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IN VITRO ACTIVITY OF CAPREOMYCIN AND CIPROFLOXACIN AGAINST SOUTH INDIAN ISOLATES OF *M TUBERCULOSIS*

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Summary. Mycobacterium tuberculosis isolated from 107 south Indian patients, including 53 isolates sensitive to Streptomycin (S), Isoniazid (H) and Rifampicin (R) and 54 resistant to SHR/HR were tested for their *in vitro* susceptibility to Capreomycin and Ciprofloxacin. Of these, 3 (6%) SHR sensitive strains and 8 (15%) SHR/HR resistant strains were probably resistant to Capreomycin. However, the difference did not attain statistical significance. Considering Ciprofloxacin, the percentage distributions of the MIC with the different categories of strains were similar, there being no difference between sensitive and resistant strains, the geometric means being 3.7 and 3.8 mcg/ml, respectively.

Introduction

The conventional regimens for the treatment of pulmonary tuberculosis are less effective in patients with multiple drug-resistant organisms than in those harbouring fully sensitive strains[1]. The currently available alternative regimens for the treatment of patients with multiple drug resistance offer limited success besides being more toxic, more expensive and often resulting in the development of acquired resistance to the other drugs used[2]. For the treatment of patients with drug-resistant bacilli, several new classes of antituberculosis agents have been studied *in vitro* and *in vivo*. These include the aminoglycosides such as Amikacin and Capreomycin, long-acting Rifamycins (e.g. Rifapentine and Rifabutin), the 4-fluoroquinolones and combinations of β– lactum agents with β– lactamase inhibitors[3,4].

We report here the *in vitro* activity of an aminoglycoside, Capreomycin and a fluoroquinolone, Ciprofloxacin on south Indian isolates of *M. tuberculosis*, sensitive and resistant to Streptomycin, Isoniazid and Rifampicin, the drugs frequently used in short course regimens.

Material and Methods

Cultures : A total of 107 *M. tuberculosis* strains isolated from as many patients were tested. These comprised 53 isolates sensitive to Streptomycin (S), Isoniazid (H) and Rifampicin (R), and 54 resistant to SHR or HR. The standard sensitive strain *M. tuberculosis* H37Rv was tested on six occasions.

Drug concentrations : Capreomycin and Ciprofloxacin were dissolved in water and incorporated in Lowenstein-Jensen (L-J) medium to give pre-inpissation concentrations of 1, 2, 4, 8, 16, 32 and 64 mcg/ml.

Sensitivity testing : A standard bacterial suspension (4 mg/ml) of the cultures, which were given code numbers to conceal their identity, was inoculated with a 3mm loop on to 2 drug-free slopes and one slope each with the different concentrations of the drugs. All slopes were incubated at 37°C and at the end of 4 weeks of incubation, the minimal inhibitory concentration (MIC) was determined using the 20-colony end point[5].

Results

MIC for Capreomycin : The standard strain, *M. tuberculosis* H37Rv, tested on 6 different occasions, gave an MIC of 16 mcg/ml on 2

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occasions and an MIC of 32 on 4 occasions for Capreomycin, the geometric mean MIC being 25.4 mcg/ml (Table 1).

The distributions of MICs for Capreomycin for the strains from patients showed that the percentages of sensitive and resistant strains with MICs of 16, 32 and 64 were fairly similar. However, 3(6%) of 53 SHR sensitive strains had an MIC of > 54 as against 8 (15%) of 54 SHR/HR resistant strains, the difference not being statistically significant. The geometric mean MICs for SHR sensitive and SHR/HR resistant strains were 36.5 and 41.4 mcg/ml respectively, the overall mean being 38.9 mcg/ml.

MIC for Ciprofloxacin : The MIC of Ciprofloxacin for *M. tuberculosis* H37Rv was 2 mcg/ml on all 6 occasions tested (Table 2). The percentage distributions of the MICs with the different categories of strains were very similar, there being no difference between sensitive and resistant cultures, and the geometric mean MICs being 3.7 and 3.8 mcg/ml, respectively. These observations suggest the probability of non-existence of cross-resistance between Ciprofloxacin and Streptomycin, Isonazid or Rifampicin.

Discussion

Although primary resistance to Rifampicin in most countries is rare at present, it may become more prevalent because of the increasing use of Rifampicin containing regimens[4]. A study from Gujarat has reported an alarming increase in

Table 1 *Minimal inhibitory concentrations of Capreomycin against south Indian isolates of* M. tuberculosis

Category of strain	No. tested	MIC* of Capreomycin (mcg/ml)							
		16		32		64		>64	
		No.	%	No.	%	No.	%	No.	%
Standard (H37Rv)	6	2	–**	4	–	6	–	0	–
SHR Sens.	53	6	*11*	34	*64*	10	*19*	3	*6*
SHR/HR Res.	54	6	*11*	30	*56*	10	*19*	8	*15*
Total patients	107	12	*11*	64	*60*	20	*19*	11	*10*

* No strain had an MIC of less than 16.

** A dash(-) indicates that no percentages is presented because the total is less than 10.

Table 2 *Minimal inhibitory concentrations of Ciprofloxacin against south Indian isolates of* M. tuberculosis

Category of strain	No. tested	MIC* of Ciprofloxacin (mcg/ml)							
		<1		2		4		8	
		No.	%	No.	%	No.	%	No.	%
Standard (H37Rv)	6	0	–	6	–**	0	–	0	–
SHR Sens.	53	2	*4*	12	*23*	29	*55*	10	*19*
SHR/HR Res.	54	1	*2*	12	*22*	31	*57*	10	*19*
Total patients	107	3	*3*	24	*22*	60	*56*	20	*19*

acquired Rifampicin resistance from, 2.8% in 1980 to 37.3% in 1986, 95% of the patients being resistant to Streptomycin, Isoniazid or both[6]. In view of the possibility of an increase in Rifampicin resistance among tuberculosis patient;. there is a felt need for investigations into newer anti-tuberculosis drugs[4].

A recent study done at this Centre on the activity of Rifapentine and Rifabutin on Rifampicin resistant strains revealed complete cross resistance between Rifampicin and Rifapentine while a partial cross resistance (78%) was observed between Rifampicin and Rifabutin *in vitro*[7].

Although Amikacin has been shown to be effective against *M. avium-intracellullare* complex, Allen and others have concluded that the activity of this drug against *M. tuberculosis* is very low and, as such, it might not be effective in the treatment of disease caused by *M. tuberculosis.*

No cross-resistance has been reported between Streptomycin and Capreomycin[8,9,10]. Very little information is available on the activity of Capreomycin on Rifampicin resistant strains. Our study has shown that 6% of SHR susceptible strains as against 1.5% of SHR/HR resistant strains had an MIC of >64 for Capreomycin. The strains with MIC of >64 could probably be considered as resistant to Capreomycin, adopting the same criteria as for Kanamycin resistance[5]. This observation suggests a role for Capreomycin in the treatment of patients with multiple drug-resistant organisms.

Interest in quinolones has grown during the last decade owing to the development of fluorinated carboxylic acid derivatives such as Norfloxacin, Perfloxacin, Ofloxacin, Enoxacin and Ciprofloxacin. These derivatives possess a broad spectrum of activity against drug resistant bacteria in general, including drug-resistant mycobacteria[11,12]. Ciprofloxacin is one of the quinolones with the lowest MIC against *M. tuberculosis*[13] and is more active than Norfloxacin and Enoxacin[12,14]. No cross-resistance has been reported between the fluoroquinolones and other anti-TB drugs[1].

The present study on 107 strains showed no significant difference in the activity of Ciprofloxacin between SHR sensitive and resistant strains, the mean MICs being 3.7 and 3.8 mcg/ml, respectively. This value agrees well with other reports on the activity of Ciprofloxacin in L-J medium[14,15,16]. The peak serum concentration of Ciprofloxacin with conventional oral doses of 500-750 mg ranges between 2 and 4 ug/ml. It may be observed that the mean MIC of Ciprofloxacin is slightly below the maximum peak serum level. Indeed, several authors have reported that the MIC of fluoroquinolones is only slightly below the peak blood levels attainable with therapeutic doses of the drug [1,17,18].

However, in pulmonary tissue the drug may attain levels in excess of those in serum which may be adequate to inhibit growth of strains with resistance to one or more primary drugs[7]. For example, Ciprofloxacin levels obtained in the prostatic tissue are 2-14 times greater than serum levels[19]. In the light of the encouraging *in vitro* finding, it appears that Ciprofloxacin might be a useful drug in the treatment of multi-drug resistant tuberculosis, if used in judicious combination with other drugs.

However, the importance of these drugs in the chemotherapy of tuberculosis including its role in the treatment of patients with multi-drug resistance can be assessed only after well-planned controlled clinical trials.

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