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IN VITRO ACTIVITY OF RIFAMPICIN, RIFAPENTINE AND RIFABUTIN AGAINST SOUTH INDIAN ISOLATES OF *MYCOBACTERIUM TUBERCULOSIS*

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Summary. *M. tuberculosis* isolates from 51 resistant and 52 susceptible to Rifampicin patients were concurrently tested for *in vitro* susceptibility to Rifampicin, Rifapentine and Rifabutin. All the 52 Rifampicin susceptible strains were susceptible to Rifapentine, the geometric mean MICs being 13.3, ug/ml for Rifampicin and 6.0 ug/ml for Rifapentine. However, the geometric mean MIC for Rifabutin was as low as 1.3 ug/ml.

All 51 Rifampicin resistant strains were also resistant to Rifapentine, indicating a complete cross-resistance between the two compounds. However, 11 (22%) of the Rifampicin resistant strains were found to be susceptible to Rifabutin. The geometric mean MICs of the 40 resistant strains were 223 ug/ml for Rifampicin and 113 ug/ml for Rifabutin. Thus, even among Rifampicin resistant strains, Rifabutin showed a 1.97 fold higher activity; the difference in the means attained statistical significance.

Introduction

Although pre-treatment resistance to Rifampicin is not common in most countries at present, it may become more prevalent because of the increasing use of Rifampicin containing regimens for the treatment of tuberculosis[1].

Rifampicin is being freely used in recent years in India for the treatment of tuberculosis and leprosy by Government institutions under the respective national programmes for control of these diseases. And also by general practitioners as well as voluntary organisations. Patients who take treatment irregularly because of poor case-holding or who do not respond to treatment with Rifampicin containing regimens are likely to have developed resistance to Rifampicin. In a study in Gujarat, an alarming increase in acquired resistance to Rifampicin, from 2.8% in 1983 to 37% in 1986 was reported[2]. Further treatment of such patients with Rifampicin resistant organisms poses a problem since the currently available reserve drug regimens are not very effective in these patients, besides being expensive and highly toxic. The long-acting Rifamycin derivatives, such as Rifapentine (Cyclopentyl Rifamycin, MDL 473) and Rifabutin (Spiro-piperidyl Rifamycin, ansamycin, LM 427) are considered superior to Rifampicin by some, based on *in vitro* investigations[3, 4, 5, 6]. Other workers have reported the activity of Rifapentine to be similar to that of Rifampicin[7,8].

A study was, therefore, undertaken for a concurrent comparison of susceptibility to Rifampicin, Rifapentine and Rifabutin, *in vitro*, of south Indian isolates of *M. tuberculosis* susceptible as well as resistant to Rifampicin.

Material and Methods

Cultures : M. Tuberculosis isolates from 103 patients (51 strains resistant to Rifampicin and 52 strains susceptible to Rifampicin) were precoded and tested.

Drug concentrations : Rifampicin, Rifapentine and Rifabutin were each dissolved in dimethyl formamide and incorporated in Lowenstein-Jensen medium to give final pre-inspissation concentrations of 1, 2, 4, 8, 16, 32, 64 and 128 ug/ml.

Susceptibility testing : A standard bacterial suspension (4 mg/ml) of each isolate was inoculated (with a 3 mm internal diameter loop)

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on 2 drug-free slopes and one slope with each concentration of the drugs. All the slopes were incubated at 37°C, The order of inoculation of the 3 drug-containing slopes was randomised. Readings were taken at the end of 4 weeks of incubation. The Minimal Inhibitory Concentration (MIC) was determined by using the 20-colony end point[10].

Definition of resistance : A strain was considered to be resistant to Rifampicin or the other two derivatives if it yielded a growth of 20 colonies or more on 64 ug/ml.[10]

Results

Rifampicin susceptibility : Of the 103 isolates tested, 51 were found to be resistant to Rifampicin (MIC > 128), confirming the earlier test results at the time of intake.

Rifampicin susceptible strains

Activity of Rifapentine : All the 52 Rifampicin susceptible strains were found susceptible to Rifapentine also (Table 1). The pattern of MIC values showed Rifapentine to be more effective. Thus, out of the 52 susceptible strains, 9 had identical MICs, 2 had a higher MIC for Rifapentine by 1 dilution (in the lower 2 and 4 ug/ml Rifapentine concentrations), while 41 had a lower MIC for Rifapentine. Considering the MICs of these 41 strains, 26 were lower by 1 dilution, 10 by 2 dilutions, 4 by 3 dilutions and 1 by 4 dilutions. Thus, in 15 out of the 52 strains, Rifapentine showed at least 4 fold higher effectiveness than Rifampicin. The geometric mean MIC of the 52 strains was 13.3 ug/ml for Rifampicin and 6.0 ug/ml for Rifapentine, showing a 2.2 times higher effectiveness with Rifapentine (95% confidence interval : 1.8-2.7). The difference between the mean MTCs, tested after carrying out a logarithmic transformation, was found to be highly significant statistically ($P < 0.001$).

Activity of Rifabutin : Among the 52 Rifampicin susceptible strains, 2 strains had an MIC identical with Rifampicin and the remaining 50 showed a lower MIC for Rifabutin. Of the latter, the MICs of 8 strains were lower by 1 dilution; 1 by 2 dilutions, 12 by 3 dilutions, 16 by 4 dilutions and 13 by 5 dilutions (Table 2). Thus, in 42 out of the 52 strains, Rifabutin showed at least 4 fold higher effectiveness than Rifampicin. The geometric mean MIC of the 52 strains was 1.3 ug/ml with Rifabutin compared to 13.3 ug/ml with Rifampicin, showing an average of 10.2 fold higher effectiveness (95% confidence interval : 7.7-13.8). The difference between the mean MICs was highly significant statistically ($P < 0.001$).

Rifampicin resistant strains

Activity of Rifapentine : All the 51 Rifampicin resistant strains were resistant to Rifapentine also, indicating a cross-resistance between the two compounds. Of these, 47 had identical MICs, while 2 strains had an MIC 1 dilution higher with Rifapentine and the remaining 2 had an MTC 1

Table 1 *MICs of Rifampicin and Rifapentine for* M. tuberculosis *isolates*

Rifampicin MIC (ug/ml)	Rifapentine MIC (ug/ml)									
	< 1	2	4	8	16	32	64	128	> 128	Total
< 1	1	1								2
2	2	3	1							6
4		1	1							2
8			6	1						7
16		1	1	9	2					13
32		1	3	9	8					21
64							1			1
128								8	2	10
> 128								2	39	41
Total	3	7	12	19	10		1	10	41	103

Table 2 *MIC of Rifampicin and Rifabutin for* M. Tuberculosis *isolates*

Rifampicin MIC (ug/ml)	Rifabutin MIC (ug/ml)									
	< 1	2	4	8	16	32	64	128	> 128	Total
< 1	2									2
2	6									6
4	1	1								2
8	7									7
16	10	3								13
32	13	6	2							21
64						1				1
128		1		4			2	3		10
>128							3	18	19	41
Total	39	11	2	4		1	6	21	19	103

dilution higher with Rifampicin (Table 1). Thus, among rifampicin resistant strains, Rifapentine did not show increased effectiveness.

Activity of Rifabutin : With Rifabutin, 11 (22%) of the 51 strains were susceptible. Of the remaining 40 strains, 22 had an identical MIC while in 18 the MIC was 1 dilution lower with Rifabutin (Table 2). The geometric mean MIC of the 51 strains was 223 ug/ml with Rifampicin compared to 113 ug/ml with Rifabutin. For the purpose of calculating the means, the MIC of >128 was taken as 256. Thus, among Rifampicin resistant strains, Rifabutin showed a 1.97 fold higher effectiveness (95% confidence interval : 1.5-2.6). The difference in the mean MICs attained high statistical significance ($P < 0.001$).

Discussion

Rifapentine and Rifabutin have been considered to be more effective than Rifampicin in Rifampicin susceptible strains based on *in vitro* investigations. Arioli et al[3] reported Rifapentine to be 2-10 fold more effective than Rifampicin in Kirchner's liquid medium while Dickinson and Mitchison[9] found a 4 fold higher activity in 7H10 agar. However, Yates and Collins[8] found the two compounds to have similar activity on LJ medium. Similar findings were also reported by Truffot et al[7]. The differences could possibly be due to protein binding as it is known that the Rifamycins bind rapidly to proteins and the binding capacity is higher for Rifapentine than for Rifampicin.[11] Unpublished findings from this Centre showed that MICs of Rifapentine and Rifampicin were five to six fold lower in 7H11 agar than on LJ medium.

Our investigation has shown Rifabutin to be more effective than Rifapentine : 41 of 52 (79%) Rifampicin susceptible strains were more susceptible by three or more dilutions to Rifabutin over Rifampicin while the corresponding proportion for Rifapentine was only 10% (5/52). Besides, a small proportion of strains resistance to Rifampicin *in vitro* were found susceptible to Rifabutin : 22% in our study, 36% of 44 by Woodley and Kilburn[6], 11% of 37 by Hawkins et al[5] and 31% of 35 by Mitchison et al[1].

The characteristic of Rifabutin as indicated by the manufacturers is that concentration of the drug in tissues is several times higher than in serum (0.39 ug/ml after 300 mg oral dose), the serum level being approximately 10 times lower than that with the same dose of Rifampicin[12]. The serum half-life of Rifabutin is 16 hours[13] and protein binding is only 25% of that for Rifampicin[14].

With increase in Rifampicin acquired resistance, Rifabutin may be useful for treating patients with Rifampicin resistant organisms. The longer half-life of Rifabutin could possibly delay the emergence of resistance in patients who are resistant to the companion drugs and probably make it particularly suitable for intermittent administration. This could be validated only after well-planned controlled clinical trials conducted

to assess the activity of Rifabutin in Rifampicin resistant tuberculosis.

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