Brief reports

Inhibition of the respiration of multi-drug resistant clinical isolates of Mycobacterium tuberculosis by thioridazine: potential use for initial therapy of freshly diagnosed tuberculosis

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Chlorpromazine and thioridazine are phenothiazines employed in the treatment of psychosis. These agents inhibited the respiration of clinical isolates of Mycobacterium tuberculosis resistant to streptomycin, rifampin, isoniazid, ethambutol and/or pyrazinamid, all first line drugs. Since any adverse reaction to thioridazine is generally less severe than to chlorpromazine, the possibility is attractive that thioridazine may have a potential in the initial management of patients with newly diagnosed tuberculosis with an as yet undetermined antibiotic susceptibility profile.

Introduction

The antibacterial activity of phenothiazines against mycobacteria has been sporadically reported over the past 35 years (Fisher & Teller, 1959, Bourdon, 1961, Molnár, Beladi & Földes, 1977, Kristiansen & Vergmann, 1986). More recently, the phenothiazine chlorpromazine, (Figure 1), has been shown to inhibit the growth of Mycobacterium tuberculosis ingested by macrophages (Crowle, Douvas & May, 1992).

Although this action is achieved by concentrations of chlorpromazine which are readily achieved in the serum of patients treated for psychoses (0.5–1.0 mg/L), the adverse neurological effects often encountered at such concentrations would prevent any practical applications of chlorpromazine for the management of tuberculosis. However, the chemically related phenothiazine, thioridazine (Figure 1) causes less severe adverse reactions (Hyttel et al., 1984). Therefore, if thioridazine could be shown to have similar antimycobacterial properties, it might be feasible to employ it for the treatment of tuberculosis, especially against strains resistant to conventional first-line antimycobacterial drugs.
Material and methods

Strains of *M. tuberculosis* were isolated from sputum, characterized by conventional methods and tested for susceptibility to streptomycin, rifampicin, isoniazid, ethambutol and pyrazinamide. The isolation was performed on Löwenstein-Jensen solid medium. The susceptibility tests were conducted with the Bactec 460 system employing liquid 12B-Middlebrook Bactec medium (Becton-Dickenson).

Thirty-three strains resistant to two or more of the above-mentioned antibiotics (Table) were tested separately with chlorpromazine (Sigma; 20 strains) or thioridazine (Sigma; 13 strains) in the presence and absence of antibiotics to which the organism was resistant. Testing of chlorpromazine and thioridazine was conducted with different isolates because of the limited amount of initial material that had been processed by the Bactec system and the need to test each isolate at multiple concentrations of either phenothiazine. The concentrations of chlorpromazine or thioridazine ranged from 1 to 64 mg/L in Bactec medium. The addition of these compounds was done in half-light since they are light-sensitive.

Relevant concentrations of antibiotics were added in accordance with the recommendations made by Interlied (1991). The cultures were subsequently incubated in the dark for a period of 7 days at 37°C.

The amount of $^{14}$C-glucose was 4 $\mu$Ci per Bactec bottle at constant specific

<table>
<thead>
<tr>
<th>Resistance to:</th>
<th>Number of isolates (range of &quot;MIC&quot; (mg/L)* of each phenothiazine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH &amp; PZA</td>
<td>3 (8-32)</td>
</tr>
<tr>
<td>INH &amp; RIF</td>
<td>3 (8-32)</td>
</tr>
<tr>
<td>INH &amp; SM</td>
<td>2 (4-32)</td>
</tr>
<tr>
<td>INH, RIF &amp; EMB</td>
<td>0</td>
</tr>
<tr>
<td>INH, RIF &amp; PZA</td>
<td>1 (32)</td>
</tr>
<tr>
<td>INH, RIF &amp; SM</td>
<td>0</td>
</tr>
<tr>
<td>INH, RIF, EMB &amp; SM</td>
<td>1 (16)</td>
</tr>
<tr>
<td>INH, RIF, EMB, SM &amp; PZA</td>
<td>10 (8-32)</td>
</tr>
</tbody>
</table>

INH, Isoniazid; PZA, pyrazinamid; RIF, rifampicin; SM, streptomycin; EMB, ethambutol.

*Lowest concentration (mg/L) of chlorpromazine or thioridazine that totally inhibited generation of $^{14}$CO$_2$. 

Table. Clinical isolates of *M. tuberculosis* assayed for response to chlorpromazine or thioridazine

Figure 1. Molecular structures of chlorpromazine (a) and thioridazine (b).
Antimycobacterial activity of thioridazine

Figure 2. The mean effects of chlorpromazine (■) and thioridazine (□) on the generation of $^{14}\text{CO}_2$ by 20 and 13 strains of $M.\text{tuberculosis}$, respectively. Standard deviations are depicted as bars.

activity. The assessment of the effects of chlorpromazine or thioridazine in the presence or absence of antibiotics was determined by comparison of the average cpm of $^{14}\text{CO}_2$ generated by triplicate control cultures and the count generated by a single experimental cultures at each concentration of chlorpromazine or thioridazine and expressed as a percentage difference. The percentage differences of individual strains calculated for each concentration of chlorpromazine or thioridazine were averaged for the respective 20 and 13 clinical isolates employed in this study.

It must be stressed that the Bactec 460 system determines only the effect of a compound on the respiration of $M.\text{tuberculosis}$ and, since respiration can continue in the presence of concentrations that totally inhibit the replication of the organism (Interlied, 1991), the MIC of the compounds cannot be deduced from the data generated by the Bactec 460 system.

Results and discussion

Figure 2 demonstrates the effect of varying concentrations of chlorpromazine on 20 strains of $M.\text{tuberculosis}$, and of thioridazine on 13 other clinical isolates of $M.\text{tuberculosis}$, all of which were resistant to two or more of the antibiotics employed.

Chlorpromazine significantly inhibited the generation of $^{14}\text{CO}_2$ in a concentration-dependent fashion even at concentrations of 2 mg/L. The inhibition was independent of the degree of multi-drug resistance exhibited by individual isolates (Table). At concentrations slightly above 32 mg/L, the generation of $^{14}\text{CO}_2$ by all clinical isolates was totally inhibited. Our data indicate that higher concentrations of chlorpromazine are required to inhibit respiration completely than are required to inhibit growth; 32 vs 16 mg/L, respectively, as would be expected (Interlied, 1991). The presence of any of the antibiotics (at varying concentrations of chlorpromazine) did not cause a reversal of resistance to these compounds for any clinical isolates. Thus the inhibition noted was identical to that produced by chlorpromazine alone (data not shown).
The thirteen strains similarly challenged with varying concentrations of thioridazine demonstrated that thioridazine was as effective as chlorpromazine (Figure 2). The inhibition observed was again independent of the degree of multi-drug resistance shown by the strain (Table) and the generation of $^{14}\text{CO}_2$ was inhibited totally at concentrations above 32 mg/L. Aliquots of 0.2 mL from cultures containing thioridazine (32 mg/L) were transferred to drug-free medium and incubated for 2 weeks, but no growth of \textit{M. tuberculosis} was detected after this period (data not shown). A concentration of 32 mg/L must accordingly be considered to be bactericidal.

The in-vitro results obtained in this study suggest that multi-drug resistant strains of \textit{M. tuberculosis} show equal susceptibility to the phenothiazines chlorpromazone and thioridazine. The antimycobacterial action of the phenothiazines is independent of the neuroleptic activity related to some of the phenothiazines (Kristiansen & Vergmann, 1986).

Primary infections of tuberculosis are of growing concern in larger cities in the USA (Anonymous, 1991) and are often accompanied by drastic increases in the resistance of \textit{M. tuberculosis} to the drugs commonly employed. This resistance has in turn contributed to the dissemination of tuberculosis. With this background, thioridazine may have a potential as an alternative in the management of freshly diagnosed infections of tuberculosis. The effective concentrations of chlorpromazine or thioridazine required in this study were quite high (greater than 32 mg/L), and are substantially greater than those achieved \textit{in vivo} (0.5 mg/L). However, the phenothiazines may be concentrated within macrophages (Crowle \textit{et al.}, 1992) and, although the vast majority of tubercle bacilli are not within macrophages, human lung tissue also concentrates the phenothiazines. Concentrations of chlorpromazine up to 98 \(\mu\text{g/g}\) of wet lung tissue are reached after treatment (Forrest, Forrest & Roizin, 1963).

Since chlorpromazine has antimycobacterial activity within the macrophage (Crowle \textit{et al.}, 1992), it is likely that thioridazine exerts a similar activity with concentrations at the level of 1 mg/L. Other phenothiazines, especially those belonging to the antihistamine group, might also merit further investigation against drug resistant mycobacteria (Williams, 1995).

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\section*{References}


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