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The potential role of Thioridazine in the treatment of tuberculosis

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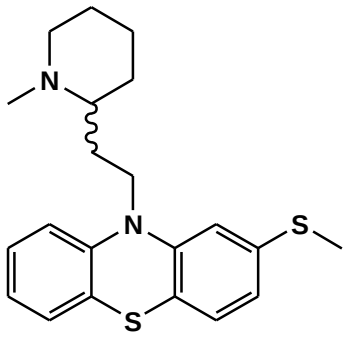
Abstract
Phenothiazines (PZNs) are a class of drugs that have significant *in vitro* activity against susceptible, polydrug- and MDR strains of *Mtb*, as well as enhancing the activity of some drugs used for first-line treatment. The PZNs have the potential as an adjunct to conventional drug therapy during the lengthy period before antibiotic susceptibility data are available. They may reduce morbidity associated with the use of Rifampicin and Streptomycin by allowing their use in lower dosages. The need for new and effective anti-TB drugs is recognized, PZNs are hope against MDR-TB, whether less toxic PZN may be exploited as an anti-TB agent. The role of PZN analogs as an adjunct drug in the therapy of tubercular and MDR-TB.

Keywords: *Mycobacterium tuberculosis*; Thioridazine, therapy, Latent TB

Introduction

The antipsychotic phenothiazine (PZN) drug Thioridazine (TZ) is active against drug-susceptible and drug-resistant *Mycobacterium tuberculosis* (*Mtb*), both in macrophages (Ordway, Viveiros *et al*. 2003) [48] as well as in murine models (van Soolingen, Hernandez-Pando *et al*. 2010) [63]. The TZ is also known to kill multidrug-resistant tuberculosis (MDR-TB). It is no longer recommended for treatment due its side effects like dry mouth, urination-difficulties, obstipationglaucoma and postural hypotension (Amaral, Boeree *et al*. 2004) [9]. Although serum concentrations above the MIC for *Mtb* (8-16 mg/L range) cannot be safely attained in humans, The TZ still has potential as an anti-TB drug because of intracellular accumulation, such that concentrations inside macrophages are at least 10-fold higher than in serum. Despite the favorable toxicity profile of TZ relative to chlorpromazine (CPZ) and other PZNs, cardiac arrhythmia associated with prolongation of the QTc interval remains a risk. The TZ has been used successfully to cure patients with XDR-TB in Argentina and as salvage therapy in similar patients in India (Amaral, Boeree *et al*. 2010) [9]. The mechanism of action of TZ is likely multifactorial, as the drug appears to act on enzymes involved in fatty acid metabolism and membrane proteins, particularly efflux pumps (Dutta, Mazumdar *et al*. 2011) [19], in addition to inhibiting type II NADH: menaquinone oxidoreductase as a PZN (Weinstein, Yano *et al*. 2005) [68]. Mechanisms of *Mtb* resistance to the PZN**s** remain to be elucidated.



Thioridazine

Globally, the emergence of multidrug-resistant strains of MDR-TB is an increasing problem that affects public health and patient care. It is estimated that 1.7 to 2.0 billion humans are infected with *Mtb*, the causative organism of human TB. An estimated 1.5 million people died from TB in 2006 (WHO. 2008) [72]. India has more new TB cases annually than any other country, ranking first among the high burden TB countries worldwide according to the *WHO Global TB Report 2006*. TB remains one of the leading infectious causes of mortality in India, resulting in 364,000 deaths annually. There were more than 1.8 million new TB cases in India in 2004, representing over one-fifth of all TB cases worldwide. The estimated incidence rate in 2004 was 168 per 100,000 people (WHO. 2006) [69, 73]. There are estimated 450,000 new

cases of MDR-TB around the world every year (WHO/IUATLD. 2000) [70]. The Global Project on Anti-TB Drug Resistance Surveillance, Report No.2, revealed that the median prevalence of MDR in strains isolated from new cases (primary drug resistance) was only 1% (range 0-14.1%), whereas the median prevalence in previously treated cases (acquired drug resistance) was 9.3% (range 0-48.2%) (Floyd *et al*. 2002). Emerging anti-TB drug resistance in India deserves serious attention as India's rate is highest among 22 high-burden countries.

Classification of Drug resistant tuberculosis

Polydrug *resistant* TB: resistance to two or more antibiotics. *Multidrug Resistance* TB (MDR): resistance to at least Rifampicin (RIF) and isoniazid (INH). *Extensively Drug Resistant* TB (XDR): resistance to at least INH and RIF (i.e. MDR-TB), a flouroqinolone and to one or more of injectable aminoglycosides (amikacin, capreomycin, kanamycin) (WHO. 2006) [69, 73]. In cases of MDR-TB is the main line of treatment is the 2nd line drugs. The problems in countries like India are:

1. Lack of laboratory procedures required for the delivery of antibiotic susceptibility data results in blind treatment of infection.
2. The 2nd line drugs are expensive and duration of treatment is considerably longer.

These factors further lead to an increase in the incidence of MDR-TB. The solution lies in providing an alternative that has the potential to treat patients effectively regardless of the antibiotic susceptibility profile of the causative organism at a cost that is affordable by the most economically deprived country.

Phenothiazines as Anti Tubercular agents: The PZN groups of drugs are tricyclic compounds that are used as antipsychotic agents. They were once the most widely used antipsychotics. They are Aliphatic derivatives: The CPZ and TZ; and Piperazine derivatives: Trifluperazine, Perphenazine and Fluphenazine (William. 2004) [71]. Piperazine derivatives are more potent as they are effective in lower doses (William. 2004) [71]. Methylene blue, the first PZN, was an aniline dye. Ehrlich had demonstrated that it had activity against *Plasmodium falciparum*, and when used to patients would cause them to become lethargic. The wide acceptance and use of CPZ in the ensuing years for the treatment of severe neuroses and psychoses yielded a few anecdotal reports suggesting that this agent had anti-TB properties. Interest in the development of these compounds as anti-TB agents did not materialize because of the by then well-known severe side effects produced by the chronic use of CPZ. The introduction of INH in the 1950s for the treatment of infections by *Mtb*, later followed by other effective compounds (Streptomycin (STR) and RIF), lessened any further interest in the use of CPZ as an anti-TB drug. Inspite of the fact that PZNs are active against *Mtb* they were never considered seriously as ATT because the lowest concentration required for significant *in vitro* inhibition of growth greatly exceeded that achievable in patients (i.e. about 0.5 mg/L) receiving a minimum dose of 600 mg/day and severe side effects that are associated with its chronic use (Leonard *et al*. 2001) [37]. The CPZ could inhibit the growth of *Mtb* that had been phagocytosed by human macrophages when CPZ was present in the medium at concentrations ranging from 0.23 to 3.6 mg/liter. *Mtb* phagocytosed within the macrophages was susceptible to concentrations of CPZ 10 times lower in the culture medium than those needed for a similar inhibition of unphagocytosed bacteria. These concentrations were within the range anticipated in patients treated with this PZN. The macrophages had the ability to concentrate the PZN, an interpretation consistent with those studies showing that CPZ was found in pulmonary tissue in concentrations in excess of 100 times those in plasma. The CPZ produces serious side effects, was not considered for use in the treatment of TB. With the worldwide resurgence of TB and increase in MDR-TB, there is an urgent need of newer agents and hence the renewed interest in PZNs as Anti-TB agents. The CPZ, the first commercially produced PZN for the treatment of psychosis, was also one of the first of the PZN series shown to have anti-TB properties. In all the PZNs, Trifluperazine appears to have most potent anti-TB action. The lowest effective *in vitro* concentration against *Mtb* is seen after the organism has been phagocytosed by human macrophages. In the absence of macrophages, the MIC is almost 10-fold higher, as a consequence of the ability of macrophages to concentrate CPZ, many times over that achieved in the medium.

Mechanism of Action: The calmodulin like protein has been demonstrated in mycobacterium and it has been observed that there is a positive correlation between the levels of CAMLP, phospholipids as well as lipids and growth. The PZNs are Calmodulin antagonists and therefore inhibit the growth of *Mtb.*

Role of phenothiazines in MDR-TB: It has been observed that all strains of *Mtb* tested so far, regardless of whether they are susceptible to all agents, or are MDR- or polydrug resistant (PDR), or even resistant to all five primary antibiotics, are equally affected by CPZ (Viveiros *et al*. 2000) [67]. The effect of CPZ and other PZNs on various strains of *Mtb*, it is clear that the PZNs are equally effective against MDR strains as they are against the susceptible strains. This is of clinical vital in treatment of MDR-TB. The CPZ increases the anti-TB activity of RIF and STR, thus permitting to give them in lower doses without sacrificing the integrity of treatment (Viveiros and Amaral. 2001) [4]. The CPZ, TZ and Trifluperazine were shown to enhance the activity of RIF and STR when used in combination at concentrations that are minimally effective when used separately against clinical strains of PDR-TB (Viveiros and Amaral. 2001) [4]. They however have no augmenting activity against ethambutol (EMB) in any strain and INH in PDR-strains.

Choice among Phenothiazines: The extensive use of CPZ for the treatment of psychosis during the past 40 years provided abundant evidence for this compound producing severe side effects when used over long periods. More serious side effects include: Cholestatic disease, Acute liver injury, Keratopathies, Conjunctivitis, Phototoxicity, Photoallergy (dermal), Agranulocytosis

These severe side effects associated with the chronic use of CPZ makes it suitable for use in the treatment of TB. The TZ on the other hand is a compound with fewer side effects. It may cause transient mild retinopathy. Other than that the most common side effect is drowsiness. In experimental mice, chronic use of TZ has resulted in cardiotoxicity. The same effect has not been noticed

in humans except in cases of overdose or co administration with other potentially cardio toxic drugs. Regardless of this, a careful monitoring of cardiac function during TZ treatment is strongly recommended. The use of TZ as the PZN of choice for the treatment of freshly diagnosed pulmonary TB is further reinforced by the observations that this compound enhances the in vitro activity of RIF and STR against PDR-TB (Viveiros and Amaral. 2001) [4]. The use of PZNs for treatment of an active pulmonary TB infection caused by antibiotic-susceptible *Mtb* strains provides no advantage over the use of present therapies. The concentration of PZNs needed to kill or even inhibit mycobacterial replication when the bacterium is outside the macrophage is far beyond that which can be achieved in the patient so it cannot be used for the treatment of a cavitary pulmonary *Mtb* infection of moderate to severe status (Duggal *et al*. 2008) [24].

Discussion

Ongoing research has generated several new drugs, which are in various stages of preclinical and clinical assessment (Ginsberg. 2010; Dutta and Karakousis 2012) [29, 18]. Despite this progress (Ma *et al*. 2010; Nuermberger *et al*. 2010) [38, 47], the global TB drug pipeline is insufficient to address the imminent but unmet medical needs for new anti-TB drugs to treat 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 (EMB and STR may cause permanent defects) (van den Boogaard *et al*. 2009; Mdluli *et al*. 2014) [62, 43]. Additionally, to minimize the selection of resistant strains, it is highly desirable that the genes encoding the novel drug targets display low mutation frequencies (Gillespie. 2002) [28]. 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 (Palomino and Martin. 2013). The “repurposing” of the old antipsychotic PZN, TZ has been considered as an adjunctive therapy for MDR- and XDR-TB cases (Thioridazine. Tuberculosis (Edinb) 2008; Boeree. 2011; Amaral *et al*. 2010; Amaral *et al*. 2012; Thanacoody. 2011) [60, 12, 9, 6, 59]. The TRZ has broad-spectrum antibacterial (nonantibiotic) activity (Amaral *et al*. 2001; Amaral *et al*. 2006) [4, 7], including against various drug sensitive and drug-resistant *Mycobacterium* spp. at 6-12.5 mg/ml *in vitro* (Bettencourt *et al*. 2000). However, the MIC against *Mtb* in macrophages has been reported to be in the range of 0.1e3.6 mg/ml (Ordway *et al*. 2003; Martins *et al*. 2007) [48, 5, 41], due to intracellular concentration of the drug (Ordway *et al*. 2003) [48]. 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 INH, which targets primarily actively multiplying bacilli by inhibiting the mycolic acid synthesis pathway, and the transcriptional inhibitor RIF, which targets primarily growth restricted bacilli (Xie *et al*. 2005) [74], TRZ has been shown to target both slowly replicating and logarithmically growing bacilli (Sohaskey. 2008; Sohaskey. 2011; Salie *et al*. 2014) [56, 57, 55] in an in vitro hollow fiber system (Musuka *et al*. 2013) [45], likely due to its multiple mechanisms of action. In this issue of TB (de Knegt. 2014) [17], De Knegt and colleagues showed concentration and time-dependent bactericidal activity for TRZ against both actively-replicating and slowly-replicating *Mtb*. Furthermore, relatively high concentrations of TRZ showed synergy with INH and RIF. In the case of INH, this resulted in elimination of mycobacteria and prevention of INH-resistant mutants, consistent with similar findings in a mouse model of TB infection (Dutta *et al*. 2014) [21, 22]. Previously, Viveiros *et al*. reported that TRZ enhances the activity of RIF and STR when used in combination at minimally effective concentrations against clinical strains of poly-drug resistant *Mtb* (Viveiros and Amaral. 2001) [4]. Due to its pleiotropic effects, TRZ may provide strategies for multi-target drug development for combination chemotherapy (Dutta *et al*. 2010; Dutta *et al*. 2011; Rao *et al*. 2008) [20, 44, 53]. 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 (Boshoff *et al*. 2004; Yano *et al*. 2006) [13, 75]. In addition, TRZ targets the Rv3160c-Rv3161c operon, which may be required for the detoxification of TRZ, members of the SigB sigma 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 (Mehra *et al*. 2010) [20, 44]. Several of these targets have been shown to be essential for *Mtb* persistence in the infected host (Dutta *et al*. 2010; Dutta *et al*. 2014) [20, 44, 21, 22]. Increased activity of efflux pumps of mycobacteria can prevent antibiotics from reaching their intended target, leading to an MDR phenotype (Adams *et al*. 2011; Dartois. 2014) [2, 16]. TRZ has been shown to have efflux pump-inhibiting activity against mycobacteria both in vitro and ex vivo (Rodrigues *et al*. 2008) [54]. For example, it is an effective inhibitor of the intrinsic efflux pump system, which is considered responsible for intrinsic resistance to erythromycin (Rodrigues *et al*. 2008) [54]. Machado and colleagues have demonstrated that overexpression of such efflux pumps favors accumulation of mutations in INH targets and that INH resistance can be reduced by TRZ exposure in *Mtb* (Machado *et al*. 2012). The PZNs also inhibit bacterial access to calcium by inhibiting the activity of calcium-dependent ATPase and, hence, drug transport processes, resulting in accumulation of PZN 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 (Amaral *et al*. 2007) [5, 41]. At high concentrations, TRZ can cause cardiac side effects, specifically prolongation of the QT interval (Thanacoody. 2011) [59]. Pharmacokinetic data in mice suggest that TRZ is not toxic at humanequivalent doses (25 mg/kg), but daily dosing above 50 mg/kg resulted in acute mortality of mice. TRZ was found to accumulate in murine lung tissue relative to serum (Dutta *et al*. 2014) [21, 22]. 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 (Dutta *et al*. 2013) [23]. In fact, monotherapy with human-equivalent doses of TRZ in mice showed limited activity, and a doseeresponse curve was not observed. The addition of TRZ to INH displayed modest synergy and prevented selection of INH-resistant mutants (Dutta *et al*. 2014) [21, 22]. 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 106 bacilli reduced lung bacillary loads by 0.7 log10 after 310 days of treatment, however complete sterilization was not achieved (Martins *et al*. 2007) [5, 41]. 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 reduced lung bacillary counts by 0.2 and 0.4 log10, respectively, relative to untreated controls. In mice infected with MDR-*Mtb*. 2 months of treatment with TRZ alone at daily doses of 32 and 70 mg/ kg reduced the lung bacillary counts by 0.1 and 0.2 log10, respectively (van Soolingen *et al*. 2010) [63]. Although it remains to be shown that TRZ has sterilizing activity against *Mtb* persisters, we found a trend towards improved bacillary clearance in TRZ-treated mice, as evidenced by lower relapse rates relative to mice receiving the standard combination regimen (Dutta *et al*. 2014) [21, 22]. It has been suggested that TRZ may have activity against persisters, as dormant *Mtb* in the in vitro hollow fiber system (Musuka *et al*. 2013) [45] and the Wayne model of progressive hypoxia is killed by TRZ, perhaps by targeting *Mtb* respiration (Black *et al*. 2014) [11]. TRZ has been used in the clinical treatment of patients with MDR-TB. Thus, Abbate and colleagues successfully treated XDR-TB patients with the combination of TRZ and other antibiotics in Buenos Aires, Argentina (Abbate *et al*. 2012) [1, 6]. In addition, TRZ has been used for compassionate purposes in the treatment of XDR-TB patients failing other treatment regimens in India (Udwadia *et al*. 2011) [61].

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 (Kwan and Ernst. 2011; Pawlowski *et al*. 2012). 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 (Dutta and Karakousis. 2014; Horsburgh and Rubin. 2011) [21, 22, 32]. LTBI is believed to represent the immunological control of a paucibacillary population of nonreplicating and slowly metabolizing bacilli (Nuermberger *et al*. 2004) [46] residing within caseous granulomas. The microenvironment within such granulomas may include hypoxia, nutrient limitation, and acidic pH (Gomez and McKinney. 2004) [30]. Importantly, these “dormant” bacilli exhibit phenotypic antibiotic tolerance, exhibiting reduced susceptibility to INH, while retaining susceptibility to RIF. Thus, clinical studies have shown that reactivation rates are similar following 9 months of daily INH treatment as with 4 months of daily RIF treatment (Jasmer *et al*. 2002) [34]. 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 (Primm *et al*. 2000; Dahl *et al*. 2003) [52, 15], and a switch to utilization of fatty acids as a source of carbon and energy through the glyoxylate cycle, 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 (Manabe and Bishai. 2000) [40]. Previous studies have shown that mice immunized via the aerosol route with *M. bovis* BCG are able to effectively limit bacillary growth following subsequent aerosol challenge with virulent *Mtb* and do not succumb to infection (Zhang *et al*. 2009; Zhang *et al*. 2011) [76, 77]. Importantly, the relatively small bacillary population established in this model exhibits greater susceptibility to RIF relative to INH, mirroring the anti-TB susceptibility profile observed in human LTBI (Horsburgh and Rubin. 2011) [32].

In the current issue of TB (Amandeep and Sadhna. 2014) [3], Singh *et al*. have used this model to study the activity of TRZ alone and in combination with the standard LTBI regimens, INH or RIF. 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. The synergy of TRZ with INH or RIF against *Mtb in vitro* (Amandeep and Sadhna. 2014) [3]. 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 (Flynn and Chan. 2004; McMurray. 2001) [26, 42], and believed to be important for control of bacillary growth during LTBI. Alternative animal models, including rabbits (Subbian *et al*. 2013) [58] and nonhuman primates (Flynn *et al*. 2003) [27], 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 (Pan *et al*. 2005) [50] characterized by tissue hypoxia (Harper *et al*. 2012), as observed in larger animal models (Via *et al*. 2008) [64]. Because of these favorable features, this mouse strain has been used in several recent studies to test the efficacy of standard anti-TB drugs and novel anti-inflammatory therapies (Lanoix *et al*. 2014; Irwin *et al*. 2014; Vilaplana *et al*. 2013) [36, 33, 65]. Using C3HeB/FeJ mice, we have developed a novel paucibacillary model, which exhibits the pathological hallmark of human TB lesions (Dutta *et al*. 2014) [21, 22]. 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 (RIF þ pyrazinamide > RIF>INH). 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 (Dutta *et al*. 2014) [21, 22].

The WHO group 5 drug classification refers to anti-TB drugs with unclear efficacy or untapped potential (Chang *et al*. 2013) [14]. 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 alone and in combination with the standard regimen should be tested in CD4 knockout mice or nude mice, as HIV-infected patients stand to benefit most from novel anti-TB therapies. The structure toxicity relationship may lead to better-tolerated analogs. Structural modification of the PZN core is possible in a manner that does not affect the ability of the PZN derivatives to inhibit *Mtb*, but abolishes undesirable dopamine and serotonin receptor binding (Salie *et al*. 2014) [55]. Further in vitro and animal studies are urgently needed to guide the future study of PZNs in clinical trials.

Conclusion

In contrast to atypical antipsychotics, which increase the risk of metabolic syndrome, type-2 diabetes and thus secondary infections including TB, mainly MDR-TB, phenothiazines (PZNs) may prove useful for the treatment of TB infections in certain patients. They particular useful in MDR-TB, can be used as an adjunct to conventional therapy during initial treatment of the patients. The length of treatment is anticipated to be weeks, side effects associated with chronic thioridazine therapy are not anticipated. The efficacy of PZNs as anti-TB agents especially against phagocytosed bacteria, it is necessary to conduct further study to determine the role of PZNs in the treatment of TB especially, MDR-TB.

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