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Review

Potential Therapy of Multidrug-resistant and Extremely Drug-resistant Tuberculosis with Thioridazine

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Abstract. *Multidrug-resistant tuberculosis (MDRTB) infections that continue to increase in frequency globally have progressed to become extremely drug-resistant tuberculosis (XDRTB). The therapeutic problems associated with MDRTB pale in comparison to those for XDRTB where mortality is high. This mini-review highlights the evidence that supports the use of the phenothiazine neuroleptic thioridazine for the therapy of XDRTB. Although thioridazine does produce some serious side-effects, the poor prognosis associated with an XDRTB infection of a patient that presents with AIDS merits that the use of thioridazine for therapy of XDRTB is seriously considered. A recommended protocol is presented.*

Pulmonary Infection with Extremely Drug-resistant Tuberculosis (XDRTB). The problem: No Effective Anti-XDRTB Drugs Available

Pulmonary tuberculosis is an intracellular infection produced by the human steadfast *Mycobacterium tuberculosis*. Whereas this infection is effectively managed with isoniazid (INH) and rifampin, the infections produced by strains that are resistant to these drugs (multidrug-resistant tuberculosis, MDRTB) are highly problematic for therapy, have poor prognoses, and if the MDRTB-infected patient presents with AIDS, mortality may reach as high as 90% within the first year of diagnosis, regardless of aggressive therapy that may involve at least five anti-TB drugs (1). Although the global rate of new cases of MDRTB continues to rise (2), the problem of MDRTB pales in comparison to the one created by pulmonary tuberculosis infections produced by XDRTB strains. XDRTB strains are resistant to almost all available anti-TB compounds and infections produced by these strains may essentially be considered terminal (3). As of the time writing, there are no new drugs available for the effective therapy of XDRTB pulmonary infections, and, as far as we are aware, none are in the pharmaceutical pipeline either.

Urgent problems require urgent solutions and MDRTB and, more critically, XDRTB are urgent problems that must be addressed. Until one or more drugs that can effectively cure an XDRTB infection are available, we propose that the use of the neuroleptic thioridazine is considered for the management of these highly antibiotic-resistant infections. Our argument on behalf of this proposal is thus presented.

Pulmonary tuberculosis is an intracellular infection of the macrophages of the human lung. Unlike the neutrophil, which is equipped *via* its lysosomal system to readily kill most bacteria, the macrophage of the human lung does not kill *Mycobacterium tuberculosis* which is readily phagocytosed and encapsulated within the newly formed phagosome. The intracellular nature of the infection means that if an antibiotic is to cure the patient of a pulmonary tuberculosis infection, the drug must penetrate the macrophage, penetrate the phagosome, reach a concentration that is compatible with its *in vitro* ability to inhibit replication (bacteriostatic) or kill the bacterium (bactericidal), while passessing through the macrophage it must retain its activity. Although an excess of a hundred compounds that have *in vitro* activity against antibiotic-susceptible and antibiotic-resistant strains of *M. tuberculosis* were identified in 2008-2011, none were shown to have activity at the phagosomal site where the organism normally resides; moreover, many are precluded from use due to their high toxicity. There is little optimism for any new and effective agent for the foreseeable future.

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Phenothiazines: Potential Anti-XDRTB Agents

Phenothiazines are heterotricyclic compounds from which 95 % of all medicinal compounds have their origins. The first phenothiazine, methylene blue, was created by Paul Erhlich (4), which was shown to have antibacterial activity in the late 19th century (5). However, because the substanced was also shown to have neuroleptic activity in mammals (6) and Erhlich's 'salvasan' already seemed to be a promising antibacterial agent, interest in methylene blue was essentially limited to its ability to tranquilize (7). It took more than half a century for chemists to decolorize methylene blue and retain its neuroleptic properties (8). The methylene-derived compound chlorpromazine was, and remains, an extremely effective neuroleptic globally used for the therapy of psychoses. As a consequence of this extensive use, anecdotal reports on the antimicrobial properties of chlorpromazine sporadically appeared in the literature, particularly with respect to the observation that psychotic patients who also presented with pulmonary tuberculosis and were treated with this drug, appeared to have their infections ameliorated or even cured (9). Because at the time these observations were made, antituberculosis therapy was effective with isoniazid and rifampin, interest in the development of anti-TB drugs from a phenothiazine never arose. Moreover, global use of chlorpromazine soon demonstrated the severe and frequent side-effects produced by this agent (10). However, with the advent of MDRTB in the early 1990s, and the escalating frequency of MDRTB worldwide (2), the prior sporadic anecdotal reports promoted renewed interest in this agent, resulting in a number of *in vitro* studies demonstrating that chlorpromazine significantly inhibited the replication of antibiotic-susceptible *M. tuberculosis* (11-14) as well as antibiotic resistant strains (15-17). However, because the *in vitro* activity of CPZ took place at concentrations which are clinically irrelevant, that is, they are beyond reach (the maximum plasma concentration that can be safely achieved in the patient is ca. 0.5 mg/l), chlorpromazine could not be considered for the management of antibiotic-resistant pulmonary tuberculosis. Moreover, there still remained the problem of frequent severe side-effects. About the time that MDRTB began to be noted as a severe problem in New York City, the activity of chlorpromazine against intracellular *M. tuberculosis* was demonstrated by Crowle *et al.* (18) at concentrations in the medium, within those clinically achievable. These studies provided impetus for the further study of phenothiazines as potential anti-TB agents with special attention being paid to a derivative of chlorpromazine that was as effective as chlorpromazine in its neuroleptic properties and shown to be equal to chlorpromazine in its *in vitro* activity against both antibiotic-susceptible and -resistant strains of *M. tuberculosis* (15-17). This agent, thioridazine, was soon shown to have the ability to enhance the killing of intracellular bacteria (19-21) and, of greater importance, to effectively promote the killing of intracellular MDRTB (22) and XDRTB (23) by non-killing human macrophages. The demonstration that thioridazine was able to effectively cure mice of severe pulmonary tuberculosis infection (24) was soon confirmed (25). Others also showed that derivatives of chlorpromazine were also as effective (26). Undoubtedly, thioridazine has promise as a viable anti-MDRTB/XDRTB agent, and this has recently been recognized by Thanacoody (27).

Mechanism by which TZ Enhances the Killing of Intracellular MDRTB

Phenothiazines inhibit the binding of Ca^{++} to proteins involved in the regulation of calcium for example, calmodulin (28) and calcium-dependent enzymes (29, 30) many of which are involved in glycolysis by eukaryotes (31, 32). The demonstration that mycobacteria contain a calmodulin-like protein whose binding of Ca^{++} could be inhibited by a phenothiazine (33, 34), contributed to the idea that the *in vitro* activity of thioridazine or any other phenothiazine against *M. tuberculosis* was manifested by denying access to Ca^{++}. However, unlike eukaryotes, the glycolytic and gluconeogenic enzymes of bacteria are not dependent upon Ca^{++} for their activity. Because phenothiazines are also known to inhibit a large variety of enzymes non-specifically, attention has recently been focused on the identification of the key enzymes of mycobacteria that are directly affected by phenothiazines (35-38). To this extent, direct inhibition of type-II NADH-menaquinone oxidoreductase, the key enzyme in the electron transport chain, has been shown to be completely inhibited by a phenothiazine (37). Moreover, therapy of mice infected with *M. tuberculosis* with the phenothiazine resulted in cure (24).

The *in vitro* activity of thioridazine, or any other phenothiazine, on *M. tuberculosis* takes place at concentrations which are 30 to 50 times the maximum plasma concentration that can be achieved in the patient. The reason that concentration of the agent in the culture medium that is of the order 0.1 mg/l can enhance the killing of intracellular *M. tuberculosis* has been attributed to the ability of macrophages to concentrate the agent to a level comparable to that which is bactericidal *in vitro* (18, 19-21). This assumption is based on the ability of macrophages rich in lysosomes to concentrate the drug (39-42). How the drug is actually concentrated remains unknown. However, on the surface of eukaryotic cells such as the macrophage are proteins which recognize noxious agents that penetrate the plasma membrane, bind to the agent and extrude it to the outside of the cell (43). Among these transporters is P-glycoprotein (Pgp1 or ABCB1) the product

of the human *MDR1* (*ABCB1*) gene (44, 45). It is supposed that when a bacterium is phagocytosed, because the process is one of invagination, the Pgp1 transporter that is still present in the plasma membrane that encapsulates the bacterium, then transports the noxious agent from the cytoplasm into the lumen of the phagosome, resulting in the concentration effect.

Although the above hypothesis is attractive, the ability of phenothiazines to enhance the killing of intracellular bacteria may lie in their ability to inhibit K^+ and Ca^{++} transport processes (46-51). Phenothiazine sensitive transport processes depend on energy derived from the hydrolysis of ATP (53). As was mentioned previously, the phenothiazine affects the hydrolysis of ATP, a calcium-dependent process, by denying access to Ca^{++}.

Similarly, phenothiazines also inhibit K^+ transport processes (51). The killing activity of macrophages has been shown to depend upon Ca^{++} and K^+ transport processes (52-55) and because these processes are inhibited by phenothiazines (46-51) and by ouabain (56), and both phenothiazines and ouabain enhance the killing of intracellular mycobacteria (57, 58), the enhanced killing is considered to be due the inhibition of K^+ and Ca^{++} transport processes (57, 58). The mechanism by which the killing is activated in non-killing human macrophages, by agents that inhibit K^+ and Ca^{++} transport, has been described (57-59) and involves the acidification of the phagolysosome and the subsequent activation of the hydrolases that degrade and kill the bacterium (59).

As a consequence of the above, we have developed strategy that targets the macrophage for enhanced killing as opposed to targeting the microbe itself. The advantage of this strategy is that it by-passes the expected mutational responses of the microbe that leads to resistance to any new agent and also by-passes whatever existing resistance the microbe may have. Because the agents that are known to affect K^+ and Ca^{++} transport processes have been extensively studied, the use of existing inhibitors of K^+/Ca^{++} as lead compounds for the development of new compounds may provide an effective route for the rapid development of the desired enhancers of intracellular killing.

Why Has Thioridazine Not Been Seriously Considered for the Therapy of Pulmonary Infections of MDRTB?

Thioridazine has been used successfully for over 40 years in the therapy of psychosis. Due to its misuse for the therapy of dementia and other mental non-psychoses disorders, it has been restricted to the therapy of psychoses (60). Thioridazine is a piperidine antipsychotic drug belonging to the phenothiazine drug group and was previously widely used for the treatment of schizophrenia and psychosis. It is available from various companies under the names Mellaril®, Novoridazine, and Thioril. Due to concerns about cardiotoxicity and retinopathy at high doses this drug is not commonly prescribed, being reserved for patients who have failed to respond to, or have contraindications for, more widely used antipsychotics. A serious side-effect of thioridazine therapy is the potentially fatal neuroleptic malignant syndrome. Thioridazine exerts its actions through central adrenergic-blocking, dopamine-blocking and minor anticholinergic activity. Other serious side-effects produced by high doses include prolongation of the QT interval (time between heart beats) which may put the patient at risk if the prolongation is high (61), and leads to ventricular tachycardia, which may lead to ventricular fibrillation and sudden death. Although undoubtedly these side-effects are quite serious, they are not very different from those produced by other neuroleptics (62) and they do only occur infrequently (62). Although the most frequent side-effect is somnolence, others, such as phototoxicity (63) and agranulocytosis, may take place at low frequencies. Nevertheless, because the frequency of side-effects of thioridazine and other neuroleptics has now been associated with genetic factors (64), where a thioridazine study is actually conducted may significantly influence the frequency of thioridazine-induced cardiac changes reported.

Recommended Therapy for Compassionate Reasons

Patients infected with XDRTB have poor prognoses even when treated with the recommended regimen of at least seven drugs (65, 66). When co-infected with HIV and presented with AIDS, mortality is very high (67). If there is a need for compassionate therapy, namely if the patient has been managed with the recommended regimen of at least five anti-TB drugs and the prognosis is very poor, mortality certain, and the probability of thioridazine-induced cardiopathy is low, thioridazine may be considered for the therapy of an XDRTB-infected patient who is also presented with AIDS. However, prior to therapy, the patient should be thoroughly evaluated for cardiac function and for the first 48 hours of therapy, the use of an ambulatory cardiac monitor is highly recommended.

The initial dose given for the therapy of psychosis is 50 mg/day for mild cases to as high as 600-800 mg/day for severe psychosis. We would recommend starting the patient with 25 mg/day for one week after which time the dose may be doubled to 50 mg/day and this daily dose maintained for a further two to three weeks. At the end of this period, the patient should be evaluated for cardiac functions. Sputum should be collected for acid-fast staining and culture in order to determine if the therapy has yielded any positive effects. The daily dose may be increased to 75 mg/day and maintained for the next three weeks. Repeat check of cardiac functions, collection of sputa, etc. are needed to ensure that cardiopathy has not taken

place and for the determination of the effectiveness of therapy (criteria for effective therapy: acid-fast-negative; TB culture negative). If therapy is proven effective, the patient should be maintained on 75 mg/day for the next five months, with periodic evaluations for cardiac function/sputa assay. Clinical, radiological and laboratory criteria employed for the evaluation of cure are those currenly in use worldwide, as described by the Center for Disease Control and Prevention (CDC), USA, American Thoracic Society (68).

An Argentinian group led by Eduardo Abbate has used the aforementioned protocol to successfully treat 10 out of 12 XDRTB patients that for more than 1 year had not responded to any combination of antibiotics used as a second-line of defence for therapy of XDRTB (69). During therapy, patients were monitored for cardiac functions and none were observed to present with any cardiopathy. These results have, with the support provided by intensive bench studies, cited in this review, resulted in the creation of an organized programme that is now in operation in Peru and other parts of South America. This programme employs thioridazine in combination with three antibiotics, none of which are known to cause cardiopathy. The programme is not a clinical trial for this would require many months of planning and activation, and because patients with XDRTB cannot afford to wait, they are receiving therapy on the basis of 'compassionate therapy' given that prior therapies have failed and progression of disease towards certain mortality is anticipated. At the time of writing, it is too early to report any results.

Authors have been informally advised that there is serious consideration for the use of thioridazine in combination with antibiotics by the WHO, CDC and African Health Agencies. Others, such as Martin Boeree of the Netherlands, are considering similar introduction into existing clinical trials in Africa. The reader is urged to read the most recent references (70-71) for additional information. The use of thioridazine for the therapy of XDRTB in Argentina in combination with linezolid and moxifloxacin, antibiotics to which TB in the seventeen non-AIDS patients was initially resistant, yielded complete cures in eleven patients; four have responded well and are still in follow-up, and two discontinued therapy due to adverse responses to the combination of drugs (72). Therefore, these most recently published studies show that thioridazine is effective for therapy of XDRTB. Given that thioridazine is cheap, its use in countries that are economically disadvantaged and where MDRTB and XDRTB infections are prevalent makes therapy affordable. Moreover, monotherapy of XDRTB patients in India resulted in patients regaining their appetite and weight, and symptoms associated with the infection were either reduced or ablated (73), suggesting that this form of therapy provides a 'salvage pathway' that improves the quality of life of the patient with XDRTB patient.

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