

# Thioridazine: an Old Neuroleptic effective against Totally Drug Resistant Tuberculosis



## Tioridazina: um Velho Neuroleptico eficiente contra a Tuberculose Totalmente Resistente às Drogas

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### ABSTRACT

Globally, tuberculosis infections continue to increase their resistance to antibiotics. Multidrug resistant tuberculosis infections (MDR TB) have progressed to extensively drug resistance status (XDR TB) and the latter have evolved in some parts of the world to totally drug resistant (TDR TB) infections. MDR TB is difficult to treat successfully, and when therapy is ineffective, a single case can cost almost \$500,000. When the infection is XDR TB therapy is mostly unsuccessful and is accompanied with high mortality. TDR TB-a yet to be defined infection, is resistant to all forms of therapy and mortality is almost certain.

We have, over a period of 14 years, studied thioridazine (TZ) an old neuroleptic that we have shown to: i) have *in vitro* activity against all antibiotic resistant forms of *Mycobacterium tuberculosis*; ii) have activity against intracellular *Mycobacterium tuberculosis* regardless of its antibiotic resistance status; iii) cure the infected mouse of an antibiotic susceptible and MDR TB infections; and, iv) when used in combination with antibiotics used for therapy of tuberculosis, would render the organism significantly more susceptible. These studies have guided our Argentinian colleagues to treat successfully XDR TB infections with thioridazine in combination with three antibiotics to which the infection was initially resistant.

This mini review will describe our further work and the mechanisms by which TZ alone and in combination with antibiotics cures an XDR TB infection and why it is expected to cure TDR TB infections as well. The concepts presented are totally new and because they focus on the activation of killing by non-killing macrophages where *Mycobacterium tuberculosis* normally resides during infection, and coupled to the inhibition of efflux pumps which contribute to the antibiotic resistant status, effective therapy of any antibiotic resistant TB infection is possible.

Because TZ is cheap and therefore affordable to any economically disadvantaged country, and will produce no harm when appropriate measures are taken, it is the ideal drug for immediate use in countries that have high frequencies of MDR, XDR and TDR TB infections.

### RESUMO

A ocorrência de formas de tuberculose resistente aos antibióticos continua a aumentar a nível global. A tuberculose multiresistente (MDR-TB) evoluiu para tuberculose extensivamente resistente (XDR-TB) e esta última tornou-se já, em algumas partes do mundo, totalmente resistente aos medicamentos disponíveis (TDR-TB). O tratamento da MDR-TB é difícil e quando a terapia se revela ineficaz, o custo de um único caso de MDR-TB pode chegar a cerca de 500.000 USD. Nos casos de XDR-TB, a terapia é, na maior parte dos casos, ineficaz e a infecção é acompanhada de elevada mortalidade. TDR-TB, uma infecção cuja definição ainda não está estabelecida, é resistente a todas as formas de terapia e a mortalidade associada é quase certa.

Os estudos realizados pelo nosso grupo ao longo dos últimos 14 anos sobre a tioridazina (TZ), um fármaco há muito utilizado como neuroleptico, mostraram que este agente possui as seguintes propriedades: i) tem atividade *in vitro* contra todas as formas de *Mycobacterium tuberculosis* resistente aos antibióticos; ii) tem atividade contra o *Mycobacterium tuberculosis* intracelular, independentemente da sua resistência aos antibióticos; iii) é capaz de curar ratinhos infectados com tuberculose, quer a forma sensível aos antibióticos, quer MDR-TB, e; iv) quando utilizada em combinação com antibióticos utilizados para a terapia da tuberculose, torna o microorganismo significativamente mais susceptível. Estes estudos serviram de orientação para os nossos colegas argentinos, que utilizaram a tioridazina para tratar com sucesso casos de XDR-TB, combinando esta molécula com três antibióticos aos quais a infecção era inicialmente resistente.

Esta revisão descreve algum do nosso trabalho adicional e os mecanismos pelos quais a TZ, quer sózinha, quer em combinação com antibióticos, leva à cura de uma infecção XDR-TB e as razões pelas quais se espera que seja também capaz de curar TDR-TB. Os conceitos apresentados são inovadores e dado que se concentram na activação da capacidade bactericida dos macrófagos, nos quais *M. tuberculosis* reside normalmente durante a infecção, em conjunto com a inibição das bombas de efluxo que contribuem para a resistência aos antibióticos, tornam possível a terapia eficaz de qualquer forma de tuberculose resistente aos antibióticos.

Uma vez que a TZ é um fármaco barato e portanto, acessível em qualquer país economicamente desfavorecido, e não produz efeitos secundários se forem tomadas as medidas adequadas, é a droga ideal para o uso imediato em países com elevadas frequências de MDR, XDR e TDR-TB.

### INTRODUCTION

Globally, tuberculosis infections continue to increase their resistance to antibiotics.<sup>1</sup> Multidrug resistant tuberculosis infections (MDR TB) have progressed to extensively drug resistance status (XDR TB)<sup>2</sup> and the latter have evolved in some parts of the world to totally drug resistant (TDR TB) infections.<sup>3</sup> MDR TB is difficult to treat success-

fully, and when therapy is ineffective, a single case in the USA can cost almost \$500,000.<sup>4</sup> When the infection is XDR TB, therapy is mostly unsuccessful and is accompanied with high mortality. TDR TB-a yet to be defined infection, is believed to be resistant to all forms of therapy and mortality is almost certain.<sup>3</sup>

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Pulmonary tuberculosis (TB) infection is caused by *Mycobacterium tuberculosis* (Mtb). Primarily it is an intracellular infection of the pulmonary macrophage by Mtb that may lie dormant for many decades. Infection normally takes place from inhaling micro-droplets that contain the infecting organism Mtb expelled by a subject that is presenting with active tuberculosis disease. Active disease is defined by the breaking out of the organism from its macrophage prison and is therefore the infectious component of the disease. The pulmonary cell that houses Mtb is a macrophage that is part of the alveolar structure. Because the pulmonary macrophage does not kill the organism, the subject may remain infected for life. How and why the organism breaks out of its macrophage prison is still not well understood. Suffice to say that with the exception of co-infection with HIV and presentation of AIDS, 5 to 10% of Mtb infected subjects progress to active disease. It is important to note that it is active disease which is reported to national and international health agencies (example W.H.O.) since due to extensive use of BCG vaccination world-wide, a TB infection becomes problematic to define serologically as a consequence of the immune responses induced in the BCG vaccinated subject. Although the actual number of infections is not known, approximately 2 or more billion people are believed to be infected worldwide.<sup>1</sup>

Therapy of TB differs with respect to the antibiotic susceptibility status of the infection. Antibiotic susceptible infections are readily resolved with isoniazid (INH) and rifampicin. However, therapy will take place over a period of many months. Depending upon the therapeutic programme and the extent of damage to the infected lung, therapy may be administered for as long as two years. Due to this prolonged period of therapy, the opportunity for selection of spontaneous mutations that cause antibiotic resistance takes place-leading to the development MDR, XDR and TDR Mtb strains.<sup>5</sup> Therapy of MDR and XDR TB is progressively more problematic and failure is accompanied with increasing frequency of mortality.<sup>5</sup>

At the current moment there are really no singularly effective compounds for therapy of MDR and XDR TB. And of course, TDR TB is beyond current effective therapy. It should be stated that for a drug to be effective for therapy of any TB infection, it must be active at the site of infection, namely, the phagolysosome that imprisons the bacterium. Although hundreds of compounds have been shown to have *in vitro* activity against Mtb, precious few are shown to be active at the intracellular sites of infection. And most of these have presented with serious toxicity to the patient. However, there is one compound that has been shown to have *in vitro* activity against Mtb,<sup>6</sup> promotes the killing of intracellular Mtb by non-killing macrophages,<sup>7</sup> cures the mouse of an antibiotic susceptible<sup>8</sup> and MDR TB<sup>9</sup> infections, improves the quality of life of the XDR TB patient<sup>10</sup> and has been shown to cure the XDR TB infected subject when used in combination with antibiotics to which the offending organism was resistant<sup>11</sup> This compound is the old neuroleptic thioridazine (TZ). It is cheap, and when used with care, will

not produce any significant toxicity or pathology.<sup>10-18</sup> The mechanism by which TZ cures an XDR TB infection and why it should also cure a TDR TB infection is the subject of this mini review.

### How does TZ enhance the killing activity of non-killing macrophages?

Chlorpromazine (CPZ), the parental phenothiazine of TZ, has *in vitro* activity against antibiotic susceptible Mtb.<sup>6,19</sup> However the concentrations that effectively inhibit the replication of the organism are in the range of 15 to 25 mg/L and given that the maximum safe level that can be achieved in the chronically treated psychotic subject is ca. 0.5 mg/L of plasma, the compound appears to be 'dead in the water' with respect to its potential as an anti-TB drug. Nevertheless, because Crowle and his group demonstrated that the concentration of CPZ in the medium required to inhibit the replication of intracellular Mtb was within clinical range,<sup>19</sup> interest in chlorpromazine took place during 1990's when tuberculosis infections nearly quadrupled in New York City with more than half exhibiting an MDR phenotype and outbreaks continued despite rigorous responses by New York City/New York State health agencies.<sup>20-24</sup> Therefore, because TZ is a much milder neuroleptic producing fewer serious side effects, although by no means an innocuous drug,<sup>6,13-19,24</sup> it was studied for *in vitro*,<sup>6</sup> *ex vivo*<sup>7</sup> activities and ability to cure the Mtb infected mouse.<sup>8,9</sup> Briefly, although the concentrations of TZ required for *in vitro* activity against all encountered strains of Mtb regardless of their antibiotic resistance profile were well beyond those that could be achieved in the patient,<sup>6</sup> at concentrations within those that can be readily achieved in the therapy of the psychotic subject, TZ enhanced the killing of newly phagocytosed Mtb,<sup>7</sup> MDR Mtb<sup>7</sup> and XDR TB.<sup>25</sup> The mechanism by which enhanced killing took place involved the inhibition of K<sup>+</sup> and Ca<sup>++</sup> transport by TZ from the phagolysosome that contained the phagocytosed mycobacterium, thereby permitting the accumulation of H<sup>+</sup> to a level sufficient to activate dormant hydrolases<sup>26-30</sup> and subsequent degradation of the phagocytosed bacterium.<sup>31</sup> These studies resulted in the development of a new concept for therapy of tuberculosis that involved the targeting of the macrophage for enhancement of its ability to kill an intracellular bacterium rather than targeting the bacterium itself,<sup>26-30</sup> therefore by-passing the predictable mutational response of the organism to a new drug. Because we had previously shown that combinations of TZ and antibiotics rendered Mtb more susceptible to the antibiotics,<sup>32</sup> Abbate and his group successfully treated XDR TB patients with combinations of TZ and antibiotics.<sup>11</sup> Because of adequate patient preparation and evaluation of the patient for any change in cardiac function associated with administration of TZ, none of the patients presented with prolongation of QT interval or any other cardiopathy associated with TZ administration.<sup>11</sup> Lastly, due to the activation the killing machinery of the macrophage by TZ, one can reasonably expect the killing of intracellular XDR strains of Mtb.

## Why will TZ cure the TDR TB patient?

Most clinical isolates of bacteria that present with an MDR phenotype owe their phenotypic resistance to over-expressed efflux pumps.<sup>33-35</sup> Although efflux pumps have been shown to be over-expressed by antibiotic resistant Mtb strains,<sup>36-43</sup> only recently have they been characterised genetically and physiologically.<sup>44</sup> With respect to therapy of XDR TB patients with TZ and combination of antibiotics to which the infection was initially resistant,<sup>11</sup> and because it is well established that TZ is an effective inhibitor of efflux pumps of mycobacteria,<sup>38,39,42,44,45,46</sup> it is reasonable to expect that the administration of TZ will render inactive any over-expressed efflux pump that contributes to the XDR phenotype of the infecting bacteria. Therefore, and one would predict that TZ would be equally effective against intracellular TDR TB whose phenotype of resistance is in part or wholly due to over-expressed efflux pumps. The activa-

tion of the killing machinery of the macrophage by TZ and the inhibition of over-expressed efflux pumps that contribute to resistance of TDR TB predicts that the protocols used for the therapy of XDR TB developed at the Institute of Hygiene and Tropical Medicine/Universidade Nova de Lisboa, will cure TDR TB patients. Moreover, as shown recently, TZ inhibits a large number of essential genes of Mtb<sup>47</sup> and also kills dormant Mtb.<sup>48</sup> These two latter features of TZ are expected to also contribute to effective cures of the TDR TB patients.

## CONFLICTS OF INTEREST

Not stated.

## FUNDING SOURCES

Not stated.

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