SERUM CONCENTRATIONS OF RIFAMPICIN AND ISONIAZID IN TUBERCULOSIS

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ABSTRACT

Ninety four patients, 1-13 years of age suffering from different types of tuberculosis were investigated for serum rifampicin (RIF) and isoniazid (INH) concentrations using microbiological and fluorimetric methods, respectively. Of these, 64 (68.1%) had pulmonary primary complex (PPC); 20 (21.3%) progressive primary disease (PPD) and 10 (10.6%) tuberculous meningitis (TBM). Patients with PPC, PPD and TBM were given two-drug (6HR), three drug (2HRZ, 4HR) and four drug (2SHRZ, 4HRE, 3HE) regimens, respectively. RIF and INH were administered in a dose of 12 and 10 mg/kg/day, respectively. After 10-12 days of continuous therapy, their serum concentrations were estimated at 0, 2, 4, 6, 8 hours for RIF and 0, 1, 3, 5, 7 hours for INH. For RIF, the time to achieve maximum concentrations (T_{max}) was 2 hours, range of mean of maximum concentration (C_{max}) 3.38 to 3.88 μg/ml, terminal half life elimination (T½) 3.03 to 3.81 hours and area under serum concentration curve (AUC) 0-8 hours 24.7 to 28.3 μg/ml hours in different forms of tuberculosis. INH had a T_{max} of 1 h, C_{max} 4.38 to 8.17 μg/ml, $T_{½}$ 4.0 to 4.98 hours and AUC 0-7 hours 34.1 to 57.5 μg/ml hours. The concentrations achieved at 7-8 hours with these dosages were much above those required for therapeutic efficacy (minimum inhibitory concentration), being 50 to 250 times for RIF and 35-60 times for INH. We recommend pharmacokinetic studies with lower doses of RIF and INH for less toxic, equally effective and cheaper antitubercular chemotherapy.

Key words: *Rifampicin, Isoniazid, Tuberculosis.*

Isoniazid, rifampicin and pyrazinamide are the commonly used drugs for the treatment of tuberculosis in children and form an important part of short course regimens. However, the problem of hepatotoxicity and cost is significant, particularly when they are used in higher dosage. The prescribed dosages of isoniazid and rifampicin in children are usually in the range of 10-20 mg/kg/day with toxic reactions occurring when the dosages are on the higher side-15-20 mg/kg/day(1,2). Also, the serum and tissue concentrations achieved are relatively high as dosage given on weight basis is almost double in children as compared to adults(3,4). Thus logically, by using lower dosages it should be possible to achieve the desired serum and tissue drug concentrations at lesser cost and toxicity. However, these dosage recommendations are solely based on clinical impressions and not on pharmacokinetic studies. Very few such studies are available in children(5,6). Seth *et al.*(7,8) and Beotra *et al.*(9) have undertaken pharmacokinetic studies in tuberculosis in Indian children.

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Received for publication: November 22, 1991; Accepted: February 18, 1993

The present study was designed to estimate the serum concentrations of rifampicin and isoniazid in different types of tuberculosis incorporating different drug-regimens.

Subjects and Methods

Ninety four patients of both sexes in the age group 1-13 years were enrolled for the study from the Pediatric Tuberculosis Clinic and the indoor pediatric wards of the All India Institute of Medical Sciences, New Delhi. Those patients were selected who did not have any other severe systemic disease involving hepatic and renal systems in particular, and those who did not receive any antitubercular drugs in past. Except for the TBM patients who were receiving anticonvulsant and decongestant therapy, all others did not receive any other drug 7 days prior to the commencement of pharmacokinetic study. They were diagnosed as suffering from the three different types of tuberculous disease commonly seen, representing the complete spectrum of severity of disease in children–the pulmonary primary complex (PPC) representing the mild type, tuberculous meningitis (TBM) the severe type, while progressive primary disease (PPD) the intermediate or moderate type. Of these, 64 (68.1%) belonged to PPC group, 20 (21.3%) to PPD and 10 (10.6%) had TBM (*Table I*). Patients with PPC were given two-drug containing regimen 6HR where isoniazid (H) and rifampicin (R) were given dialy for 6 months. Three-drug regimen 2HRZ, 4HR was given to PPD patients where H, R and pyrazinamide (Z) were given daily in the first two months of the intensive phase of therapy followed by H and R daily for the next 4 months. Nine-month duration four-drug regimen 3SHRZ, 4HRE, 3HE was allocated to TBM patients, who received daily doses of streptomycin S, H, R and Z in the first 2 months, R and ethambutol (E) in the next 4 months, followed by H and E daily for rest of the 3 months of the therapy. The drug doses were calculated as mg/kg of body weight per day and were: H-10, R-12, Z-30, S-20 and E-20. Ethambutol was administered in this dose as Seth *et al.*(10) has shown no evidence of ocular toxicity in patients on ethambutol therapy. Proper administration of drug dosages and precise sampling of patients was ensured by admitting the study patients of Pediatric Tuberculosis clinic in Pediatric Ward for one day. Rifampicin was admini-

TABLE I–*Age and Sex Wise Distribution of Patients*

Disease Group	Age groups in years (Male-Female)									
	1-<3		3-<5		5-<10		10-13		Total	
	M	F	M	F	M	F	M	F	M	F
PPC (n=64)	-	-	6	3	22	16	6	11	34	30
PPD (n=20)	-	1	2	3	4	5	2	3	8	12
TBM (n=10)	4	3	1	1	1	-	-	1	6	4
Total (n=94)	4	3	9	7	27	21	8	15	48	46

stered orally on empty stomach followed one hour later by isoniazid by oral route. Food was withheld for three hours after drug intake. Due to hepatic enzyme induction property of rifampicin, blood sampling of patients was initiated only after a period of 10-12 days of starting therapy by which time the serum concentrations of the drugs stabilized. Seth *et al.*(7,8) and Beotra *et al.*(9) have established that peak serum concentration of rifampicin is attained at 2 hours and isoniazid at 1 hour of drug intake in Indian children with tuberculosis. This observation has helped designing the blood sampling-time for rifampicin and isoniazid in such a way as to achieve the maximum (peak) serum concentrations of both the drugs at the same sampling time to avoid collecting an extra blood sample for the purpose. Hence, isoniazid was administered after 1 hour of taking rifampicin so that 2 hour blood sample for rifampicin was also the one hour sample for isoniazid. Accordingly, blood samples were taken at 0 (or 24) hour, *i.e.,* just before administration of next due dose, at 2, 4, 6 and 8 hours of rifampicin intake which corresponded to 0 (or 23), 1, 3, 5 and 7 hours of isoniazid intake. For ethical reasons each child was subjected to blood-sampling only twice. The samples were centrifuged and rifampicin and isoniazid concentrations were estimated in serum by microbiological(11) and fluorimetric(12) methods, respectively.

Maximum (peak) serum concentration (C_{max}), time to attain maximum serum concentration (T_{max}), terminal half-life of elimination ($T_{½}$) and area under the serum concentration time curve (AUC) were the pharmacokinetic parameters calculated. AUC was calculated in the interval 0-8 hours for rifampicin and 0-7 hours for isoniazid according to the trapezoidal rule(13). This study was approved by the Ethics Committee of the All India Institute of Medical Sciences, New Delhi.

Results

Except for T_{max}, all other parameters have been expressed as mean values. T_{max} for rifampicin was observed at 2 hours and C_{max} ranged from 3.38 ± 0.95 µg/ml in PPD (2HRZ, 4HR) to 3.88 ± 0.78 µg/ml in PPC (6HR) group. Rifampicin concentration in serum at 0 hour (or 24 hours), *i.e.,* just before administration of the next due dose of the drug, ranged from 0.21 ± 0.11 µg/ml in TBM (2SHRZ, 4HRE, 3HE) to 0.40 ± 0.41 µg/ml in PPD group, $T_{½}$ from 3.03 to 3.81 hours and AUC 0-8 hours from 24.7 to 28.3 µg/ml hours in the different disease states (*Tables II & III, Fig. 1*). The T_{max} of isoniazid was seen at 1 hour and C_{max} ranged from 4.38 ± 1.89 to 8.17 ± 4.76 µg/ml in different types of tuberculosis. Isoniazid concentration at 0 hour (or 23 hours) ranged from 0.33 ± 0.38 to 0.84 ± 0.05 µg/ml, $T_{½}$ from 4.2 to 4.98 µg/ml and AUC 0-7 hours 34.1 to 57.5 µg/ml hours in different disease states (*Tables IV & V, Fig. 2*).

Discussion

For a drug to be effective its serum concentration must exceed its minimum inhibitory concentration (MIC). Serum rifampicin and isoniazid concentrations are a good basis for evaluation of efficacy of a rational antitubercular regimen. Unfortunately, in the pediatric population, the recommended doses of the antitubercular drugs are based not upon well designed pharmacokinetic studies, but rather on clinical observations. Animal studies and those in adults have shown that the serum and tissue concentrations achieved by oral administration of rifampicin and isoniazid are much above their MIC(14). Also, the concentrations

TABLE II–*Serum Rifampicin Concentration in Different Types of Tuberculosis and Regimens*

Type of tuberculosis and regimen	Serum rifampicin concentration (μg/ml) at different time intervals (hours) (Mean ± SD)				
	0	2	4	6	8
PPC					
6HR	0.26±0.3 (18)	3.88±0.78 (29)	2.58±0.72 (32)	1.43±0.69 (28)	1.02±0.87 (29)
PPD					
2HRZ, 4HR	0.40±0.41 (5)	3.38±0.95 (5)	2.37±0.72 (11)	1.26±1.03 (12)	1.24±0.90 (7)
TBM					
2SHRZ, 4HRE, 3HE	0.21±0.11 (4)	3.86±1.73 (5)	1.60±1.4 (4)	1.25±0.30 (3)	1.01±1.00 (3)

Figures in parentheses are number of samples;
PPC = Pulmonary primary complex; PPD = Progressive primary disease; TBM = Tuberculous meningitis; H = Isoniazid; R = Rifampicin; Z = Pyrazinamide; S = Streptomycin; E = Ethambutol.

TABLE III–*Pharmacokinetic Parameters of Rifampicin*

Type of tuberculosis and regimen	C_{max} (μg/ml) (mean)	T_{max} (hours)	$T_{1/2}$ (hours) (mean)	AUC 0-8hours (μg/ml hours) (mean)
PPC				
6HR	3.88	2.0	3.03	28.3
PPD				
2HRZ, 4HR	3.38	2.0	3.81	26.2
TBM				
2SHRZ, 4HRE, 3HE	3.86	2.0	3.24	24.7

PPC = Pulmonary primary complex; PPD = Progressive primary disease; TBM = Tuberculous meningitis; C_{max} = Maximum serum concentration; T_{max} = Time to achieve maximum concentration; $T_{½}$ = Terminal half-life of elimination; AUC = Area under serum concentration-time-curve; H = Isoniazid; R = Rifampicin; Z = Pyrazinamide; S = Streptomycin; E = Ethambutol.

achieved in children are higher because doses given on weight basis are almost double as compared to adults(3,4). The minimum tuberculostatic concentrations (MIC) for isoniazid and rifampicin are 0.025 to 0.05 μg/ml and 0.005 to 0.02 μg/ml, respectively. In the present study, the mean rifampicin serum concentrations ranged from 3.38 to 3.88 μg/ml 2 hours (peak levels) and from 0.21 to 0.4 μg/ml at 0 (or 24 hours) in

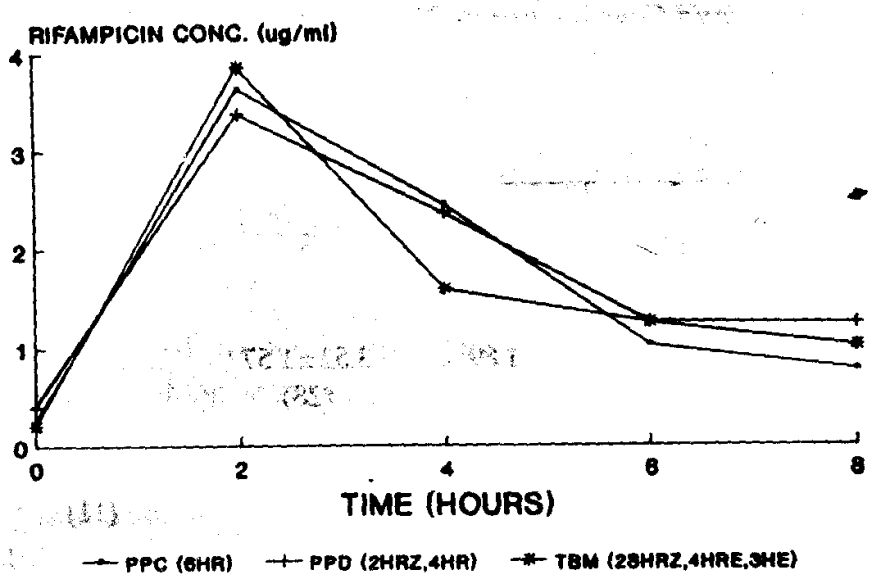

Fig. 1. Serum rifampicin concentrations (mean) in different types of tuberculosis.

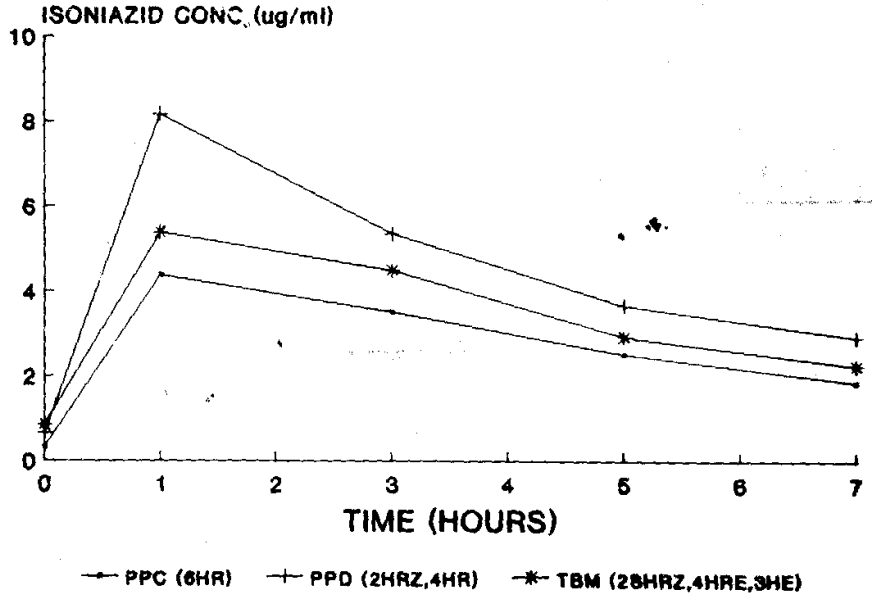

Fig. 2. Serum isoniazid concentrations (mean) in different types of tuberculosis.

different disease and regimen groups (*Table II*). These values are 170 to 200 times the MIC value of rifampicin at peak concentration time (2 hours) and 10 to 20 times the MIC at 0 or 24 hours of drug administration. Similarly, isoniazid concentrations (*Table IV*) are at least 90 to 160 times of its MIC at the peak concentration time (1 hour) and 7 to 17 times at 0 or 23 hours of drug administration in different disease and regimen groups. The higher C_{max} value of isoniazid seen to PPD group with three-drug regimen–2HRZ, 4HR as compared to regimens of other disease groups (*Fig. 2*) is attributed, in part, to the influence of pyrazinamide on the biotransformation of

TABLE IV–*Serum Isoniazid Concentration in Different Types of Tuberculosis and Regimens*

Type of tuberculosis & regimen	Serum rifampicin concentration (μg/ml) at different time intervals (hours) (Mean ± SD)				
	0	1	3	5	7
PPC					
6HR	0.33±0.38 (18)	4.38±1.89 (22)	3.52±1.57 (28)	2.51±1.22 (21)	1.86±1.04 (22)
PPD					
2HRZ, 4HR	0.65±0.18 (14)	8.17±4.76 (5)	5.36±3.09 (8)	3.66±2.63 (11)	2.92±1.82 (7)
TBM					
2SHRZ, 4HRE, 3HE	0.84±0.50 (4)	5.38±3.64 (8)	4.5±3.77 (5)	2.92±3.01 (4)	2.25±1.29 (5)

Figures in parentheses are number of samples.
PPC = Pulmonary primary complex; PPD = Progressive primary disease; TBM = Tuberculous meningitis; H = Isoniazid; R = Rifampicin, Z = Pyrazinamide; S = Streptomycin; E = Ethambutol.

TABLE V–*Pharmacokinetic Parameters of Isoniazid*

Type of tuberculosis & regimen	Cmax (μg/ml) (mean)	Tmax (hours)	T½(hours) (mean) (mean)	AUC 0-8hours μg/(ml hours)
PPC				
6HR	4.38	1.0	4.98	34.1
PPD				
2HRZ, 4HR	8.17	1.0	4.20	57.5
TBM				
2SHRZ, 4HRE, 3HE	5.38	1.0	4.55	43.8

PPC = Pulmonary primary complex; PPD = Progressive primary disease; TBM = Tuberculous meningitis; Cmax = Maximum serum concentration; Tmax = Time to achieve maximum concentration; $T_{1/2}$ = Terminal half-life of elimination; AUC = Area under serum concentration-time-curve; H = Isoniazid; R = Rifampicin; Z = Pyrazinamide; S = Streptomycin; E = Ethambutol.

isoniazid. Pyrazinamide suppresses the acetyl-isoniazid formation, thereby increasing the serum concentration of isoniazid in slow acetylators as observed by Vivien *et al.*(16) showed that majority of Indian children are slow acetylators for isoniazid inactivation. Even in the TBM group with four-drug regimen which includes streptomycin and pyrazinamide besides isoniazid and rifampicin, the isoniazid concentration seen

at 1 hour (Cmax) is more than that seen in PPC group with 6HR regimen. Since most patients of TBM also receive anticonvulsant and decongestant therapy and are malnourished, the effect of all or one of these factors on pharmacokinetics of antitubercular drugs is difficult to elucidate with the present study design. Streptomycin and isoniazid have been reported to have synergistic action(17). Thus, along with pyrazinamide streptomycin also, to some extent, exerts its influence on increasing the serum concentration of isoniazid.

Hobby(18) reported the effects of rifampicin in combination with other antitubercular drugs in his *in vitro* studies. He showed that antimycobacterial action of rifampicin may be enhanced, and the emergence of rifampicin-resistant cells prevented, by the addition of just subinhibitory (sub-MIC) amount of isoniazid, streptomycin or ethambutol. Naito *et al.*(19) found that rifampicin exerted some degree of synergism in combination with streptomycin, isoniazid, ethambutol or thiacetazone.

The serum half-life of rifampicin and isoniazid in the present study did not show significant difference among the different disease and regimen groups (*Tables III & V*). The AUC for rifampicin and isoniazid, indicative of the comparative bioavailability of the drug, corresponded with adequate serum concentrations achieved by both drugs. The observation substantiates the fact that serum concentrations achieved and bioavailability of both drugs are high in relation to their MIC when given in presently recommended doses.

Results of the present study show a sustained serum concentration much above the MIC, of both rifampicin and isoniazid even 24 hours after administration of a single dose of the drug when given as 12 mg and 10 mg/kg of body weight per day, respectively. These observations and those of Hobby(17) and Naito *et al.*(19) suggest that rifampicin and isoniazid pharmacokinetic studies should be undertaken in children with tuberculosis using these drugs in much lower but therapeutically effective doses. Necessity of similar studies on other antitubercular agents should not wisely be overlooked. The International Union Against Tuberculosis and Lung Disease has advocated daily dose of 5 mg/kg of isoniazid and 10 mg/kg of rifampicin in children in all forms of tuberculosis(20). Pediatric pharmacokinetic profiling is needed to authenticate this recommendation. Since drugs like rifampicin and pyrazinamide are rather expensive and drug-compliance a major problem in the developing countries, such an approach may significantly reduce the cost of therapy. Based on such studies, suitable low-cost antitubercular regimens should be formulated as applicable to children in developing countries.

Acknowledgement

The authors wish to express their gratitude to the Indian Council of Medical Research for financial support.

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