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Short communication

In vitro and ex vivo activity of thioridazine derivatives against *Mycobacterium tuberculosis*

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Abstract

Thioridazine (TZ) has previously been shown by us to have in vitro and ex vivo activity against antibiotic-susceptible and multidrug-resistant *Mycobacterium tuberculosis* (MDRTB). Because current therapy of MDRTB is highly problematic even when all five 'first line of defence' drugs are employed, there is a need for effective antituberculosis drugs. New derivatives of TZ were synthesised and their in vitro activity against a reference strain of *M. tuberculosis* was evaluated with the aid of the BACTEC 460 system. Derivatives that presented significant activity were evaluated by ex vivo studies and were shown to enhance the killing of intracellular *M. tuberculosis*.

Keywords: Thioridazine; Derivatives; *Mycobacterium tuberculosis*; Multidrug resistance; In vitro activity; Ex vivo activity

1. Introduction

Previous studies have shown that thioridazine (TZ) has in vitro activity against antibiotic-susceptible and multidrug-resistant *Mycobacterium tuberculosis* (MDRTB) strains [1–3]. Recently, others have shown that derivatives of this phenothiazine are also active against this organism [4]. However, because *M. tuberculosis* is an intracellular infection, demonstration of in vitro activity must be followed by studies that evaluate the compound's ability to kill the organism in situ [5]. This principle was employed in the past and studies demonstrated that TZ enhanced the killing of intracellular *M. tuberculosis* at a concentration below that present in the plasma of a TZ-treated patient [1]. However, because the side effects of TZ range from very mild (somnia is common) to the very severe (Torsade de Pointes, although very rare) [5–8], TZ has not been seriously considered as an alternative for the therapy of MDRTB infections [9]. Use of TZ for the management of an antibiotic-susceptible *M. tuberculosis* infection is not justified since the 'first line of defence' drugs are sufficiently effective. However, because therapy of MDRTB is highly problematic even with the use of four to five first-line drugs, there is a direct need for new compounds. We have therefore used TZ as a lead compound for the synthesis of derivatives that could be first screened for in vitro activity against *M. tuberculosis*, after which those derivatives that have significant in vitro activity could be evaluated for activity against intracellular *M. tuberculosis*.

2. Material and methods

Mycobacterium tuberculosis H37Rv was grown in BACTEC 460 medium until it reached maximum growth index (GI). From this, an inoculum was obtained and transferred to a fresh bottle of BACTEC 460 medium and the turbidity was adjusted to a 0.5 McFarland scale [2]. Aliquots

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of 0.1 mL were transferred to BACTEC 460 vials containing concentrations of each TZ derivative ranging from 0.0 mg/L to 100 mg/L. Parallel to these cultures and serving as additional controls for comparative purposes, aliquots were similarly transferred to BACTEC 460 medium containing varying concentrations of TZ ranging from 1.0 mg/L to 50 mg/L. An additional control containing 0.2 mg/L isoniazid was conducted in parallel to establish that no change in the susceptibility of the reference bacterium had taken place. In addition, an absolute control containing 1/100 of each culture as required by the method for the determination of a minimum inhibitory concentration was conducted [2]. All cultures were maintained at 37 °C until the absolute control (no drug) reached a GI of 999.

TZ derivatives were prepared by novel syntheses (to be reported elsewhere). Owing to protection of intellectual property, their structures are not presented.

TZ derivatives with in vitro activity against *M. tuberculosis* H37Rv were evaluated for toxicity using the Trypan Blue exclusion method as previously described. Exclusion of the dye is a property of living cells, hence a substance that causes <10% of the cell population to stain blue during a 3-day culture period is considered non-toxic at the cellular level [1,9]. In addition, their activity against phagocytosed *M. tuberculosis* was determined [1]. The methods employed for isolation of monocytes from human whole blood, preparation of monocyte-derived macrophages and evaluation of compounds for activity against intracellular *M. tuberculosis* have been described in detail elsewhere [1] and will not be described again.

3. Results and discussion

The results obtained in this study show that all of the TZ derivatives tested in the BACTEC 460 system have activity against the *M. tuberculosis* reference strain H37Rv and a few (#1867, #1870 and #1875) have similar activity to that of TZ (Table 1). Evaluation of the active derivatives for potential toxicity showed that at concentrations equivalent to that associated with toxicity for TZ [1,9], no significant toxicity was detected (Trypan Blue exclusion by over 90% of lymphocytes).

If a compound is to be useful as an antituberculosis agent it must have activity against intracellular *M. tuberculosis*. Because our previous studies demonstrated that TZ at a clinically relevant concentration of 0.1 mg/L enhanced the killing of phagocytosed *M. tuberculosis* [1], derivatives that had the greatest in vitro activity were selected for evaluation of similar killing activity. As shown in Fig. 1, all of the derivatives at a concentration of 0.1 mg/L enhanced the killing of intracellular *M. tuberculosis*. Furthermore, in contrast to the killing effect of TZ where it takes 3 days for TZ to kill all of the phagocytosed *M. tuberculosis* (data not presented), compounds #1867, #1870 and #1875 killed all of the phagocytosed organisms within 1 day.

Table 1
Growth inhibition of *Mycobacterium tuberculosis* by thioridazine derivatives

Compounds	MIC_{50} (mg/L)
Thioridazine	2.5
Isoniazid	0.08
#1550	>20
#1686	20
#1687	20
#1532-2	20
#1688	20
#1689	20
#1819	>20
#1820	>20
#1821	>20
#1867	5
#1868	20
#1869	>20
#1870	10
#1871	>20
#1872	20
#1873	>20
#1874	>20
#1875	5
#1929	>20

MIC_{50}, minimum inhibitory concentration for 50% of the organisms.

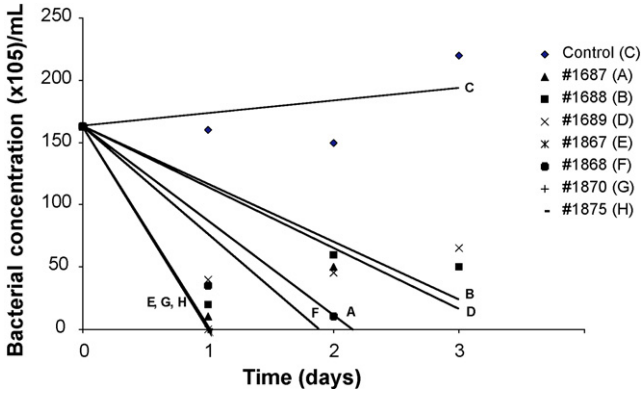

Fig. 1. Killing of phagocytosed *Mycobacterium tuberculosis* by thioridazine derivatives at 0.1 mg/L. Monocyte-derived macrophages obtained from the peripheral blood of healthy donors were infected with *M. tuberculosis* (10 bacteria:1 macrophage) and incubated for 0, 1, 2 and 3 days. Following incubation, cells were lysed and the released phagocytosed mycobacteria were plated. After 3–4 weeks of incubation, colony-forming units were obtained and the bacterial concentration was determined.

The results obtained in this study demonstrate the ex vivo potential of new non-toxic derivatives of TZ against *M. tuberculosis*. Further evaluation of these new derivatives for in vivo toxicity and effectiveness in rendering the *M. tuberculosis*-infected mouse free of this infection are absolutely necessary before these compounds can be seriously considered for the therapy of a pulmonary tuberculosis infection.

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