TRIFLUOPERAZINE AND CEF-ALLICIN FROM GARLIC (*ALLIUM SATIVUM*) AS POTENTIAL NEW ANTITUBERCULAR DRUGS ACTIVE AGAINST DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Trifluoperazine (TFP) and a compound called CEF-allicin purified from garlic (*Allium sativum*) possess antitubercular activity against both drug susceptible and resistant clinical isolates of *Mycobacterium tuberculosis*. They are bactericidal in nature with multiple sites of primary action. This new use for known drug TFP was based on our observation that mycobacteria have calmodulin like protein which regulates their metabolism and a calmodulin antagonist has antitubercular activity.The minimum inhibitory concentration (MIC) of TFP against *M. tuberculosis* was 4-5 µg/ml. It inhibited considerably by 6hrs, the synthesis of total lipids from ^{14}C-acetate and proteins and DNA as judged by the uptake of ^{14}C-glycine and ^{3}H-thymidine respectively by the bacilli. With 50 clinical isolates from our hospital at Delhi, the MIC was 4µg/ml, for 40% and 8µg/ml, for 50% of the isolates susceptible as well as resistant to one or more of the five drugs isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide. The MIC of CEF-allicin was 25µg/ml, for both *Mycobacterium tuberculosis* and isoniazid resistant clinical isolate TRC-C 1193. It inhibited in 6hrs or less the synthesis of total lipids completely and proteins and DNA of *M. tuberculosis* from its labeled precursors almost completely.

KEY WORDS : Trifluoperazine, allicin, garlic, *Allium sativum*, *Mycobacterium tuberculosis*, antitubercular drugs, multi drug resistance.

INTRODUCTION

In view of the increased incidence of tuberculosis caused by multi drug resistant (MDR) mycobacteria, the need for the development of new antitubercular drugs preferably with structure different from that of the existing ones and active against MDR *Mycobacterium tuberculosis* needs to be hardly emphasized. If the drugs are also active against *M. avium*, which causes tuberculosis in some HIV patients, it is an added advantage. We have found that two drugs trifluoperazine and a garlic compound possess the above advantages and are potential antitubercular drugs.

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ANTITUBERCULAR ACTIVITY OF TRIFLUOPERAZINE

Demonstration of calmodulin like protein (CAMLP) and its relation to growth

CAMLP was demonstrated by us in a number of mycobacteria (pathogenic and nonpathogenic and slow and fast growing) like *M. smegmatis*, *M. phlei*, *M.tuberculosis* H_{37}Rv and H_{37}Ra and *M. bovis* BCG (1). We called this protein calmodulin like protein because it functionally stimulated bovine brain 3', 5'-cyclic nucleotide phosphodiesterase, immunologically reacted with antibody to bovine brain calmodulin and was heat and acid stable which are some of the important properties of eukaryotic calmodulin. This protein has been purified and its gene sequenced, (Prasad Reddy et al communicated). There was a positive correlation between the growth of mycobacteria and their intracellular content of CAMLP, total lipids, and total and individual phospholipids (1). This inter-

esting correlation prompted us to find out whether a calmodulin antagonist would inhibit their growth.

Effect of Trifluoperazine on the growth of *Mycobacterium Tuberculosis* H37Rv

As a calmodulin antagonist, we preferred trifluoperazine because it inhibited the incorporation of ^{32}P into the total phospholipids of mycobacteria (1) and it is in use from a long time as a drug for psychotic disorders. TFP inhibited the growth of *M. tuberculosis* $H_{37}Rv$ in vitro when tried alone with minimum inhibitory concentration (MIC) of 4-5µg./ml. TFP at 10 times MIC killed large number of actively growing (10 days) tubercle bacilli. TFP was bactericidal in nature(2).

Effect of Trifluoperazine on single drug resistant Clinical Isolates

The MIC of TFP against streptomycin resistant clinical isolate (TRC-S0111) was 8µg/ml. But the MIC of TFP against isoniazid resistant isolate (TRC-C1193) was 8µg/ml when grown as a shake culture and 15µg/ml on solid medium. It appears that the MIC was lower with higher oxygen tension(2).

Mechanism of Action of Trifluoperazine

In a time course study (3), even within 6 hrs of exposure to TFP, which is less than the generation time of 14 hrs of the tubercle bacilli, there was considerable inhibition of the incorporation of ^{14}C-acetate into total lipids (63%) and uptake of ^{14}C-glycine (74%) and ^{3}H-thymidine (52%) by the whole cells and by 48 hrs the inhibition was 87%, 97% and 74% respectively. When TFP was added to the bacilli already taking up and metabolizing the precursors at a later point of time (3 hrs for labeled acetate and thymidine and 12 hrs for glycine) it inhibited completely their uptake. So the onset of TFP action on the mycobacteria is very rapid (3-6hrs). TFP has multiple sites of primary action on the synthesis of lipids, DNA, proteins and probably glycine derived carbohydrates.

Effect of Trifluoperazine on *M. Avium*

TFP inhibited the growth of clinical isolates (at San Diego, USA) of *M. avium* in vitro and in the monocyte derived macrophages at MIC of 20 and 30µg/ml.

Effect of Trifluoperazine on multi drug resistant clinical isolates of *M. Tuberculosis*

We tested 50 isolates of which 29 were susceptible (to all five) and 21 resistant to one or more of the five drugs isoniazid, rifampicin, streptomycin, ethambutol and pyrazinamide (5). The MIC of TFP for 40% of both susceptible and resistant isolates was as low as 4µg/ml. while that of 50% of each of them was 8µg/ml.

In a collabrative study with one of us (PSM) Selvakumar et al. (6) also tested at Tuberculosis Research Centre, Chennai 34 isolates out of which 19 were sensitive (to all the four) and 15 resistant to one or more of the four drugs isoniazid, rifampicin, streptomycin and ethambutol. The MIC was 8µg/ml for 16% susceptible and 30% resistant isolates and 16µg/ml for 63% susceptible and 86% resistant isolates with mean MIC of 16µg/ml. The mean MBC (minimum bactericidal concentration) was 64µg/ml and 32µg/ml for the susceptible and resistant isolates respectively. This means that the mean MIC:MBC ratio was lower (1:2) for resistant isolates but higher (1:4) for susceptible isolates. According to them the bactericidal activity (BA) was much higher for drug resistant isolates than for drug sensitive isolates. The better results in our Delhi study could be due to one or more of the three reasons. (i) Since TFP is known to be unstable, we used fresh solution each time. In the Chennai study it was not stated how the TFP solutions were made. (ii) We used a medium (Youmans and Karlsons) not as rich in nutrients as the Middlebrook 7H9 medium of Chennai study. (iii) There could be variations in the susceptibility of the isolates of the two regions to TFP.

The MIC of TFP is no doubt high. But the requirement is its efficacy when tried in combination with other antitubercular drugs. We have taken advantage of the fact that tuberculosis patients with psychotic problems have to be given one antipsychotic drug also besides regular antitubercular therapy. Instead of some other drug if TFP is given to such patients there may be the dual benefit of relief of psycnotic symptomes and antitubercular activity. Trial is in progress at our place and the results are encouraging (Gupta et al.).

In another collaborative study (with PSM) Katoch et. al. (7) Observed that TFP had anti

M. leprae activity also. TFP inhibited in vitro ATP synthesis (taken as indicator of limited growth or metabolic status) considerably (87%) at 5 µg/ml and almost completely (98.5%) at 10µg/ml.

Antitubercular activity of CEF-allicin from garlic

Rao et. al. (8) were the first to demonstrate that allicin prepared from the ethanol extract of garlic inhibited the growth of *M. tuberculosis* at a higher concentration of 400µg/ml, while Delaha and Garagusi reported that the chloroform extract of garlic juice exhibited antitubercular activity against 17 species of mycobacteria, typical and atypical with MIC ranging from 1.34 to 2.68 mg/ml. (see ref. 8). No garlic compound was ever tried so far on any drug resistant mycobacteria.

Isolation of CEF-allicin from garlic

Ratnakar and Murthy (9) prepared allicin from garlic by a modification of the method of Rao et. al. (8). It was further purified over a silica gel column and elution with chloroform. This is called CEF-allicin (9). We did not determine its structure.

Antitubercular activity of CEF-allicin

Allicin, which was initially prepared, inhibited the growth of the susceptible laboratory strain and the isoniazid resistant strain TRC-C1193 at a concentration of 70µg/ml. After further purification, CEF-allicin was inhibitory to both the susceptible and isoniazid resistant isolates at a concentration of 25µg/ml. This is the lowest MIC reported so far for any garlic compound (9). It is bactericidal in nature.

Mechanism of antitubercular action

In a time course study, by 6 hrs the incorporation of ^{14}C-acetate into the total lipids of *M. tuberculosis* was almost completely (99%) inhibited by allicin (10 times MIC). With in the same time, the uptake of ^{3}H-thymidine and ^{14}C-glycine by the whole cells was inhibited by 86% and 74% respectively. Thus CEF-allicin seems to be a promising antitubercular drug with remarkable multiple sites of action affecting the synthesis of lipids, DNA and proteins (possibly even glycine derived carbohydrates) in less than 6 hrs.

MIC of CEF-allicin is very high, but it would be useful to find out if its MIC level would be lower when tried in combination with other antitubercular drugs. Further, if it is well tolerated at its MIC level (in combination with other drugs) the mere higher number of µgs of MIC should not be a consideration. It is the activity at a tolerated dose that is relevant.

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