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Effect of trifluoperazine on in vitro ATP synthesis by *Mycobacterium leprae*

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Abstract

The effect of trifluoperazine (TFP), a calmodulin antagonist, was investigated on in vitro ATP levels of human derived *Mycobacterium leprae*. *M. leprae* were obtained from biopsies from multi-bacillary forms of leprosy and were incubated in a modified Dubos medium system which supports limited in vitro synthesis of *M. leprae*. This incubation was carried out in the absence and presence of different concentrations of trifluoperazine. Samples for estimation of bacillary ATP levels were taken at day 0 and at 14 days of incubation. TFP inhibited ATP levels in *M. leprae* and this inhibitory effect was marginal at 2.5 μg ml^{-1} (35% inhibition), highly significant at 5 μg ml^{-1} (87% inhibition) and almost total at 10 μg ml^{-1} (98.5% inhibition). This compound appears to have potential as an anti-leprotic drug and also as a broad spectrum anti-mycobacterial agent in view of its anti-tubercular activity reported earlier.

Keywords: Trifluoperazine; Leprosy; Anti-mycobacterial

1. Introduction

Although calmodulin-like protein (CALMP) has been reported to be present in bacteria [1–5], its role in bacteria was unknown. Systematic studies initiated by Murthy and colleagues [6–9] not only demonstrated the presence of CALMP in many species of mycobacteria (*Mycobacterium smegmatis* and *M. bovis* BCG [6,7], *M. phlei* [8], *M. tuberculosis* H37 Rv and Ra [9]), but also indicated its role in the metabolism of their lipids, especially phospholipids, as well as their growth [6–9]. Based on the above studies, Ratnakar and Murthy [10,11] found that the calmodulin antagonist and anti-psychotic drug trifluoperazine (TFP) inhibited the in vitro growth of *M. tuberculosis* H37 Rv (minimum inhibitory concentration (MIC) of 4 μg ml^{-1}) and of the clinical isolates resistant to isoniazid (strain TRC C 1193, MIC 8–15 μg ml^{-1}) and streptomycin (strain SO111, MIC 8 μg ml^{-1}). Trifluoperazine (TFP) was observed to be the most potent in the six phenothiazine group of calmodulin antagonists regarding its in vitro anti-tubercular activity [12]. They also found that TFP possesses multiple sites of action on the synthesis of macromolecules by incorporation of [^{14}C]acetate into lipids, [^{3}H]thymidine into DNA,

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[^{14}C]glycine into proteins and glycine derived carbohydrates [13] and ^{32}P into phospholipids [7]. After exposure to anti-tubercular drugs, there was a decrease in the content of CALMP in *M. tuberculosis* H37 Rv and H37 Ra [9].

Demonstration of anti-tubercular activity has been an important lead which prompted us to find out whether trifluoperazine could inhibit the growth of *M. leprae*. In this communication we report that in vitro TFP inhibits the viability of clinical isolates of *M. leprae*.

2. Materials and methods

2.1. Chemicals

Trifluoperazine was purchased from Sigma Chemical Company, USA. ATP reagents were from Bio-Orbit, Bromma (Sweden). *M. leprae* were derived from untreated lepromatous (LL) human cases.

2.2. Assay of in vitro ATP levels of M. leprae

M. leprae were obtained from three human lepromatous (LL) cases. Bacilli were harvested and purified by the technique of Dhople and Storrs [14] as modified by Katoch et al. [15,16]. From a bacillary suspension, smears were prepared, stained for acid fast bacilli (AFB) by the Ziehl-Neelsen technique and bacillary counts were determined [17]. These bacilli were incubated in a modified Dubos medium system comprising asparagine, glycerol and gelatin, pH 6.2 at 30°C [18,19] and ATP levels were measured by a firefly bioluminescence assay reported earlier [16]. Briefly, the bacilli were harvested and ATP extracted by the Tris-boiling method [16]. ATP estimation was carried out in a LKB-1251 (Pharmacia-LKB, Sweden) Luminometer using the conditions reported earlier [16]. The assay conditions were: 200 μl of the sample with proportional volumes of reagents (LKB 1243-200) and Tris-EDTA buffer. One minute integral counts were taken and the ATP concentration was calculated using standard ATP from LKB. Three ATP estimations were done from the same specimen and mean values (calculated as pg per million bacilli) were taken. ATP levels (increase or decrease) were compared with the initial levels in the same experiment. The pH was rechecked after addition of the drug and was not altered.

2.3. Effect of trifluoperazine on the ATP levels of M. leprae

Trifluoperazine was incorporated into the medium in concentrations of 2.5 μg, 5.0 μg, and 10 μg ml^{-1}. The effect of the drug was assessed by determining the ATP levels (pg per 10^6 cells) in the presence and absence of drug. Percent inhibition was calculated by comparison with untreated control ATP levels at 14 days.

3. Results and discussion

Trifluoperazine was observed to reduce the ATP levels of in vitro incubated *M. leprae* derived from human leprosy cases (Fig. 1). The inhibition was observed to be marginal (35% mean) at 2.5 μg ml^{-1} but highly significant at 5 μg ml^{-1} (mean 87%) and almost total at 10 μg ml^{-1} (mean 98.5%) after 14 days of incubation. In the absence of growth of *M. leprae* in any acceptable in vitro medium system, an alternative in vitro drug screening approach includes the measurement of intracellular ATP levels [18–22] as an indicator of metabolic status [22] or limited growth [18,20]. By comparing with colony forming units for cultivable mycobacteria and growth in the mouse foot pad for *M. leprae*, ATP content has been established as an sensitive index of viability for various mycobacteria [14–16,21,23–25]. Using such a system the anti-leprosy activity of recognized anti-leprosy compounds have been investigated in our earlier studies [18]. The inhibitory activity of trifluoperazine as observed in this study is significant and is in the same range as that reported for *M. tuberculosis* by Ratnakar and Murthy [11]. In the case of *M. tuberculosis* these investigators reported multiple sites of action. Results suggest, though do not prove directly, the presence of CALMP in *M. leprae*. Inhibition by TFP suggests that CALMP may have a role in the metabolism of *M. leprae* possibly in a way similar to that in *M. tuberculosis*. The mechanism of action may also be similar to that in *M. tuberculosis* by inhibiting the

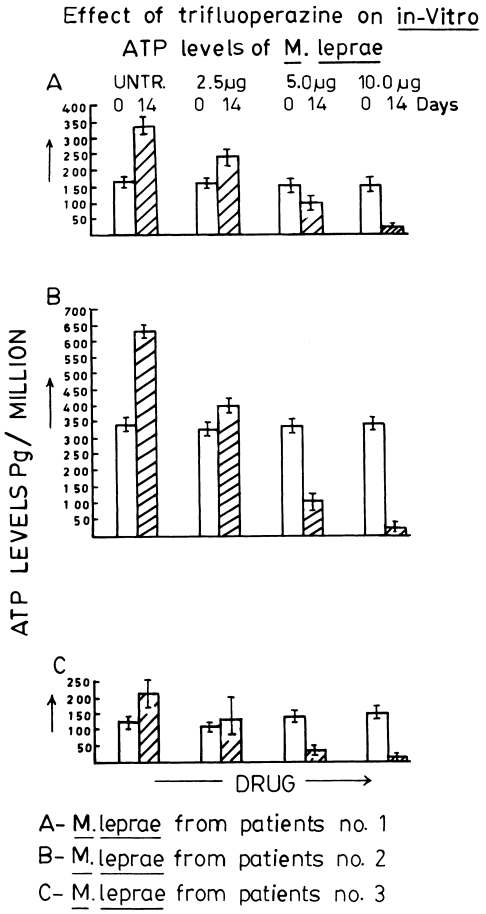

Fig. 1. The effect of trifluoperazine on ATP levels of *M. leprae*. ATP biomass estimated as pg per million cells (mean ± S.D.) depicted as clear bars at day 0 and compared at 14 days (shaded bars) with controls having no drug. A,B,C are three strains of bacilli harvested from three patients.

synthesis of lipids (including phospholipids), DNA, proteins and carbohydrates [13]. Its effect in the presence of the other anti-leprosy drugs like dapsone, clofazimine and rifampicin as well as newer anti-leprosy drugs is to be tried. The use of TFP in combination with other anti-leprosy drugs has to be explored first in animals and then in humans.

Rao et al. were the first to extend the limited studies of TFP with drug resistant clinical isolates of mycobacteria [10,11] to a larger number of clinical and environmental isolates [26,27]. According to them, TFP inhibited the in vitro growth of *M. avium* (MIC 10 µg ml^{-1}). We have also observed that TFP was very effective against single and multidrug resistant clinical isolates of *M. tuberculosis* indicating the possible advantage of TFP for drug resistant *M. tuberculosis* (unpublished). Murthy and others [11–13,23,24] suggested that trifluoperazine or its derivatives or other calmodulin antagonists could be useful in the multi-drug regimen for tuberculosis patients.

The effects of trifluoperazine on *M. leprae* as observed in this study, as well as its reported effect on cultivable mycobacteria, suggest this compound to be a broad spectrum anti-mycobacterial compound. These findings in various mycobacteria including *M. tuberculosis, M. leprae* and *M. avium* need to be investigated by further in-depth studies for their ultimate clinical application.

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