy period was discontinued, mice given the isoniazid-RLZ or isoniazid-RPT combination showed clearance in the lungs of the CSU93 strain and only a few detectable Erdman bacilli. Animals treated with isoniazid and RIF or isoniazid and RBT also showed clearance, but to a much lesser degree. A similar pattern of clearance was seen in the spleen. Bacteria in mice disseminate to this organ and can usually be

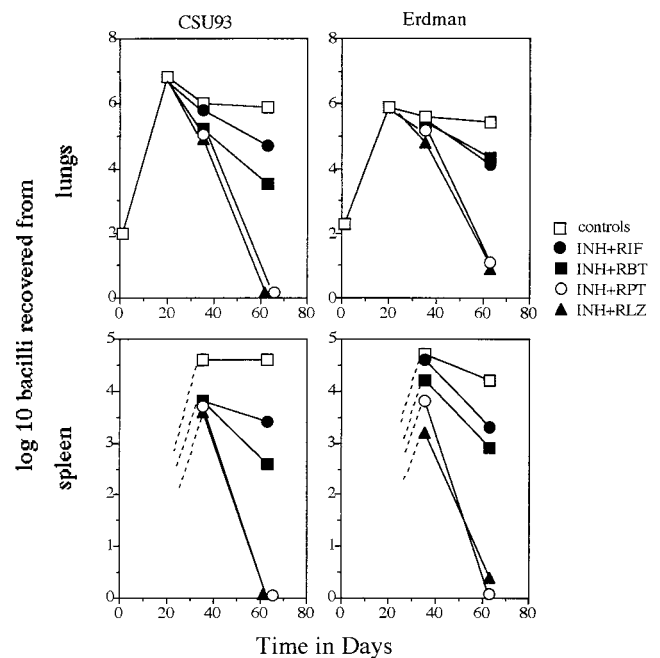


FIG. 1. Growth of *M. tuberculosis* CSU93 and Erdman following aerosol exposure of C57BL/6 mice and the effects of therapy (given from days 21 to 56). INH, isoniazid. All drugs were given at a dose of 25 mg/kg/day. Data are expressed as mean values ( $n = 4$ ; standard errors of the mean, omitted for clarity, did not exceed 0.45).

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detected a few weeks into infection. Bacteria were detectable in all mice at day 35 (2 weeks into therapy), but these were then cleared in a pattern similar to that seen in the lungs, again with isoniazid-RLZ and isoniazid-RPT being the most-effective drugs. These animal model data therefore support the evaluation of these regimens as intermittent therapy in clinical trials.

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