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Tentative susceptibility testing breakpoint for the neuroleptic drug thioridazine, a treatment option for multi- and extensively drug resistant tuberculosis

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ABSTRACT

Introduction: New drugs against multi-(MDR) and extensively drug (XDR) resistant tuberculosis are urgently needed. While new candidate drugs are being developed, reinvestigation of already approved drugs available for other indications could be of value. The objective of this study is to determine tentative drug susceptibility testing strategies and breakpoints for thioridazine, a well-known and well-tolerated neuroleptic drug, which has been shown to be effective against drug resistant tuberculosis both *in vitro* and *in vivo*.

Methods: By testing the minimal inhibitory concentration (MIC) on Middlebrook 7H10 media, the wild-type distribution of thioridazine was established for *Mycobacterium tuberculosis* ($n = 51$) and this distribution was compared to the MICs of M/XDR strains ($n = 67$).

Results: A tentative epidemiological cut off (ECOFF) of thioridazine at 16 mg/L was suggested. Even though such concentrations are not clinically achievable in serum, thioridazine is concentrated intracellularly and concentrations of only 0.1 mg/L has been shown to kill *M. tuberculosis* residing inside cells. MICs above the wild-type (MIC > 16 mg/L) were found in 4/67 (6%) of the M/XDR strains suggesting that resistance mechanisms against thioridazine may already be present in resistant clinical strains.

Conclusions: In view of the difficulties obtaining clinical outcome data for single drugs in the case of tuberculosis since combination therapy is mandatory, the tentative ECOFF may be considered a tentative clinical breakpoint, but the findings should be validated by others. The data from this study strengthens the use of thioridazine as a treatment option for M/XDR tuberculosis, although its proper place in the therapeutic arsenal should ideally be confirmed in clinical trials.

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Introduction

The alarming increase of multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB) has not been matched by the development of new anti-TB drugs. Recently, it has been suggested that the neuroleptic drug thioridazine may take a place in the therapeutic arsenal [1]. Thioridazine has been used safely for the treatment of psychiatric disorders for more than 40 years and has shown

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both *in vitro* (extra- and intracellular) and *in vivo* (mice and men) activity against *Mycobacterium tuberculosis* [1–3].

Several modes of action have been described, such as interaction with bacterial respiration, blocking of calcium binding to proteins, e.g., calcium-dependent ATPases, as well as cell wall damage through a hitherto unidentified mechanism [4]. Inhibition of efflux pump activity has also been shown. For example, thioridazine can block the ethidium bromide efflux activity, which is correlated to the efflux of several antibiotics [5]. This may explain that a synergistic effect of thioridazine together with standard anti-TB drugs such as pyrazinamide and rifampicin has been shown *in vitro* and *in vivo* (mice) [1,3]. Since the antimicrobial activity of thioridazine involves different pathways, it has been speculated that resistance owing to single-step mutations is unlikely. Nevertheless, if thioridazine is introduced for widespread use in M/XDR-TB patients, previous experience suggests that drug resistance might also eventually occur to this drug. However, as far as this study is concerned, appropriate antimicrobial susceptibility testing (AST) methods have not been defined and a breakpoint to which AST should be related has not been established.

For other bacterial and fungal pathogens, the setting of breakpoints for AST is based on the establishment of wild-type minimum inhibitory concentrations (MICs) in combination with pharmacokinetic and pharmacodynamic (PK/PD) information, as well as clinical outcome data as recommended by, e.g., the EUCAST (www.eucast.org). The wild-type distribution of MICs is defined by testing the MICs for a large number of consecutive "wild type" strains. The MICs form (with few exceptions) a normal (Gaussian) distribution, and the highest MIC in the wild-type distribution has been labeled the epidemiological cut off or ECOFF. Strains with MICs above the ECOFF are likely to harbor acquired mutational resistance mechanisms. For TB, combination therapy is the rule, and clinical and PD data for individual drugs is therefore difficult to obtain, and in such cases, susceptibility testing breakpoints will have to rely mainly on wild-type MIC distributions and ECOFFs [6]. Even though this concept has not been widely used for TB, the authors of this study have recently published wild-type MIC distributions and tentative ECOFFs for both first- and second-line anti TB drugs [7–12].

The aim of the present study is to suggest a tentative breakpoint for testing the susceptibility of *M. tuberculosis* to thioridazine by: (i) establishing the wild-type MIC distribution of thioridazine for *M. tuberculosis;* and (ii) comparing the MICs of wild-type *M. tuberculosis* strains to the MICs of M/XDR strains.

Materials and methods

Strains

To determine the wild-type MIC distribution for thioridazine, 51 consecutive clinical *M. tuberculosis* strains from the mycobacteriology laboratory at Karolinska University Hospital in Stockholm, Sweden, were tested. Moreover, 100 non-consecutive strains were tested from the strain collection at the Swedish Institute for Infectious Disease Control (SMI). Among these strains, 67 were MDR, including 16 XDR, and the remaining 33 exhibited other resistance patterns or were susceptible to anti TB drugs as previously determined by the Bactec 460 or 960 methods or the proportion method on Middlebrook 7H10 media.

MIC determinations

MIC determinations were performed in two different rounds as described in detail previously [7]. Shortly, by the use of a 96-stick replicator, bacterial suspensions were inoculated onto 14-cm petri dishes containing Middlebrook 7H10 agar medium and thioridazine (Sigma) in a 2-fold serial dilution ranging from 0.002 to 512 mg/L. The plates were incubated at 37 °C for three weeks and then read visually by comparing the growth for each strain with a 1:100 diluted growth control. The MIC was determined as the lowest concentration with less growth than the 1:100 diluted control, hence representing >99% inhibition. The pan-susceptible *M. tuberculosis* control strain H37Rv was tested in duplicate in both rounds.

Results and discussion

The MIC of thioridazine for *M. tuberculosis* H37Rv was consistently 8 mg/L, which indicates excellent intra- and inter-assay reproducibility. Out of 51 consecutive clinical *M. tuberculosis* strains, 48 had an MIC of 8 mg/L and three had an MIC of 16 mg/L, thus showing an unusually narrow wild-type MIC distribution with an ECOFF of 16 mg/L (Fig. 1). Similar MIC range (8–32 mg/L) has also been reported previously by using the Bactec 460 method for 13 *M. tuberculosis* strains showing various resistance patterns [2]. Using the absolute concentration method, van Ingen et al. obtained thioridazine MICs of 4 mg/L for eight *M. tuberculosis* strains regardless of their susceptibility to other anti TB drugs [2].

Thioridazine has been shown to effectively kill drug sensitive and MDR-TB in mice [3] and to cure 10/12 untreatable XDR-TB patients with a daily dosage of 75 mg as compassionate therapy [1]. However, the clinical effect of the drug in comparison and in combination with the other drugs which are

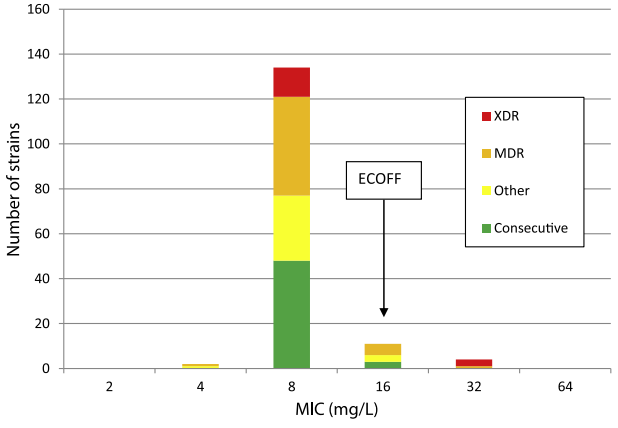

Fig. 1 – MIC distributions of consecutive (wild-type, green), MDR (orange), XDR (red), and other non-consecutive strains. The highest MIC within the wild-type i.e. the tentative epidemiological cut off (ECOFF) is indicated by an arrow. Strains with MICs above the ECOFF may harbor resistance mechanisms.

necessary for treatment is very difficult to assess. Clinical trials are under way, and if these results are also favorable, it seems plausible that the wild-type strains are possible to treat with therapeutic dosages.

The MICs of 67 M/XDR strains, as well as 33 other non-consecutive strains were in the same range as the wild-type (4–16 mg/L). One MDR and three XDR strains displayed MICs of 32 mg/L, i.e., above the ECOFF. Even if wild-type strains could be treated with normal dosages (i.e., 75 mg/day), this is not necessarily true for strains with MICs above the ECOFF, which could have resistance mechanisms. These mechanisms could be general, i.e., affecting different drug classes, and one possible explanation is that these M/XDR strains during anti-TB therapy have gained an increased efflux pump activity [13] in order to decrease the level of antibiotics from the cell and that these efflux pumps also affect thioridazine (even though thioridazine itself is an efflux pump inhibitor), but this remains speculative and has to be proven. However, it is still interesting to note that, irrespective of the mechanism, resistance to thioridazine is likely to occur and may even already be present among highly resistant strains. Thus, thioridazine needs to be introduced with great care in combination with other drugs effective against *M. tuberculosis* in order to avoid the selection of drug-resistant mutants.

Serum concentrations at similar level as the wild-type MICs are not possible to achieve in humans *in vivo*, mainly owing to a dose-dependent risk of prolonged QT interval and severe ventricular arrhythmias [4]. The risk of cardiac toxicity was observed in psychiatric patients and typically when daily doses of more than 800 mg/day were given [4]. However, serum concentrations of 0.5–1 mg/L are fully achievable, and it has been shown that thioridazine is concentrated at least 10-fold inside the vacuoles of the macrophage, i.e., the natural habitat of *M. tuberculosis,* and that even the low concentration of 0.1 mg/L can kill intracellular *M. Tuberculosis* [2,4].

In conclusion, serum concentrations of thioridazine cannot be compared directly with the obtained MICs of the wild-type strains to predict the killing of *M tuberculosis in vivo*, but an MIC above the ECOFF (i.e., above the wild-type MICs) could indicate the presence of acquired resistance mechanisms. A tentative ECOFF of 16 mg/L for drug susceptibility testing on Middlebrook 7H10 medium is suggested to differ between wild-type and non wild-type strains. The ECOFF may serve as a tentative clinical breakpoint, but more clinical data is needed. The findings of this study need to be confirmed by other investigators. Although the results are promising, more clinical studies, ideally randomised controlled trials are warranted to determine the exact role for thioridazine in the treatment of TB.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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