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Sterilizing Activity of Thioridazine in Combination with the First-Line Regimen against Acute Murine Tuberculosis

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We recently reported that in lung tissue, thioridazine accumulates at high concentrations relative to serum levels, displaying modest synergy with isoniazid and reducing the emergence of isoniazid-resistant mutants in mouse lungs. In this study, we sought to investigate the sterilizing activity of human-equivalent doses of thioridazine when given in combination with the "Denver regimen" against acute murine tuberculosis. We found a trend toward a positive impact of thioridazine on the bacterial clearance and lowering relapse rates of the combined standard TB chemotherapy.

Active tuberculosis (TB) in humans comprises a mixed population of rapidly multiplying bacilli and sporadically replicating or nonreplicating persisters, which require prolonged treatment to prevent clinical relapse, posing a major obstacle to global TB eradication. Strategies involving new drugs and shorter regimens, as well as new applications for existing FDA-approved drugs, are urgently needed to combat the TB epidemic. Efflux pumps, crucial for *Mycobacterium tuberculosis* survival and persistence under antimicrobial stress, are now known to contribute to intrinsic or acquired resistance (1). Therefore, efflux pump inhibitors, which are already in clinical practice for other medical indications, may be useful as novel chemotherapeutics against *M. tuberculosis* (2). The efflux pump inhibitor and antipsychotic drug thioridazine (TRZ), which is inexpensive, readily available, and relatively safe, has shown activity against drug-sensitive and drug-resistant strains *in vitro* (3, 4), *ex vivo* (5), and *in vivo* (6, 7), and in extensively drug-resistant (XDR)-TB patients when used in combination with antibiotics to which the strains were initially resistant (8). Although we found that TRZ is ineffective as monotherapy in the mouse model of TB, the drug exhibited modest synergy during coadministration with isoniazid (INH), reducing the emergence of INH-resistant mutants (9). As TRZ accumulates at high concentrations relative to serum levels in lung tissue (9), we hypothesized that treatment with TRZ in combination with the standard first-line anti-TB (Denver) regimen (10–12) may accelerate the eradication of bacilli from the lungs of mice acutely infected with *M. tuberculosis*, reducing the time required to prevent microbiological relapse.

All animal-related procedures were approved by the Johns Hopkins University (JHU) School of Medicine Animal Care and Use Committee. A total of 141 female BALB/c mice aged 4 to 6 weeks (Charles River Labs, Wilmington, MA) were aerosol infected with *M. tuberculosis* H37Rv (JHU), using the inhalation exposure system (Glas-Col, Terre Haute, IN) calibrated to deliver ~10^4 CFU per mouse lung in two consecutive runs. After aerosol infection, the mice were randomized into treatment groups, as outlined in Table 1. Two weeks postinfection, the mice were treated daily (5 days/week) orally with human-equivalent doses of INH (10 mg/kg), rifampin (RIF) (10 mg/kg), and pyrazinamide (PZA) (150 mg/kg) with or without TRZ (25 mg/kg) (9, 13) for up to 6 months. For the first 2 months of treatment, mice were given RIF, INH, and PZA, and for the remaining 4 months they were given only RIF and INH to mirror the Denver regimen. The RIF dose preceded that of the other drugs (INH-PZA/INH-PZA-TRZ) by at least 1 h to prevent pharmacokinetic antagonism (14, 15). Mice were scheduled for sacrifice on the day after infection, on the day of treatment initiation, and at the indicated time points after treatment to determine the numbers of CFU implanted in the lungs, pretreatment baseline lung CFU counts, and posttreatment lung CFU, respectively (Table 1). Treatment was discontinued for groups of 10 mice after completion of 4, 5, or 6 months of antibiotic treatment for the assessment of relapse. Relapse was defined as the presence of mycobacterial colonies upon plating of entire undiluted lung homogenates.

Animal body weights and lung and spleen weights were recorded at the time of sacrifice. The lungs of sacrificed animals were examined grossly for visible lesions, and small, randomly selected sections were formalin fixed for histopathology. The remainder of each lung was homogenized in 2.5 ml phosphate-buffered saline (PBS). Lung homogenates were plated on selective 7H11 plates (BD, Baltimore, MD) for CFU enumeration.

CFU data were derived from five mice per group. Log-transformed CFU were used to calculate means and standard deviations (SDs). Comparisons of data among experimental groups were performed by *t* test. A difference was considered statistically significant at a P value of <0.05.

One day postinfection, the mean (±SD) lung CFU counts were $\log_{10}$ 4.37 ± 0.06 and 4.37 ± 0.09 in aerosol runs 1 and 2, respectively. Thirteen days later, on the day (day 0) of treatment initiation the mean lung CFU count was 8.28 ± 0.14 $\log_{10}$. The untreated mice became moribund by 3 weeks postinfection and were euthanized in accordance with animal care regulations. No spontaneous mortality was recorded in the treated groups during the entire study period. In the initial phase, the standard regimen of RIF-INH-PZA reduced the lung CFU counts to 5.72 ± 0.16 and 3.73 ± 0.15 $\log_{10}$ after 1 and 2 months of treatment, respectively,

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TABLE 1 Bacillary burden in the lungs of acutely infected mice during treatment and relapse rates

Group[a]	Log_{10} CFU count (mean ±SD) at[b]:								Relapse rate (no. positive culture/total no. of mice [%]), assessed 3 mo after completion of treatment for:		
	D13	D0	M1	M2	M3	M4	M5	M6	M4	M5	M6
Untreated	4.37 ± 0.07	8.28 ± 0.14									
RIF-INH-PZA			5.72 ± 0.16	3.73 ± 0.15	1.82 ± 0.26	0.37 ± 0.23	0 ± 0	0 ± 0	9/10 (90)	2/10 (20)	0/10 (0)
RIF-INH-PZA-TRZ			5.28 ± 0.19	3.41 ± 0.3	1.31 ± 0.12	0 ± 0	0 ± 0	0 ± 0	6/10 (60)	0/10 (0)	0/10 (0)
Total no. of mice	6	5	20[c]	10	10	10	10	10	20	20	20

[a] Drug doses: 10 mg/kg isoniazid (INH), 10 mg/kg rifampin (RIF), 150 mg/kg pyrazinamide (PZA), and 25 mg/kg thioridazine (TRZ). For the two treated groups, PZA was given for the first 2 months.
[b] D, day; M, month.
[c] All 10 untreated mice died within 3 weeks.

whereas TRZ in combination with RIF-INH-PZA showed very modest synergistic activity, reducing bacterial burden by an additional 0.439 log_{10} ($P = 0.005$) and 0.31 log_{10} ($P = 0.07$) relative to RIF-INH-PZA after combination treatment for 1 month and 2 months, respectively. During the continuation phase, the addition of TRZ to RIF-INH resulted in slightly greater killing activity at 3 months of treatment compared to RIF-INH (mean lung CFU counts, 5.28 ± 0.19 and 5.72 ± 0.16, respectively; $P = 0.004$). At 4 months after treatment initiation, all lungs of mice receiving the RIF-INH-TRZ regimen were culture negative, remaining so at month 5 and month 6. However, the lungs of mice receiving RIF-INH remained culture positive at month 4 (0.43 ± 0.13 log_{10}), thereafter becoming culture negative at month 5 and month 6. In mice treated with the Denver regimen, relapse rates of 90%, 20% (2 CFU isolated in each mouse lung), and 0% were observed after completion of treatment for 4 months, 5 months, and 6 months, respectively, whereas mice treated with the Denver regimen plus TRZ showed relapse rates of 60%, 0%, and 0% after completion of treatment for 4 months, 5 months, and 6 months, respectively.

To our knowledge this is the first preclinical study to investigate the sterilizing activity of TRZ when used in combination with the standard first-line regimen against acute murine TB. TRZ given at human-equivalent doses was safe and well tolerated for the entire treatment period (9, 16). When TRZ was added to the Denver regimen, we observed a trend toward enhanced clearance of bacilli in the lungs of acutely infected mice relative to the control regimen alone. Our findings might be explained by the previously reported synergy of TRZ with the cell wall-active agent INH (9), enhancing the killing of actively multiplying bacilli, and with the transcriptional inhibitor RIF, accelerating the clearance of persisters (17). Dormant *M. tuberculosis* in an *in vitro* hollow fiber system (18) and in the Wayne model of progressive hypoxia (19) shows susceptibility to TRZ, which targets *M. tuberculosis* respiration (12). Furthermore, TRZ appears to induce the killing of intracellular bacilli by macrophages (20). The sterilizing activity of TRZ given in combination with multidrug-resistant (MDR) and XDR regimens deserves further study in preclinical animal models. In addition, the role of TRZ against latent TB infection should be investigated (13, 21).

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