Indian J Med Res 100, July 1994, pp 1-4

Early bactericidal action of pulsed exposure to rifampicin, ethambutol, isoniazid & pyrazinamide in pulmonary tuberculosis patients

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Accepted April 26, 1994

The bactericidal action of two therapeutic regimens on *Mycobacterium tuberculosis* was assessed by viable counts in serial sputum samples in 49 pulmonary tuberculosis patients being treated with rifampicin (R), etbambutol (Emb), isoniazid (I) and pyrazinamide (Z) together in a single dose thrice weekly ($REmbIZ_3$) or with REmb and IZ on alternate days ($REmb_3IZ_3alt$). In both groups of patients, there was a significant reduction ($P \leq 0.02$) in the colony forming units (cfu) of *M. tuberculosis* per ml of sputum during the first two days of treatment itself. This early bactericidal action (EBA) as well as the reduction in counts during the subsequent days of treatment were similar ($P > 0.2$) for both $REmbIZ_3$ and $REmb_3IZ_3alt$ regimens indicating that splitting up REmbIZ into REmb on one day and IZ on the next day in short course chemotherapy (SCC) regimens may not affect the bactericidal action of the regimens.

Key words Early bactericidal activity - pulsed exposure - short course chemotherapy

In most of the short course chemotherapy (SCC) regimens of 6-8 months' duration, four drugs, *viz.*, rifampicin (R), isoniazid (I), pyrazinamide (Z) and streptomycin (S) or ethambutol (Emb) are given together, usually for two months initially, in a single dose either daily or intermittently[1]. Since the amount of the drugs to be consumed by the patients in a single dose is large in such regimens, patient compliance may be adversely affected. Further, murine studies have suggested that there could be antagonism between I and R when these two drugs are given together most probably due to pharmacological interaction[2,3]. These interactions could be overcome by splitting the 4 drugs into: two, 2-drug combinations (REmb & IZ), giving each combination on alternate days, thus making each 2-drug combination intermittent. A controlled clinical trial is in progress at the Tuberculosis Research Centre, Madras to study whether administration of the two 2-drug combinations on alternate days would be as effective as all 4 drugs given together.

In an earlier study, using an *in vitro* system, we found that R, Emb, I and Z together thrice weekly had as good early bactericidal action (EBA) on *Mycobacterium tuberculosis* strains as REmb and IZ on alternate days[4]. Although *in vitro* models[5,6], macrophage systems[7-9] and murine tuberculosis models[10] can be used to study the bactericidal action of such regimens on *M. tuberculosis*, it would be more appropriate to study this in patients with pulmonary tuberculosis[11].

The present study was undertaken in patients being treated for pulmonary tuberculosis to determine the bactericidal action on *M. tuberculosis* when REmb and IZ are given on alternate days, in comparison with REmbIZ given together thrice weekly, by carrying out viable counts in serial sputum samples.

Material & Methods

Study subjects : Adult, sputum culture positive, pulmonary tuberculosis patients with no history of previous treatment were included in the study. Patients with drug-resistant organisms initially were excluded.

Regimens : The patients were randomly allocated to receive R, Emb, I and Z together in a single dose thrice weekly ($REmbIZ_3$), or REmb and IZ on alternate days ($REmb_3IZ_3alt$). From the total number of patients randomly allocated to the two regimens, those with negative cultures on day 0 (before start of treatment), and those who failed to attend or whose cultures were contaminated at any of the subsequent time points (days 2, 4, 9 and 16 after starting treatment) were excluded from the analysis leaving 21 patients in the $REmbIZ_3$ group and 28 in the $REmb_3IZ_3alt$ group.

The dosages were the same for both the regimens, namely, rifampicin 450 mg, ethambutol 1000 mg and pyrazinamide 1.5g for patients weighing 40.0 kg or less, and 600 mg, 1200 mg and 2.0 g, respectively, for patients weighing 40.1 kg or more. The dosage of isoniazid was 600 mg, irrespective of body weight.

Sputum collection : Sputum was collected overnight in sterile wide mouth containers by the patients and brought to the Centre on day 0 (before start of treatment) and on days 2, 4, 9 and 16 after starting treatment. The samples were given code numbers to conceal their identity before they were processed in the laboratory.

Sputum viable count : The number of colony forming units (cfu) of *M. tuberculosis* per ml of sputum sample was determined following the method of Jindani *et* al[11]. Briefly, within 1 h of receiving a coded sample in the laboratory, it was homogenized by shaking first with large glass beads and then with 10 per cent dithiothreitol (Sputolysin). The mixture was centrifuged at 3000 rpm for 1.5 min, and the deposit resuspended in sterile distilled water. From this, viable counts were set up on 7H11 medium plates made selective with polymyxin B, carbenicillin, trimethoprim and amphotericin B[12]. At the end of 4 wk of incubation, the number of colonies in the plates was counted using a colony counter and the cfu per ml of neat sputum was obtained.

Table I. Mean $\log_{10}$ cfu/ml of *M. tuberculosis* in serial sputum samples of patients treated with $REmbIZ_3$ and $REmb_3IZ_3alt$

Treatment	Mean $\log_{10}$ cfu/ml ± SD on (day)				
	0	2	4	9	16
$REmbIZ_3$ (n=21)	6.99±1.64	5.58±1.65	4.59±2.13	3.17±1.91	2.79±1.77
$REmb_3IZ_3alt$ (n=28)	6.29±2.08	5.39±2.05	4.89±1.83	3.581±1.98	2.76±1.60

Using the methods described above, in another experiment, cfu/ml was determined in sputum samples collected from 19 untreated pulmonary tuberculosis patients on two consecutive days to find out the intrapatient variation.

Analysis of data : The mean $\log_{10}$ cfu/ml of *M. tuberculosis* in the sputum samples in the two groups of patients before start of treatment (day 0) and on days 2, 4, 9 and 16 during treatment were calculated. In less than 10 per cent of the occasions there were no detectable colonies in the viable count. To facilitate handling of data, a count of 1 was uniformly added resulting in a log value of 0 for a negative culture. The change per day in 0-2 days and 2-16 days were computed for each patient and an RBD analysis undertaken in patients on each of the two regimens. The difference between the two regimens in the differences was also tested using a t-test for independent observations.

Results

The mean $\log_{10}$ cfu/ml of *M. tuberculosis* in the sputum samples of the two groups of patients ($REmbIZ_3$ and $REmb_3IZ_3alt$) before start of treatment (day 0) and on days 2, 4, 9 and 16 during treatment are presented in Table I. In the two groups of patients, the log,, cfu/ml in sputum on day 0 were fairly similar, *viz.*. 6.99 and 6.29, respectively ($P > 0.2$).

In the $REmbIZ_3$ group, on day 2, after one dose of $REmbIZ_3$ the mean count became 5.58. This reduction was significant ($P < 0.01$). By day 16 the count reached 2.79. Similarly, in the $REmb_3IZ_3alt$ group, by day 2, after the patients had received one dose of REmb and one dose of IZ, the mean count became 5.39, and this reduction was also significant (P=0.02). By day 16, the count was reduced to 2.76.

In the $REmbIZ_3$ regimen, the early bactericidal action (EBA), estimated as the change in $\log_{10}$ cfu/ml/day during the first two days (0-2 days) of treatment was 0.71 (Table II), and the rate of reduction in counts during the subsequent days (2-16 days) was 0.20; the former was significantly higher than the latter ($P < 0.05$). The corresponding values in the $REmbIZ_3$alt regimen were 0.45 and 0.19, and were not significantly different ($P > 0.1$). The EBA of $REmb_3IZ_3$alt regimen (0.45) was not significantly different from that of $REmbIZ_3$ regimen (0.71) (P=0.36), and the reduction in counts during the subsequent days (0.19 and 0.20, respectively) was very similar. These results indicated that the bactericidal action on *M. tuberculosis* in pulmonary tuberculosis patients for $REmbIZ_3$ and $REmb_3IZ_3$alt regimens may not be different.

In the experiment carried out to determine intrapatient variation, the $\log_{10}$ cfu/ml of *M. tuberculosis* in two consecutive sputum samples from 19 untreated pulmonary tuberculosis patients were 7.21 (SD 1.49, range 4.08 - 8.74) and 7.38 (SD 1.57, range 3.28 - 9.04), respectively. By using ANOVA it was seen that the variation between patients was substantially greater (SD 2.12) than the variation between duplicate samples from the same patients (SD 0.44) providing evidence of consistent differences between patients.

Discussion

The results of this study in tuberculosis patients support the preliminary evidence obtained by us from an earlier *in vitro* study[4]. In the *in vitro* experiment, the bactericidal action of pulsed exposure to R, Emb, I and Z together thrice weekly and split up into REmb and IZ on alternate days was studied using cultures of *M. tuberculosis* H37Rv, 2 isolates of *M. tuberculosis* sensitive to these drugs as well as 4 isolates resistant to one or more drugs. Both the treatment procedures had equally good bactericidal action on *M. tuberculosis* strains in the *in vitro system* and the differences were not statistically significant ($P > 0.1$). In the present *in vivo* study also $REmbIZ_3$ and $REmb_3IZ_3$alt had similar ($P > 0.3$) early bactericidal action. The EBA of the $REmb_3IZ_3$alt regimen was not smaller than that of the $REmbIZ_3$ regimen.

Table II. Mean change in $\log_{10}$ cfu/ml/day of *M. tuberculosis* in sputum of patients treated with $REmbIZ_3$ and $REmb_3IZ_3$

Treatment	Mean change in $\log_{10}$ cfu/ml/day±SD between days		
	0-2	2-16	0-16
$REmbIZ_3$ (n=21)	0.71±0.95	0.20±0.17	0.26±0.11
$REmb_3IZ_3$alt (n=28)	0.45±0.93	0.19±0.15	0.22±0.15
P =	0.36	0.83	0.29

There was also no evidence of increased EBA of $REmb_3IZ_3$alt regimen compared to the $REmbIZ_3$ regimen, excluding any antagonism between I and R in EBA in the present study. In the murine model experiments carried out by Grosset and associates,[2,3] the bactericidal activities of IRZ and IR were similar whereas that of RZ was significantly greater. The relapse rate was significantly smaller in the group of mice treated with RZ in the continuation phase than in the groups treated with IRZ (or IR). These results in the murine model had suggested that antagonism occurred between I and the combination RZ. In the present study, no antagonism could be seen in the EBA; whether it would be reflected in the sterilizing effect and relapse rates of the regimens remains to be seen.

The reduction in the rate of elimination of viable bacilli after the first week of treatment seen in the study by Jindani *et al*[11] was observed in the present investigation also. As observed by them, the early faster rate of elimination is probably determined by the inherent bactericidal activity of the drug regimens against the large number of actively growing *M. tuberculosis* in the cavitary walls which are comparable to logarithmic phase cultures *in vitro,* whereas the later slower rate is determined by the almost dormant physiologic state of the surviving organisms independent of the drug regimens employed.

The standard deviation for EBA (change in log,, cfu/ml/day between days 0 and 2) in the present study was 0.95 with the $REmbIZ_3$ regimen and 0.93 with the $REmb_3IZ_3$alt regimen (coefficient of variations being 134 and 207%, respectively) which were much larger than those observed in similar studies

with other antituberculosis drugs and combinations carried out by others in Nairobi[11] and in Hong Kong[13]. This variation was 0.19 (60 d.f.) in the Nairobi study and 0.38 (84 d.f.) in the Hong Kong study. It was considered by the latter group of workers[13] that the larger variation between Hong Kong patients than between the Nairobi patients could be due to characteristics of the pulmonary lesions in these patients. The much larger variation seen in the present study is due to the gross heterogeneity in the response to treatment in the patients in our study. Because of the large standard deviations observed in the present study, even a decreased activity of 36 per cent with the $REmbI_3IZ_3$alt regimen in the first 2 days was not found to be statistically significant. A computation of 'post-facto power' calculation revealed that the power of the test with the present sample size was less than 50 per cent. A similar finding was noticed also between the two regimens in the reduction between 2-4 days, again due to the large standard deviations.

To summarise, the results of the present study suggest that splitting up REmbIZ into REmb on one day and IZ on the next day in SCC regimens may not affect the EBA of the regimens. On the other hand, this will help in reducing the bulk of the drugs to be consumed and may help in compliance. Treatment of patients with these two regimens is in progress at this Centre and the results of this study will reveal whether splitting up REmbIZ into REmb and IZ will help in reducing adverse reactions. Further, EBA is not an indicator of the sterilising ability of regimens. The sterilising ability and effectiveness of a regimen in chemotherapy can be estimated only based on sputum conversion rates and relapse rates[11] which will soon be available from the on-going clinical trial at the Centre.

Acknowledgment

The authors thank Prof. D.A., Mitchison for his critical observations on the results of this study, Sh. P.R. Somasundaram for his help in the preparation of this paper, Dr P. Venkatesan for helping with the statistical analysis, Smt. Alamelu Narayanan, Sh. K.J. Ilampurnan and Smt. Gomathy Samuel for technical assistance and the Clinic and Nursing Staff of Tuberculosis Research Centre. Madras, for organising the sputum collection and drug administration.

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