Thioridazine for treatment of tuberculosis: Promises and pitfalls

Noton K. Dutta a, *, Petros C. Karakousis a, b, *

a Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA
b Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

SUMMARY

The articles by De Knegt et al. and Singh et al. in a recent issue of this Journal address one of the current debates regarding the potential role of thioridazine in the treatment of tuberculosis. This commentary presents a summary of the available evidence, and, emphasizing the need for further research, asks the question: “How far can we go in repurposing thioridazine?”

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1. Should thioridazine be included in World Health Organization (WHO) group 5 drugs for the treatment of drug-resistant tuberculosis (TB)?

Ongoing research has generated several new drugs, which are in various stages of preclinical and clinical assessment [1,2]. Despite this progress [3,4], the global TB drug pipeline is insufficient to address the imminent but unmet medical needs for new anti-TB drugs to treat multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. Additional sustainable research efforts are also required to identify new drug combinations in order to simplify or shorten TB treatment to 2 months or less, thereby improving medical adherence and preventing new cases of MDR and XDR TB from occurring. Ideally, these new drug regimens should be cost-effective, easily adopted in the field, and have activity in a broad range of individuals, including those with liver compromise (many anti-TB drugs are hepatotoxic), HIV (to avoid drug interactions), and in children (ethambutol and streptomycin may cause permanent defects) [5,6]. Additionally, to minimize the selection of resistant strains, it is highly desirable that the genes encoding the novel drug targets display low mutation frequencies [7].

One strategy for accelerating the discovery of novel regimens is to search for existing compounds, which are in current clinical use for the treatment of other diseases, but which may also exhibit anti-TB properties [8]. Recently, “repurposing” of the old antipsychotic phenothiazine, thioridazine (TRZ) [9], has been considered as an adjunctive therapy for MDR- and XDR-TB cases [9–13].

TRZ has broad-spectrum antibacterial (antimicrobial, non-antibiotic) activity [14–16], including against various drug-resistant and drug-resistant Mycobacterium spp. at 6–12.5 μg/ml in vitro [17]. However, the MIC against Mycobacterium tuberculosis (Mtbc) in macrophages has been reported to be in the range of 0.1–3.6 μg/ml [18,19] due to intracellular concentration of the drug [18]. Thus, clinically acceptable dosing of TRZ in an infected patient might result in an inhibitory effect in situ (within infected macrophages) similar to that observed in vitro. Unlike the first-line drugs isoniazid, which targets primarily actively multiplying bacilli by inhibiting the mycolic acid synthesis pathway [20,21], and the transcriptional inhibitor rifampin, which targets primarily growth-restricted bacilli [22], TRZ has been shown to target both slowly replicating and logarithmically growing bacilli [23–25] in an in vitro hollow fiber system [26], likely due to its multiple mechanisms of action.

In this issue of Tuberculosis [27], De Knegt and colleagues showed concentration and time-dependent bactericidal activity for TRZ against both actively-replicating and slowly-replicating Mtbc. Furthermore, relatively high concentrations of TRZ showed synergy with isoniazid and rifampin. In the case of isoniazid, this resulted in elimination of mycobacteria and prevention of isoniazid-resistant mutants, consistent with similar findings in a mouse model of TB infection [28]. Previously, Viveiros et al. reported that TRZ enhances the activity of rifampin and streptomycin when used in combination at minimally effective concentrations against clinical strains of poly-drug resistant Mtbc [29].

Due to its pleiotropic effects, TRZ may provide strategies for multi-target drug development for combination chemotherapy [30–32]. TRZ appears to act on enzymes involved in fatty acid metabolism, efflux proteins (emrE-encoded), oxido-reductases, and proteins (ndh-encoded) involved in aerobic respiration, which overlap with the targets of conventional anti-TB drugs [30,33,34]. In addition, TRZ targets the Rv3160c-Rv3161c operon, which may be required for the detoxification of TRZ, members of the SigB sigma

* Corresponding authors. Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA.
E-mail addresses: ndutta1@jhmi.edu (N.K. Dutta), petros@jhmi.edu (P.C. Karakousis).

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factor regulon, which plays a crucial role in protecting the pathogen against cell envelope damage, and Rv2745c, a transcription factor that regulates ATP-dependent proteolysis [35]. Several of these targets have been shown to be essential for *Mtb* persistence in the infected host [36,37]. Increased activity of efflux pumps of mycobacteria can prevent antibiotics from reaching their intended target, leading to an MDR phenotype [38,39]. TRZ has been shown to have efflux pump-inhibiting activity against mycobacteria both in vitro and ex vivo [40]. For example, it is an effective inhibitor of the intrinsic efflux pump system, which is considered responsible for intrinsic resistance to erythromycin [40]. Machado and colleagues have demonstrated that overexpression of such efflux pumps favors accumulation of mutations in isoniazid targets and that isoniazid resistance can be reduced by TRZ exposure in *Mtb* [41]. Phenothiazines also inhibit bacterial access to calcium by inhibiting the activity of calcium-dependent ATPase and, hence, drug transport processes, resulting in accumulation of phenothiazine within the cell. This process contributes to acidification of the phagolysosome and the subsequent activation of its hydrolases, thereby inhibiting the replication of the bacterium [42]. At high concentrations, TRZ can cause cardiac side effects, specifically prolongation of the QT interval [13]. Our recent pharmacokinetic data in mice suggest that TRZ is not toxic at human-equivalent doses (25 mg/kg), but daily dosing above 50 mg/kg resulted in acute mortality of mice [28]. TRZ was found to accumulate in murine lung tissue relative to serum [28]. Interestingly, enhanced tissue concentration of TRZ was not associated with greater bactericidal activity in mouse lungs, in which the infection is primarily intracellular, relative to that in guinea pig lungs, in which the infection is predominantly extracellular [28,43]. In fact, monotherapy with human-equivalent doses of TRZ in mice showed limited activity, and a dose–response curve was not observed. The addition of TRZ to isoniazid displayed modest synergy and prevented selection of isoniazid-resistant mutants [28]. Our findings are consistent with previous studies. Martins et al. found that TRZ (16 mg/kg) initiated 30 days after intraperitoneal infection of BALB/c mice with 10⁸ bacilli reduced lung bacillary loads by 0.7 log₁₀ after 310 days of treatment, however complete sterilization was not achieved [44]. Similarly, van Soolingen et al. showed that daily dosing of TRZ at 32 mg/kg and 70 mg/kg for 2 months in the mouse resulted in bacillary clearance in TRZ-treated mice, as evidenced by lower relapse rates relative to mice receiving the standard combination regimen [46]. It has been suggested that TRZ may have activity against persisters, as dormant *Mtb* in the *in vitro* hollow fiber system [26] and the Wayne model of progressive hypoxia [23,24] is killed by TRZ, perhaps by targeting *Mtb* respiration [47]. TRZ has been used in the clinical management of patients with drug-resistant TB. Thus, Abbate and colleagues successfully treated XDR-TB patients with the combination of TRZ and other antibiotics in Buenos Aires, Argentina [48]. In addition, TRZ has been used for compassionate purposes in the treatment of XDR-TB patients failing other treatment regimens in India [48,49].

2. A potential role for TRZ in treating latent TB infection (LTBI)

Up to one-third of the world’s population is latently infected with *Mtb*, representing a vast potential reservoir for subsequent reactivation disease, particularly in the setting of the HIV pandemic. While immune-competent persons with latent TB infection (LTBI) have a 10% lifetime risk of developing active TB, this risk is dramatically increased in HIV-co-infected persons to 10% annually, with the risk of TB reactivation rising as the CD4 cell count declines [50,51].

LTBI is a clinical syndrome characterized by a delayed-type hypersensitivity response to intradermal injection of *Mtb*-derived purified proteins in the absence of clinical and radiographic findings of active disease [52,53]. LTBI is believed to represent the immunological control of a paucibacillary population of non-replicating and slowly metabolizing bacilli [54] residing within caseous granulomas [55]. The microenvironment within such granulomas may include hypoxia [56], nutrient limitation, and acidic pH [57]. Importantly, these “dormant” bacilli exhibit phenotypic antibiotic tolerance, exhibiting reduced susceptibility to isoniazid [58], while retaining susceptibility to rifampin. Thus, clinical studies have shown that reactivation rates are similar following 9 months of daily isoniazid treatment as with 4 months of daily rifampin treatment [59]. Although significant progress has been made in understanding the genetic requirements and metabolic adaptations of *Mtb* during host infection, including the role of the stringent response [60,61] and a switch to utilization of fatty acids as a source of carbon and energy through the glyoxylate cycle [62], the molecular pathways underlying LTBI remain largely undefined.

A major obstacle in our understanding of LTBI is the lack of tractable animal models. In particular, unlike human LTBI, the classical mouse model of TB infection is characterized by a high bacillary burden, with progressive lung pathology and early mouse death [63]. Previous studies have shown that mice immunized via the aerosol route with *Mycobacterium bovis* BCG are able to effectively limit bacillary growth following subsequent aerosol challenge with virulent *Mtb* and do not succumb to infection [64,65]. Importantly, the relatively small bacillary population established in this model exhibits greater susceptibility to rifampin relative to isoniazid, mirroring the antitubercular susceptibility profile observed in human LTBI [53].

In the current issue of *Tuberculosis* [66], Singh et al. have used this model to study the activity of TRZ alone and in combination with the standard LTBI regimens, isoniazid or rifampin. While TRZ alone was ineffective, the authors found that TRZ in combination with either first-line drug had significantly greater sterilizing activity than that of either drug alone. These data are largely consistent with those of De Knecht et al., who similarly showed synergy of TRZ with isoniazid or rifampin against *Mtb* in vitro [27]. Are the results of Singh et al. convincing enough to pursue a clinical trial of TRZ for LTBI treatment? [66] One unanswered question is to what extent the mouse model used is predictive of LTBI in humans. In particular, a major deficiency of the Swiss-albino mouse model is that TB lung lesions in this model lack caseation necrosis, which is the pathological hallmark of human TB granulomas [57,68], and believed to be important for control of bacillary growth during LTBI [55]. Alternative animal models, including rabbits [69] and nonhuman primates [70], faithfully represent many features of human LTBI but are not widely available. The ideal model may combine the economy and superior tractability of mice with the establishment of a paucibacillary infection and tissue necrosis observed in larger animal models.

Recently, there has been significant interest in the C3HeB/FeJ mouse strain, which lacks expression of *Ipr1* and develops necrotic TB granulomas [71] characterized by tissue hypoxia [72], as observed in larger animal models [73]. Because of these favorable features, this mouse strain has been used in several recent studies to test the efficacy of standard antitubercular drugs and novel anti-inflammatory therapies [74–76]. Using C3HeB/FeJ mice, we have...
developed a novel paucibacillary model, which exhibits the pathological hallmark of human TB lesions [77]. Although this model differs from human LTBI in that prior vaccination is required for immune-based control and establishment of a paucibacillary, asymptomatic infection, it appears to be useful nonetheless for drug screening in that it faithfully recapitulates the hierarchy of sterilizing activities of LTBI regimens (rifampin + pyrazinamide > rifampin > isoniazid). Therefore, we believe it would be worthwhile to test the activity of TRZ in this model, which can address issues related to drug penetration into necrotic lung lesions and drug activity under tissue hypoxia [77].

The WHO group 5 drug classification refers to anti-TB drugs with unclear efficacy or untapped potential [78]. TRZ may have unrealized potential in the treatment of drug-susceptible and drug-resistant active TB and LTBI. Importantly, it is not patent-protected and is relatively inexpensive (a few hundred dollars/kilo), even in the most resource-limited settings. However, more work remains to be done. It is important to determine if TRZ displays synergy with other second-line drugs for treatment of drug-resistant TB and whether it prevents the selection of mutants resistant to these drugs. In addition, the contribution of the immune system in TRZ-mediated killing remains to be explored. Specifically, the activity of TRZ in vivo and in normal human volunteers should be tested in CD4 knockout mice or nude mice, as HIV-infected patients stand to benefit most from novel anti-TB therapies. Finally, a better understanding of the structure-toxicity relationship may lead to better-tolerated analogs. Structural modification of the phenothiazine core is possible in a manner that does not affect the ability of the phenothiazine derivatives to inhibit Mtb, but abolishes undesirable dopamine and serotonin receptor binding [25]. Further in vitro and animal studies are urgently needed to guide the future study of phenothiazines in clinical trials.

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