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In vitro susceptibility of clinical isolates of *Mycobacterium tuberculosis to* cefadroxil—a cephalosporin antibiotic

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The bactericidal activity (BA) of cefadroxil, a semisynthetic cephalosporin antibiotic, against *M. tuberculosis* H37Rv was studied in Middlebrook 7H9 medium, Cefadroxil showed good BA (average fall of viable counts = $\log_{10}$ 0.32 colony forming units/ml/day) against the log phase culture of *M. tuberculosis* H37Rv. Its minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were found to be 15 µg/ml or less. The MIC of cefadroxil for 29 clinical isolates of *M. tuberculosis* and a laboratory strain, *M. tuberculosis* H37Rv was also determined by agar dilution method using Middlebrook 7H11 agar as a screening procedure. The MIC of cefadroxil was found to be 10 µg/ml or less for *M. tuberculosis* H37Rv and 16 (55.1%) of 29 clinical isolates tested. The MIC for 3 of 10 drug sensitive and 9 of 19 drug resistant isolates was 40 or more, a concentration much higher than the peak plasma concentration (28 µg/ml) attained in human beings. The higher MIC observed in 12 of 29 clinical isolates irrespective of their susceptibility pattern requires further studies to assess the usefulness of cefadroxil in the treatment of tuberculosis.

Key words Cefadroxil - cephalosporin - *M.tuberculosis* - susceptibility

Multi-drug resistant tuberculosis (MDR-TB) is being frequently reported[1]. Failure to cure MDR-TB with the currently available drugs might result in its spread in the community. There is therefore an urgent need to identify new and potent drugs to effectively treat such patients and thereby interrupt the chain of transmission in the community.

Cefadroxil is a semisynthetic cephalosporin antibiotic active against Gram-positive and Gram-negative bacteria. It is resistant to inactivation by the beta-lactamases produced by these bacteria[2] and is highly bactericidal with low toxicity. Cefadroxil can be administered orally. The peak plasma concentration attained after single doses of 500 and 1000 mg are 16 and 28 µg/ml, respectively. Measurable levels are present up to 12 h after the administration of the drug. The drug has a very low urinary excretion rate; absorption is not affected by simultaneous intake of food; and is widely distributed in body tissues. The drug can be administered twice or even once a day[3]. As these pharmacokinetic characteristics of cefadroxil are promising, a study was undertaken to determine the *in vitro* susceptibility of clinical isolates of *M. tuberculosis* to cefadroxil.

Material & Methods

Strains : Clinical isolates of *M. tuberculosis* were obtained from the patients attending the Tuberculosis Research Centre, Chennai. A total of 29 isolates were selected including 10, which were sensitive to streptomycin, isoniazid, rifampicin and ethambutol and 19 which were resistant to one or more of these drugs. The laboratory reference strain *M. tuberculosis* H37Rv was included as control. All the clinical

isolates and the reference strain were coded before setting up the drug susceptibility test.

Bactericidal activity (BA) of cefadroxil against M. tuberculosis H37Rv : BA was studied by the procedures described by Dickinson and Mitchison[4]. In brief, the log phase culture of *M. tuberculosis* in Middlebrook 7H9 liquid medium (Difco, USA) was adjusted to contain 10^6 bacilli per ml of fresh medium and cefadroxil (Lupin, India) was added to a final concentration of 15 and 30 µg/ml. The viable counts were estimated on days 0, 3 and 7 by inoculating serial 10 fold dilutions of the culture on Lowenstein-Jensen (LJ) medium. The drug free medium served as control. The growth was recorded at the end of 4 wk and expressed as log,, colony forming units (cfu)/ml.

BA was defined as the average fall of viable counts in $\log_{10}$ cfu per ml of culture per day when exposed to the given concentration of the drug for 7 days[5].

Minimal bactericidal concentration (MBC) was defined as the lowest concentration of the drug which killed more than 99 per cent of the bacterial population in the initial inoculum.

Screening of clinical isolates against cefadroxil : The clinical isolates of *M. tuberculosis* were screened for their susceptibility to cefadroxil by the agar dilution method as described by Canetti *et al* [6]. In brief, the log phase culture of *M. tuberculosis* in Middlebrook 7H9 liquid medium was diluted to contain 10^6 bacilli per ml and 0.1 ml of serial 10 fold dilutions of the culture suspension were inoculated onto drug-free and drug containing Middlebrook 7H11 agar (Difco, USA) plates. The concentration of cefadroxil tested ranged from 5 to 40 µg/ml. Viable counts were estimated after 4 wk of incubation at 37°C.

Minimal inhibitory concentration (MIC) was defined as the lowest concentration of drug which inhibited more than 99 per cent of the population in control culture.

Results & Discussion

The viable counts of *M. tuberculosis* H37Rv in 7H9 liquid medium without and with different concentrations of cefadroxil on days 0, 3 and 7 are shown in Table I. It can be seen that cefadroxil has bactericidal activity of 0.3 1, 0.32, respectively with 15 and 30 µg/ml. MIC/MBC was found to be less than 15 µg/ml which is much below the peak plasma level (28 µg/ml) attained in man. On the basis of these results, the susceptibility of clinical isolates of *M. tuberculosis* to cefadroxil was determined by agar dilution method using Middlebrook 7H11 agar medium as a screening procedure.

The distribution of MIC of cefadroxil for clinical isolates of *M. tuberculosis* and the reference strain is given in Table II. The MIC for the reference strain was found to be 10 µg/ml or less when tested on 6 occasions. The MIC for 7 of 10 drug sensitive isolates and 9 of 19 drug resistant isolates was also found to be 10 µg/ml or less. However, 3 of 10 drug sensitive isolates and 9 of 19 drug resistant isolates were inhibited only at 40 µg/ml or more implying resistance to cefadroxil. The higher MIC required to inhibit these strains, was thought to be due to the inactivation of cefadroxil by the beta-lactamases produced by *M. tuberculosis* as several species of mycobacteria are known to produce different types of beta-lactamases[7]. We tested the inactivation of cefadroxil by substituting cefadroxil as a substrate to

Table I. Viable counts of *M. tuberculosis* H37Rv in 7H9 medium without and with different concentrations of cefadroxil on days 0, 3 and 7

Conc. of drug (µg/ml)	$\log_{10}$ cfu/ml on day			BA
	0	3	7	
0 (control)	5.66	5.88	7.42	-
15	5.66	4.50	3.48	0.31
30	5.66	4.20	3.45	0.32

BA, bactericidal activity

Table II. MIC of cefadroxil against clinical isolates

Strains	No. tested	MIC (µg/ml) in 7H11 agar				
		5	10	20	40	>40
H37Rv (reference)	6	4	2	0	0	0
Clinical (sensitive)	10	7	0	0	1	2
Clinical (resistant)	19	7	2	1	1	8

penicillin G in the beta-lactamase assay as described by Backelin *et al* [8]. We found that the cefadroxil was not hydrolysed by the cefadroxil sensitive and resistant strains obtained in this study. This suggests that mechanism(s) of resistance other than the inactivation of the drug might play a role in the cefadroxil resistance in *M. tuberculosis.*

In the past many cephalosporins and penicillins, which were approved for clinical use, were screened for their activity against *M. tuberculosis* H37Rv[9]. Over 600 derivatives of cephalosporin C were screened for activity against *M. tuberculosis* H37Rv and their structure-activity relationships were studied by Misiek *et al* [10]. As a consequence of the poor activity displayed by these compounds, *M. tuberculosis* is generally not included in the screening of new derivatives of cephalosporins for antimycobacterial activity. However, availability of many beta-lactamase resistant cephalosporins prompted many workers to screen them against mycobacteria including *M. tuberculosis.* Heifets *et al* [11] reported that cephalosporins such as ceforanide, ceftozoxime, cephapirin and cefataxime showed promise in their activity against *M. tuberculosis* while, cefamandole and cephalothin were ineffective.

In the present study about 60 per cent of the clinical isolates of *M. tuberculosis* were inhibited at a concentration of the drug which is less than the peak plasma concentration attained in human beings and about 50 per cent of the drug resistant isolates were susceptible to this drug. In view of these findings and considering the good pharmacokinetic characteristics of cefadroxil, *in vitro* studies on synergestic action against *M. tuberculosis* of cefadroxil with other antitubercular drugs, isoniazid and rifampicin, and *in vivo* studies using mouse and guineapig models might throw some insight into the suitability of cefadroxil in the treatment of tuberculosis.

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