In vitro susceptibility of *Mycobacterium tuberculosis* to trifluoperazine

N. Selvakumar, Vanaja Kumar, P. V. Krishnamurthy, R. Prabhakar and P. S. Murthy*

Tuberculosis Research Centre, V. R. Ramanathan Road, Chetput, Chennai 600 031, India
*University College of Medical Sciences, New Delhi 110 095, India

The reference strain, *Mycobacterium tuberculosis* H37Rv, 19 drug-sensitive and 15 drug resistant clinical isolates of *M. tuberculosis* were tested for their *in* vitro susceptibility to trifluoperazine (TFP), an antipsychotic drug, by broth dilution method. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of TFP against *M. tuberculosis* H37Rv were 8 and 32 mg/l, respectively. The distribution of the sensitive and resistant isolates, with respect to the MIC of TFP, was similar. The distribution of the sensitive and resistant isolates, with respect to the MBC of TFP, was different and the difference was statistically significant. The findings suggest that TFP is more bactericidal to drug-resistant isolates than to the sensitive isolates.

MULTIDRUG resistant tuberculosis (MDR-TB) is being frequently reported[1]. Failure to cure MDR-TB with the currently available antitubercular drugs results in the spread of MDR-TB in the community[2]. So, there is an urgent need to find out new and potent drugs to treat such cases and thereby prevent an emerging multi-dimensional problem. Inhibition, by drugs, of lipid synthesis in mycobacteria was considered as one of the alternative approaches to prevent the bacterial growth. The earlier studies[3-7] showed the presence of calmodulin-like protein in mycobacteria and its regulatory effect on lipid metabolism and growth. A calmodulin antagonist, trifluoperazine (TFP), was shown to inhibit the growth of *Mycobacterium tuberculosis* H37Rv and an isoniazid (INH) resistant clinical isolate of *M. tuberculosis* at a concentration of 5 and 8 mg/l, respectively in Youman's and Karlson's medium when grown as shake cultures[8]. When grown as surface cultures in the same medium, *M. tuberculosis* H37Rv was inhibited by TFP at 4 mg/l, and INH and streptomycin-resistant clinical isolates were inhibited, respectively at 15 and 8 mg/l (ref. 9). To continue further research with this drug as an antimycobacterial agent, it is essential to determine the susceptibility pattern of clinical isolates of *M. tuberculosis* to TFP. Therefore, the present study was planned to determine the *in vitro* susceptibility pattern of both drug-sensitive and drug-resistant clinical isolates of *M. tuberculosis* to TFP.

A total of 19 drug-sensitive (sensitive to the four drugs; streptomycin, isoniazid, rifampicin and ethambutol) and 15 drug-resistant clinical isolates (resistant to any one or more of the four drugs mentioned earlier) were tested. The standard laboratory reference strain, *M. tuberculosis* H37Rv, was included as control. The cultures were randomly coded and tested.

Log phase Middlebrook 7H9 (Difco, USA) culture of *M. tuberculosis* H37Rv was diluted, based on bacterial counts in a Thoma Bacteriological Counter, to contain 10^6 bacilli/ml in fresh 7H9 medium and distributed, in 9.9 ml quantities, to sterile bottles. TFP (Sigma, USA) was added in 0.1 ml quantity to attain final concentrations of 8, 16, and 32 mg/l. The control culture received 0.1 ml distilled water. The colony-forming units (cfu) were determined, on day 0, 3, and 7 on Lowenstein Jensen (LJ) slopes and expressed in $\log_{10}$ cfu/ml. Minimal inhibitory concentration (MIC) is defined as the lowest concentration of the drug, in mg/l, which inhibited more than 99% of the population in the control culture on day 7. Minimal bactericidal concentration (MBC) is defined as the lowest concentration of the drug, in mg/l, which killed more than 99% of the population in the initial inoculum. The bactericidal activity (BA) is measured as the average reduction in $\log_{10}$ cfu/ml/day when exposed to the respective concentration of the drug.

All the clinical isolates and the reference strain were grown in 7H9 medium for 7 days at 37°C. The cultures were diluted to contain 10^6 bacilli/ml of fresh medium and distributed in bottles. The drug solutions were added to attain 8, 16, 32 and 64 mg/l. The culture bottles were incubated for 7 days at 37°C. The cfu were determined on day 0 for the control cultures and on day 7 for all the cultures and the MIC, MBC and BA were determined.

Table 1. Activity of TFP against *M. tuberculosis* H37Rv

	Viable counts ($\log_{10}$ cfu/ml) on day		
Conc. (mg/l)	0	3	7
Nil	4.60	6.16	8.39
32	4.60	3.60 (0.33)*	2.66 (0.27)
16	4.60	4.94	4.23 (0.16)
8	4.60	5.58	6.03

*Bactericidal activity is given in the parentheses.

Table 2. Distribution of MIC and MBC of TFP against clinical isolates of *M. tuberculosis*

	MIC (µg/ml)				MBC (µg/ml)*			
Strain	8	16	32	64	8	16	32	64
Clinical (sensitive)	3	12	3	1	0	2	2	14
Clinical (resistant)	5	8	2	0	1	7	5	2
Total	8	20	5	1	1	9	7	16

*For one drug-sensitive isolate the value of MBC was not available. Sensitive: Sensitive to streptomycin, isoniazid, rifampicin, ethambutol. Resistant: Resistant to any one or more of the above drugs.

Table 3. Growth, in mean $\log_{10}$ cfu/ml (±SD), of drug-sensitive and resistant clinical isolates of *M. tuberculosis* in drug free and in TFP containing cultures

	Growth without TFP		Growth on day 7 with TFP (mg/l)					
Strain	Day 0	Day 7	8	16	32	MIC	MBC	MIC : MBC
Sensitive	4.76	7.09	6.36	4.23	3.00	16	64	1 : 4
(*n* = 19)	(0.59)	(0.99)	(0.56)	(1.08)	(0.87)			
BA			–	0.05	0.19			
Resistant	4.78	6.34	5.22	2.95	2.11	16	32	1 : 2
(*n* = 15)	(0.54)	(0.81)	(1.45)	(1.32)	(0.60)			
BA				0.26	0.38			

BA: Bactericidal activity.

The mean MIC, MBC and BA were determined separately for sensitive and resistant clinical isolates.

The inhibitory and bactericidal activities of TFP against the laboratory reference strain *M. tuberculosis* H37Rv are given in the Table 1. The MIC and MBC of TFP against *M. tuberculosis* H37Rv were 8 and 32 mg/l, respectively. On day 3, the BA was found to be 0.33 with 32 mg/l and on day 7, it was 0.27 and 0.16, respectively with 32 and 16 mg/l (Table 1).

The distribution of MIC and MBC of TFP against the clinical isolates is shown in Table 2. The MIC was $\leq$ 16 mg/l against 15 of 19 sensitive isolates and 13 of 15 resistant isolates. The distribution of sensitive and resistant isolates, with respect to MIC of TFP, was similar. The MBC was $\leq$ 32 mg/l against 13 of 15 resistant isolates and 64 mg/l against 14 of 18 sensitive isolates. The distribution of sensitive and resistant isolates with respect to MBC was different and this difference was statistically significant ($p < 0.001$). The MIC : MBC ratio was 1 : 4 for 10 of 19 sensitive isolates while it was 1 : 2 for 12 of 15 drug-resistant isolates. The mean MIC was 16 mg/l for both the sensitive and resistant groups (Table 3). The mean MBC was 64 and 32 mg/l, respectively for the sensitive and resistant isolates. On day 7, the BA against the sensitive isolates was 0.19 and 0.05, respectively with 32 and 16 mg/l, while it was 0.38 and 0.26 for the resistant isolates (Table 3) and the differences were statistically significant ($p < 0.01$ for 16 mg/l; $p < 0.002$ for 32 mg/l).

The MIC of TFP for a majority of the clinical isolates was 16 mg/l. At the time when the results of this study were available, Reddy *et al.* [10] reported that the MIC of TFP ranged from 3 to 25 mg/l and 6.5 to 12.5 mg/l, respectively, for the drug-sensitive and drug-resistant isolates of *M. tuberculosis.* These observations revealed that the MIC of TFP against *M. tuberculosis* is about 80 times more than that could be achieved in human blood after the administration of the recommended dosage. The plasma levels of TFP in human beings range from 80 to 200 ng/ml when the drug was administered at the recommended dosage of 75 to 100 mg per day in a single dose[11]. Because of its very low plasma levels, the therapeutic index of TFP could be very low and its *in vivo* bactericidal activity can be doubted in human trials. But, it was interesting to note that significantly greater proportion of drug-resistant isolates exhibited increased susceptibility to TFP compared to drug-sensitive isolates. This may be attributed to the decreased growth rate of drug-resistant isolates compared to drug-sensitive isolates (Table 3) or to the cell wall permeability of the organism, or to the other mechanisms operating in bacteria. Further understanding of how the drug-resistant isolates of *M. tuberculosis* are more susceptible to TFP could generate more research in drug-resistant tuberculosis.

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