RESEARCH PAPER

Blood Levels of Pyrazinamide in Children at Doses Administered Under the Revised National Tuberculosis Control Program

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Objectives: To evaluate the blood levels, pharma-cokinetics and pharmacodynamic indices of pyrazinamide (PZA) in children suffering from tuberculosis, at doses administered under the weight band system of Revised National Tuberculosis Control Program of India (RNTCP) of India.

Design: Prospective, open-label, non-randomized single-dose study.

Setting: 20 children in the age group 5-12 years attending out-patient tuberculosis clinic of a tertiary hospital.

Outcome Measures: Blood levels of pyrazinamide after single dose administration, as per the weight band system of RNTCP.

Results: Group I (*n*=7) included children who received pyrazinamide within the recommended 30-35 mg/kg dose (mean 31.9±0.8 mg/kg) and Group II (*n*=13) included those who received a dose lower than 30 -35 mg/kg (mean 28.1±0.3 mg/kg). The C_{max} (95% CI of difference 2.2, 13.2; *P*=0.008) and AUC (95% CI of difference 28.6, 208.1; *P*=0.01) were significantly lower in Group II. The duration of time for which the concentration was maintained above 25 µg ml^{-1} was 4-8 h in Group I and 3-5.5 h in Group II (95% CI of difference 0.1, 2.0; *P*=0.03). The half life, elimination rate constant, clearance and volume of distribution were comparable in the two groups. The ratios of C_{max} and AUC to MIC (25 µg ml^{-1}) in children were lower than that recommended for PZA in adults.

Conclusions: Lower blood concentrations are being attained in children receiving PZA doses under the existing weight band system of RNTCP of India. The weight bands may need to be revised and dose recommendations be based on pharmacokinetic and efficacy data in children.

Key words: *Children, India, Pharmacokinetics, Pyrazinamide, RNTCP, Tuberculosis*.

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Revised National Tuberculosis Control Program (RNTCP) of India recommends pyrazinamide (PZA) administration at 30-35 mg/kg for intermittent, thrice weekly short course chemotherapy regime for tuberculosis in adults [1]. Children are administered drugs according to a Patient-wise box system under RNTCP [1]. Although the practical advantage of this system for drug administration cannot be doubted, there is a probability that children may be getting inappropriate doses when calculated on body weight basis.

In the few clinical studies where intermittent antitubercular regimens have been administered, higher doses of PZA in the range of 50-70 mg/kg were used (2-4). A PZA concentration of 25 µg/mL is considered low for thrice weekly administration [5]. Use of low doses in intermittent therapy may lead to inadequate drug concentrations which may contribute to treatment failure, relapse and drug resistance. Higher doses on the other hand may contribute to hepatotoxicity [2,6,7). There is lack of data in Indian children on the blood levels of PZA achieved with the current patient-wise box system. We conducted this study to observe the PZA blood levels achieved in children falling under Weight band 1 and 2 of RNTCP.

METHODS

An open-label, prospective, non-randomized single dose study was conducted in children, suffering from tuberculosis attending the Tuberculosis Clinic of Lok Nayak Hospital, New Delhi, India. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from the parents/ guardians of all patients.

Twenty children in the age group of 5-12 years, newly diagnosed with pulmonary or lymph node tuberculosis, were enrolled in the study. Diagnosis of tuberculosis was based on relevant clinical history, physical examination, chest *X*-ray, Mantoux test and fine needle aspiration cytology of accessible lymph nodes, wherever required.

Patients with hematological, hepatic and renal functions within the normal range were included. Patients with severe tuberculosis requiring hospital admission, concomitant presence of any other disease, and history of concomitant or long term drug intake were excluded.

Enrolled patients were admitted one day prior to study commencement, immediately on confirmation of the diagnosis. After overnight fasting, a single dose of PZA was administered orally at 06:00 h. The PZA dose was as per the Patient-wise box system of the RNTCP guidelines for treatment of tuberculosis. Children with weight between 6-10 kg (Weight Band 1) were given PZA 250 mg and children weighing between 10 and 17 kg (Weight Band 2) were given PZA 500 mg. A standard breakfast and lunch was administered 2 and 6 h after PZA administration, respectively. Regular antituberculosis treatment began 24 h later.

Venous blood samples (2.5 mL) were collected at 0,1,2,4,6,8,12 and 24 h after PZA administration. Serum was separated within 2 h of sample collection. A 1 mL sample of serum was deproteinised and supernatant was stored at –20°C for 24 h after which the assay was performed. Pyrazinamide was estimated by the spectrophotometric method of Subbammal, *et al*. [4].

The PZA dose administered to individual patients was converted to mg/kg dose and the patients were divided into two groups. Children for whom the PZA dose was within the recommended 30-35 mg/kg range (RNTCP for intermittent therapy) were included in Group I. Whereas children for whom the PZA dose was not in the 30-35 mg/kg range were included in Group II.

A single open compartment model was used to interpret the serum concentrations of PZA using WinNonlin Professional Version 4.0 (Pharsight Corp, Mountain View, CA, USA). The calculation of peak serum concentration (C_{max}), time to attain the peak concentration (T_{max}), area under the serum concentration *vs* time curve (AUC), elimination half life ($t_{1/2}$), elimination rate constant (k_{el}), apparent volume of distribution (V_d) and oral clearance (CL) is described elsewhere (9). The minimum inhibitory concentration (MIC) of PZA for *Mycobacterium tuberculosis* for pharmacokinetic pharmacodynamic (PKPD) parameter calculation was considered as 25µg/mL [4,5]. The ratio of C_{max}:MIC and AUC:MIC were calculated.

The demographic characteristics, baseline investigations and serum PZA concentrations were compared using GEE population-averaged model. The pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-24}, AUC, $t_{1/2}$, V_d, CL) and pharmacodynamic indices (C_{max}:MIC, AUC:MIC and the time duration for which serum PZA concentration remained above 25 µg/mL) were compared using two-sample *t*-test for unpaired data. For statistical analysis *P* value of <0.05 was considered significant at a confidence interval of 95%. The results are expressed as mean (standard error of the mean).

RESULTS

All 20 subjects completed the study. In both the groups, patients were comparable in their demographic profile (***Web Table* I**). Two patients were in weight band 1, and 18 in weight band 2. When calculated in mg/kg doses, it was observed that only seven children received pyrazinamide dose in mg/kg as per the RNTCP guidelines (Group I). The mean dose of PZA was 31.9±0.8 mg/kg (30.3-35.7 mg/kg) in Group I and 28.1±0.3 mg/kg (25-29.4 mg/kg) in Group II.

The mean serum PZA concentration in Group I at the end of 1h was 38.4±1.7 µg/mL. At 2h, the PZA concentration increased to 49.4±2.8 µg/ml. After 2h, PZA concentrations declined gradually till 24h (4.8±1.0 µg/mL). The mean serum PZA concentration in Group II at 1h was 33.0±0.9 µg/ml and increased to 41.7±1.2 µg/mL at 2h. The PZA concentration declined gradually after 2h to 2.9±0.5 µg/mL at 24h (***Fig.* 1**). The PZA concentrations were significantly lower in Group II up to 6h (*P*=0.003).

Pharmacokinetics: The C_{max} was achieved at 2h in all the patients. The mean C_{max} and AUC_{0-24} was significantly less in Group II in comparison to Group I (***Table* I**). The serum PZA concentration was maintained above 25 µg/mL for 4-8 h in Group I. One patient in this group had levels above 25 µg/mL for more than 6.5 h and another patient maintained this level for more than 8 h. In Group II, the concentrations were maintained above 25 µg/mL for not more than 3-5.5 h (*P*=0.03). The C_{max}:MIC and AUC:MIC ratios were also significantly lower in Group II. A linear relationship was observed between the individual dose of PZA administered and C_{max} (***Fig.* 2**). The other pharmacokinetic parameters i.e. $t_{1/2}$, K_{el}, V_d and CL were comparable between the two groups.

DISCUSSION

The RNTCP currently recommends two forms of drug dosing for children. On one hand it recommends a dose of 30-35 mg/kg PZA to children for thrice weekly therapy. On the other hand, it advocates the Patient-wise box system which is resulting in PZA being administered in a wide dose range of 25-45 mg/kg. In our study, only 35% children received appropriate amounts of PZA in mg/kg basis. The dose per kg body weight was an important

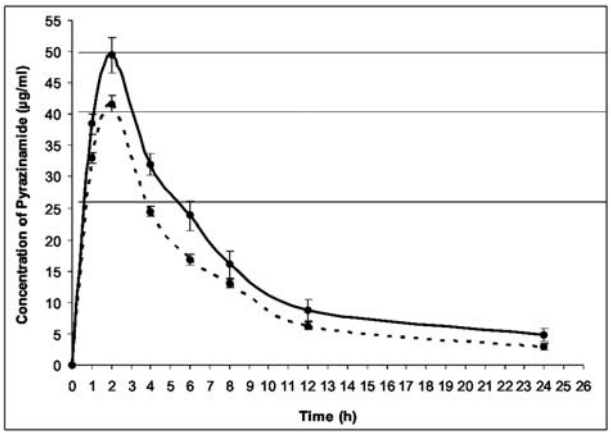

FIG. 1 *Serum concentrations of pyrazinamide over 24 h in Group I (n=7), (continuous line) and Group II (n=13) (dotted line) (values expressed as Mean ± SEM) in relation to MIC of 25, 40 and 50 µg/mL.*

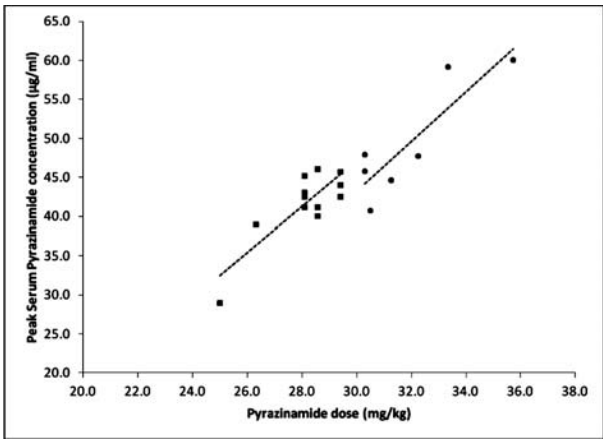

FIG. 2 *Peak Serum pyrazinamide concentration (C_{max}) in relation to the single oral pyrazinamide dose per kg body weight in Group I (n=7, circles) and Group II (n=13, squares). The dashed lines indicate a linear relation between the dose and C_{max} in Group I and II (R^2= 0.76 and 0.7, respectively).*

TABLE I PHARMACOKINETIC PARAMETERS (MEAN±SEM) OF GROUP I (PATIENTS RECEIVING APPROPRIATE mg/kg DOSE) AND GROUP II (NOT RECEIVING APPROPRIATE mg/kg DOSE) OF PYRAZINAMIDE AS PER RNTCP GUIDELINES FOR INTERMITTENT THERAPY

PK Parameter	*Group I(n=7)*	*Group II(n=13)*	*95% CI of difference*	*P value*
C_{max}, µg/mL	49.4±2.8	41.7±1.2	2.3, 13.2	0.008
T_{max}, h	2.0	2.0	–	–
$AUC_{(0-24h)}$, µg/mL h	369.5±35.3	278.4±16.0	20.4, 161.6	0.01
AUC, µg/mL h	435.0±44.2	316.6±20.7	28.6, 208.1	0.01
$t_{1/2}$, h	7.8±1.1	6.6±0.6	-1.2, 3.6	0.3
K_{el}, h^{-1}	0.12±0.03	0.12±0.01	-0.06, 0.06	0.9
V, l/kg	0.8±0.07	0.8±0.04	-0.2, 0.1	0.6
CL, l/h/kg	0.08±0.009	0.09±0.006	-0.04, 0.006	0.1
Time > 25 µg/mL, h	5.3±0.5	4.2±0.2	0.1, 2.0	0.03
C_{max}:MIC (25 µg mL^{-1})	1.98±0.1	1.67±0.05	0.09, 0.5	0.008
AUC:MIC (25 µg mL^{-1})	17.4±1.8	12.7±0.8	1.1, 8.3	0.01

SEM: standard error of the mean; CI: Confidence Interval; C_{max}: peak serum drug concentration; t_{max} : time to achieve peak serum concentration; $AUC_{(0-24\,h)}$: area under the serum concentration time curve in 24 h; AUC : area under the serum concentration time curve; $t_{1/2}$: elimination half-life; K_{el} : elimination rate constant; V : volume of distribution; CL : apparent clearance.

determinant of PZA concentrations. This has been observed previously also [8,9].

Pyrazinamide dosage recommendations for intermittent therapy are based on two important factors, MIC and lag phase [3,10]. Although at present there is less data on PKPD correlates of PZA, it has been observed that PZA blood concentration above 25 µg/mL is associated with a prolonged duration of antimycobacterial effect for daily administration [4]. However, for adequate killing with intermittent dosing, a serum concentration of 20 and 25 µg/mL have been considered as very low and low respectively [5,11]. We observed that patients who received PZA lesser than the recommended 30-35 mg/kg dose could maintain levels above 25 µg/mL for shorter duration of time. The ratios for MIC 25 µg/mL are below those suggested to be optimal [12].

For pyrazinamide, a C_{max} of 20-40 µg/mL after daily dose and 40-60 µg/mL after biweekly dose has been recommended [13]. In our study, 18 children had C_{max} between 40-60.1 µg/mL. However, the PZA

WHAT IS ALREADY KNOWN?

- Pyrazinamide is recommended in a dose range of 30-35 mg kg for thrice weekly administration in the RNTCP to be delivered based on four weight bands in children.

WHAT THIS STUDY ADDS?

- Children may receive pyrazinamide doses lower than the recommended range in weight band I and II, resulting in lower blood levels and lesser duration of time for which the pyrazinamide concentrations are maintained above the minimum inhibitory concentration.

concentration fell below 40 µg/mL within 4h of drug intake for many patients. Since the duration of time for which blood concentration stays above MIC is important and we do not know yet how much that duration should be, we cannot comment on the adequacy of the PZA concentrations achieved. A critical PZA concentration of 50µg/mL has also been defined based on inhibition of ≥ 95% wild type isolates. The *in vitro* sterilizing effect of PZA was linked to a PZA ratio of AUC: MIC with 90% maximal effect being achieved when the ratio is 209. Patient simulation demonstrated that a dose of 15-30 mg/kg achieved this ratio in the epithelial lining fluid of only 15-53% patients and dose more than 60 mg/kg performed better [14]. In view of the above findings, the AUC:MIC ratio in our study appears inadequate in all the patients.

A large inter-individual variation was observed in the blood levels as has been reported earlier also [8,11,15]. The $t_{1/2}$ was longer and CL was slower in comparison to a population pharmacokinetic study conducted in USA [11]. We are unable to explain the difference. There are many differences with both the studies, such as the PZA dose, frequency of administration, number of blood samples taken and ethnicity of the patient population. In comparison to the adult population, the $t_{1/2}$ was longer, CL was slower and V_d was larger [16]. It has been suggested that age has an inverse relationship with $t_{1/2}$ and V_d and a direct relationship with CL (16,17). The T_{max} for PZA in children has been reported to be more than 2h. However, in this study, it is 2h. This could be because there were no sampling points between 2 and 4h.

Based on pharmacokinetic studies in children, it has been suggested that PZA dose needs to be higher for children on a bodyweight basis [11]. This observation is also based on the fact that recent reports of outcome of childhood tuberculosis found a much poorer treatment response than earlier studies [18]. WHO has also recommended that intermittent therapy should not be used in children living in settings with a high HIV prevalence [19].

Higher doses than the 30-35 mg/kg thrice weekly doses followed in India under RNTCP are recommended by many professional bodies (20,21). The recent WHO guidelines also recommend the use of PZA in a daily dose of 30-40 mg/kg in children [19].

In the present study, the clinical outcome of treatment given was not assessed. Since the ultimate test of inadequate serum concentrations would be the effect on efficacy, this may be considered a limitation of the study.The present study demonstrated significantly low serum PZA levels in many children who received PZA in accordance with the RNTCP patient-wise box system. This system while easing the administration of drugs to children may not be adequate for delivering appropriate amounts of antitubercular drugs, in this case PZA.

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