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Pharmacokinetics of Pyrazinamide in Paediatric Patients of Tuberculosis Meningitis

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Abstract: Pyrazinamide (PZA) is an important component of antitubercular regimen. Its doses for paediatric patients of tuberculosis meningitis (TBM) are empirical and derived from adult doses on extrapolation basis. In order to determine the pharmacokinetics of PZA in paediatric patients of TBM a total of eighteen children, including of both sex in the age range of 3-8 yrs with the established diagnosis of TBM were administered PZA 30 mg/kg once daily dose along with other antitubercular drugs of short course regimen. On 11th day of the therapy, when the drug achieved steady state serum concentration, blood samples (two per day, from 11th to 13th day) were collected before, which constitute pre-dose (0 h) and at 1.0, 2.0, 3.0, 4.0, 8.0 and 24 h after PZA administration. PZA concentration in serum samples was determined by reverse phase high-pressure liquid chromatography. The maximal serum concentration (C_{max}), the time to achieve maximum serum concentration C_{max} (T_{max}) total clearance (Cl), volume of distribution (V_d), terminal elimination rate constant (K_{el}), elimination half-life ($t_{1/2}$), total area under serum concentration-time curve [(AUC_{0-24} and $AUC_{0-\infty}$)] were found to be 35.44±1.75 μg/ml, 2.22±0.55 h, 0.77±0.43 l/h, 10.33±4.15 l, 0.07±0.002 h^{-1}, 11.42±3.64 h, 409.19±63.72 μgml/h and 543.84±161.72 μgml/h respectively. It was observed that the C_{max} of PZA was distinctly about twice of the minimum inhibitory concentration (MIC) of PZA, 20 μg/ml and required to inhibit mycobacterium tuberculosis. We have demonstrated in the present study that the serum concentration of PZA was higher than MIC at 1.0 to 8.0 h after post dosing of it. The present study concludes that a dose of 30 mg/kg/day achieves much higher concentration of PZA as compared to MIC of PZA, irrespective of the clinical and therapeutic benefits. On the basis of results of present study, it is suggested that lowering of PZA dosage will be prudent for better patient compliance and adherence to therapy along with reduction in cost and side effects in paediatric patients.

Key words: Tuberculosis · Pyrazinamide · Paediatric · Pharmacokinetics

INTRODUCTION

Tuberculosis (TB) remains one of the main killer infections worldwide. About 2.2 million new cases of TB occur every year [1]. As per the World Health Organization report on global TB control, South East Asia accounts for approximately 40% two out of five cases of TB in world [2]. It is estimated that there are about 60 lakh active pulmonary TB cases in India and annual death rate due to TB is about 5,00,000. Of the various forms of the disease, TB in the form of tuberculosis meningitis (TBM) is a major health concern throughout the world especially in paediatric population. According to the criterion laid down by the WHO, there is no single country which has succeeded in reaching the point of control, that is, less than 1% TB positively among children in the age group 0-14 years [1-3]. Indeed, after decades of consistent decline in incidence, a resurgence of TB is occurring in developed countries. The prevalence of primary infection in child population is very high due to the high incidence of infection amongst adults who form the reservoir of TB [4].

The standard short course treatment of TB consists of isoniazid, rifampicin and pyrazinamide (PZA) plus either ethambutol or streptomycin [5]. PZA is the key sterilizing component of highly active short course antitubercular regimens in children to form the cornerstone of all first line therapy [6]. It is also valued for its powerful bactericidal effect against the metabolically active organisms commonly encountered in the sputum of adults with cavitating pulmonary TB [7]. It is one of the most frequently administered drugs for the treatment of TBM in paediatric population because of good cerebrospinal fluid penetration [8]. In TBM, the dose of

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PZA used by most paediatricians is on the higher side and the paediatric doses are derived from the pharmacokinetic studies conducted in adult patients as well as adult healthy humans [9]. The pharmacokinetics of PZA in children and adults with TB may differ from that are demonstrated in healthy adult subjects and patients. Altered absorption or elimination of PZA could compromise the efficacy or increase the toxicity of the treatment regimens by exposing to unnecessarily higher doses [10]. Treatment failures in children could lead to severe complications [11]. Despite several studies on PZA pharmacokinetics in adults patients of very few studies are available in paediatric patients [12, 13]. There is an increasing need of pharmacokinetic study for the determination of PZA in paediatric patients taking this drug along with other antitubercular drugs of short course regimen.

Therefore, the present study was undertaken to investigate the pharmacokinetics of PZA in paediatric patients making use of a highly improved and fully validated analytical technology i.e. high performance liquid chromatography (HPLC). The data derived from this study could improve our understanding of PZA pharmacokinetics and provide a rational basis for its optimum dose in the paediatric population.

MATERIALS AND METHODS

The study protocol and the informed consent form were approved by the Institutional Review Board of All India Institute of Medical Sciences, New Delhi, India. The study was conducted in Paediatrics Department of All India Institute of Medical Sciences Hospital, New Delhi, India. Written informed consent was obtained from the parents or a legal guardian of all paediatric patients before any study related procedure was conducted.

Study Subjects: The patients were selected randomly from paediatric TB clinic of All India Institute of Medical Sciences Hospital, New Delhi. Of the patients who fulfilled the exclusion and inclusion criteria and found suitable underwent a standardized diagnostic procedure. A total of eighteen (18) paediatric patients of TBM of either sex (male 10 and female 8) were participated in the present study. The demographic characters such as age, weight and height of the patients were 6.58 ± 3.12 yrs (range 3 to 8 yrs), 12.47±5.57 kg (range 8 to 17kg) and 107.17±12.14 cm (range 95 to 118 cm)] respectively. Diagnosis of TBM was based on clinical signs and symptoms, a CT (computerized tomogram) scan of the head showing evidence of TBM. The other clinical signs and symptoms were fever, vomiting, irritability, apathy, anorexia, constipation, seizures, paralysis and neck stiffness, impairment of consciousness or coma. A detailed history of contact, positive mantoux and evidence of TB (chest and/or lymph nodes) in the body were also taken in to consideration. The essential criteria consists of cerebrospinal fluids showing predominant lymphocytes, pleoytosis (75/mm), protein>60 mg%, sugar< 2/3 of blood sugar and presence of acid fast bacilli in it. Along with the essential ones, two or more of the following clinico-investigational criteria were to be met with (i) possible family history of TB, (ii) cervical lymphadenopathy> 2 cms, (iii) mantoux test (1 IU) > 10 mm (iv) positive radiological evidence of TB elsewhere in the body (v) evidence of basal exudates in CT scan (vi) histologically proved TB lymphadenitis by fine needle aspiration cytology (FNAC) and skiagram of chest suggestive of primary complex. On admission, all patients underwent a detailed neurological examination that included a fundus evaluation. Evidence for TB elsewhere in the body was observed for lymphadenitis or pulmonary involvement. A nutritional status assessment was also done by measurement of height, weight and mid arm circumference and children were classified according to the criteria laid down by Indian Association of Paediatrics, 1972 [14]. The exclusion criteria followed were (i) those received antitubercular therapy in part or were receiving therapy at the time of registration in the paediatric TB clinic or in paediatric wards (ii) those without definite diagnosis (iii) those with accompanying gastrointestinal, hepatic, renal or any other major organ disorder. During the study, no concomitant medications with propensities to influence the pharmacokinetics of PZA such as inhibitors/inducers of cytochrome P 450 enzymes were allowed. Patients were instructed not to take methylxanthine containing beverages, as well as grapefruit juice.

Study Design: After an overnight fast (Pyrazinamide tablet, Lupin Laboratories Limited, India) was administered orally in a total single dose calculated as 30 mg/kg/day bodyweight along with other antitubercular drugs of short course regimen. Food intake and water was withheld for another 2.0 hours. The blood samples were collected between 11th, 12th and 13th day of initiation of the antitubercular therapy in each patient as by this time orally administered PZA achieved their steady state concentration in blood. A total of 7 blood samples (not more than two samples per day) of 3.0 ml each were collected. After collection, the blood

samples were centrifuged (Remi Centrifuge, India) to separate serum. All serum samples were stored at $^{-1}$ 70°C until analysis.

Sample Analysis: A fully standardized and validated, reverse phase HPLC was applied to determine the PZA concentration in serum samples according to a previously described method [15]. The HPLC system (Waters, USA) consisted of two pumps of model 501, a micro^{-1} bondapak C18 column, a 481 LC UV detector and a system controller. A rheodyne manual injector (Rheodyne, Cotati, CA, USA) attached with a 50 µl sample loop was used for loading the sample. The reagents used were of analytical grade. The reagent used were sodium hydroxide (NaOH), disodium monohydrogen phosphate ($Na_2HPO_4.2H_2O$), potassium dihydrogen monophosphate (KH_2PO_4), perchloric acid ($HClO_4$), methanol, double distilled water and PZA standard (Lupin Laboratories Limited, India). The linear concentration range for PZA analysis was found to be 0.5 to 1.0 µg/ml with more than 99.9% recovery from serum. Inter-day and intra-day precision values for the quality control sample were ranged from 2.2 to 3.2 and 2.8 to 3.3% respectively. PZA was found stable in human serum for over 24 h at room temperature and for over months at-70° C. A calibration curve was constructed using response that is peak height against respective concentration. PZA concentration from serum samples was calculated using calibration curve. An UV detector at 745 nm was used for detection of PZA in the serum samples.

Sample Processing: The buffer (pH 7.4) was prepared with 18.7 ml of 0.02 M KH_2PO_4 and 80.3 ml of 0.02 M $Na_2HPO_4.2H_2O$. The mobile phase consisted of buffer (pH 7.4) and methanol (98:2), with a flow rate of 1.0 ml/min at ambient temperature. Mobile phase was thoroughly degassed for 15 min. One ml aliquot of 0.7 M perchloric acid was added to 5.0 ml glass tube containing 1.0 ml of serum and then thoroughly mixed for 10 seconds. After centrifugation at 1500 g for 10 min, 1.0 ml of the supernatant was taken and neutralized with 0.2 ml of 1M NaOH, of which 50 µl was injected. The standard curve of PZA was plotted ranging between 50 and 80 µg/ml. Unknown concentrations were derived from the linear regression analysis of the peak height ratio (analyter/internal standard) vs. standard curve. Linearity was verified using estimates of correlation coefficient. The chromatograms were recorded at a chart speed of 5 mm/min. The detection was carried out at a wavelength of 268 nm.

Pharmacokinetic Analysis: The various pharmacokinetic parameters for each subject were calculated from the serum concentration-time curves, according to one compartment model with first-order elimination kinetics using routine non-parametric equations. They were calculated as follows: the maximal serum concentration (C_{max}), the time to reach maximal serum concentration (T_{max}), total clearance (Cl) and the volume of distribution (V_d) were calculated mathematically. Terminal elimination rate constant was calculated (K_{el}) from serum concentrations mathematically fitting a straight line to the last serum concentration measurements ([ln (concentration vs. time)]) using linear regression. Area under serum concentration AUC $_{(0-24)}$ time curve was calculated from concentration at the time zero to the concentration at 24 hour by the linear trapezoidal rule. Extrapolated area under serum concentration AUC $_{(24-8)}$ againsttime was calculated from concentration at the time 24 hour to infinite time by dividing the last measurable serum concentration with K_{el}. AUC $_{(0-8)}$ was calculated as the sum of AUC $_{(0-24)}$ plus extrapolated AUC $_{(8-0)}$. The terminal half-life was calculated by using the formula $0.693/K_{el}$.

Statistical Analysis: Data were expressed as mean±SD. Pharmacokinetic values were analyzed using an analysis of variance (ANOVA) and student-t test. The criteria of statistical significance were p value less than 0.05.

RESULTS

All the patients completed the study and there was no significant protocol deviation. The adopted analytical procedure performed using HPLC method in present study is observed to be sensitive, selective and linear for the wide range of calculations of PZA concentration in serum. The demographic characteristics of the patients are presented in Table 1. Safety and tolerance of the drug was found well in all patients. For monitoring of adverse events, vital signs and clinical laboratory tests were performed in each patient. There were no reports of serious adverse event or treatment related abnormalities during the entire study or subsequent follow-ups. Short course regimen containing PZA was found to be effective during the study as evidenced by improvements in clinical signs and symptoms during clinical and physical assessment. The mean serum pharmacokinetic parameters determined after oral dose of PZA at dose 30 mg/kg/day are presented in Table 2.

Table 1: Demographic and baseline characteristics of patients of tuberculosis meningitis (n=18)

Gender: Male/Female	Number: 10/8
Age (y)	6.58±3.12
Height (cm)	107.17±12.14
Body weight (kg)	12.47±5.57

Table 2: The mean serum pharmacokinetics parameters following oral administration of pyrazinamide tablets in patients (n=18)

Pharmacokinetic parameters	Values (Mean ± SD)
C_{max} (μg/ml)	35.44±1.75
T_{max} (h)	2.22±0.55
$T_{1/2}$ (h)	11.42±3.64
C_o (μg/ml)	36.09±7.14
V_d (l)	10.33±4.15
Cl (l/h)	0.77±0.43
AUC_{0-24} (μgml/h)	409.19±63.72
$AUC_{24-\infty}$ (μgml/h)	150.34±99.50
$AUC_{0-\infty}$ (μgml/h)	543.84±161.72
K_{el} (h^{-1})	0.07±0.002

C_{max} = Maximal serum concentration after pyrazinamide administration, T_{max} = Time when C_{max} achieved, $T_{1/2}$ = Apparent terminal half-life, C_o = Serum concentration at time zero, Cl = Clearance, $AUC_{(0-24)}$ = Area under serum concentration *vs* time curve from the time zero to the concentration at 24 hour, $AUC_{(24-\infty)}$ = Area under serum concentration *vs* time curve from the time 24 h to the concentration at infinity, $AUC_{(0-\infty)}$ = Area under serum concentration *vs* time curve from the time zero to the concentration at infinity, K_{el} = Apparent terminal elimination rate constant

Table 3: Relationship between serum concentration and minimum inhibitory concentration at different time points in patients (n=18)

Time (h)	Serum concentration (μg/ml)	No. of times of MIC value
0	0.0±0.0	0.0
1	25.06±1.16	1.3
2	35.44±1.75	1.8
3	31.06±1.24	1.6
4	25.12±0.76	1.3
8	19.44±0.80	0.9
24	8.18±0.81	0.4

To correlate the relationship between minimum inhibitory concentration (MIC) and serum concentration at different time points for comparative purposes the mean serum concentration of PZA are presented in Table 3. The mean serum PZA concentration observed at different time points for paediatric patients has been presented in Fig. 1.

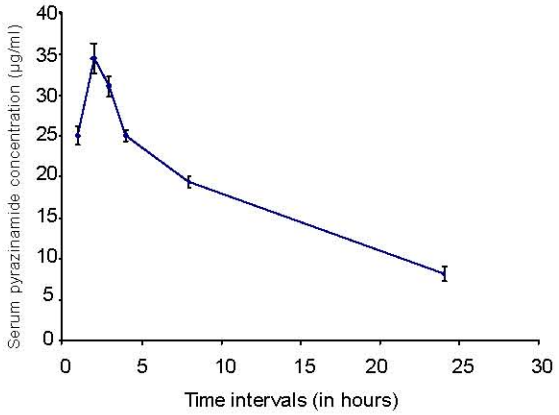

Fig. 1: Mean serum concentration of pyrazinamide at different time points (n=18)

The mean peak serum concentrations (C_{max}) of PZA was 35.44 ±1.75 μg/ml and the time to reach maximal serum concentration (T_{max}) was 2.22 ± 0.55 h. The elimination half-life ($t_{1/2}$), volume of distribution (V_d) the clearance (Cl) and elimination rate constant (K_e) were 11.42 ± 3.64 h, 10.33 ± 4.14 l, 0.77 ± 0.43 l/h and 0.07 ± 0.002 h^{-1} respectively. The area under curve ($AUC_{(0-24)}$, $AUC_{(24-\hat{a})}$) and $AUC_{(0-\alpha)}$) were found to be 409.19 ± 63.72 μgml/h, 150.34 ± 99.50 μgml/h and 543.34 ± 161.72 μgml/h respectively.

DISCUSSION

The present study shows that PZA administration in children at dose of 30 mg/kg produces significantly higher mean peak and mean serum concentration at 1.0, 4.0 and even 8.0 hour when compared with the MIC of PZA, which is required to kill the *mycobacterium tuberculosis* bacilli [7,16]. PZA is important components of short course multiple drug therapy of TB in both adults and children patients [17]. It is well absorbed from the gastrointestinal tract and widely distributed throughout the body. The daily doses for the adults are 25 to 30 mg/kg orally as a single dose [18]. In TBM, dosages used have been variable and usually on the higher side in the belief that higher levels would be achieved in CSF. Very few pharmacokinetic data are available in paediatric patients suffering from TBM [12, 13].

In order to investigate the pharmacokinetics of PZA in paediatric patients, a pharmacologically sound dosing strategy would be developed. Earlier it has been demonstrated that simultaneous administration of the antitubercular drugs (isoniazid, rifampicin and pyrazinamide) does not significantly alter the serum

levels of any one [19]. Thus the determination of PZA in patients taking antitubercular regimen could be conducted without interference of other agents. It has been observed that under experimental conditions, achievement of the serum level of a drug in the therapeutic range does not necessary mean that in this operational research one will definitely be able to achieve the same by prescribing the same dosages schedule. Since dosages in children are empirical and based on the extrapolation of studies in adults. Therefore, the aim of present study was to investigate changes in the pharmacokinetics of PZA in paediatric patients of TBM.

The importance of the present study is to use the derived data to retrieve rational dosing strategy for the TBM in the paediatric patients. This requires an integration of pharmacokinetic parameters with the pharmacodynamic characteristics. MIC of PZA, which is the most commonly used pharmacodynamic correlate. For optimal efficacy, a drug must be present at a MIC at the site of infection throughout the dosing interval, thus a precise dosing recommendation can be made on a pharmacokinetic basis [16]. This involves an examination of the time that the serum-drug concentration is above the MIC for the mycobacterium tubercle organism. With this approach a dosing interval can be ascertained at any dose level by projecting the serum drug concentration versus time curve onto the MIC for a particular organism.

Administration of PZA 30 mg/kg produced mean peak serum concentration 35.44 ± 1.75 μg/ml at T_{max} of 2.22±0.55 h. The mean serum concentration at 1.0, 2.0, 3.0, 4.0 and 8.0 hours were 25.06±1.16, 35.44±1.75, 31.66±1.24, 25.12±0.76 and 19.44 ± 0.80 μg/ml respectively and were 1.3, 1.7, 1.6, 1.3 and 1.0 times of the MIC values that is 20 μg/ml. Though, the peak concentration is achieved at 2.0 hours of the drug administration, therapeutically effective concentrations are equally achieved, the concentration even at 1.0 hour remain 1.2 to 1.7 times the MIC values signifying the onset of quick and effective activity even before 1.0 hour. As described earlier, in patients of TBM the mean peak serum concentration was found at 2.2 h of PZA administration. At peak concentration there remains a static equilibrium of the drug absorption and drug elimination. This concentration is nearly 2 times higher than the MIC of PZA, which is 20 μg/ml for *Mycobacterium tuberculosis* strain [16]. A steady rise was seen in the concentrations at 1.0 and 2.0 hour and serum concentration were constantly more than MIC as observed from 1.0 to 8.0 hours after PZA administration.

Along with isoniazid, which achieves its maximal serum concentration at 1.0 hour and rifampicin, which achieves its maximal serum concentration at 2.0 hours, PZA is able to exert its effective bactericidal action in tandem with isoniazid and rifampicin i. e. synergistic effect. The serum concentration is well above the MIC and hence the drug is able to exert its maximum bactericidal effect at an earlier time since concentration is 1.3 times the MIC at 1.0 hour. The maximum concentration achieved and the course of the concentration with the time is equally important for antimycobacterial efficacy. PZA exhibits dose dependent kinetics i. e. a proportional increase of serum concentration of PZA only up to a maximum of 3 g on oral administration [20].

Carlone *et al.* (1985) in a study on mouse macrophages harboring tubercle bacilli exposed to 30 μg/ml concentration of PZA, which is the concentration achieved at 4.0 hour in the present study, have shown the highest rates of killing of 93% for PZA and 92% for pyrazonoic acid as opposed to 59% in the controls [21]. It can be suggested that serum concentration is in tandem with the microbiological parameter i. e. sterilizing action and thus the very high rate of killing of tubercle bacilli thus signifying the adequacy and efficacy of the orally administered dose of PZA in the present study.

Since no published study on serum pharmacokinetics of PZA is available in paediatric patients of TBM. Thus, the present study results have been viewed in respect to the studies performed in adult patients of TB for the relative information. However, in strict sense, the studies in children and adults can not be compared due to varied absorption, metabolism, distribution and excretion rate of a drug. Comparisons of the present study results have been made with only those adult studies in which adult patients received PZA 30 mg/kg/day [22]. The mean time taken by PZA to achieve maximal serum concentration was 2.22 hours, which is in conformity with results obtained in adult patients of TB [22, 23].

In previous studies, in adults the oral administration of PZA produces serum concentration of about 9 to 12 μg/ml at 2.0 hours and 7 μg/ml at 8.0 hours [18, 24]. However, in present study in paediatric patients the serum concentration was persistently found higher than adult patients. It seems that unnecessarily the paediatric patients are getting exposed to a higher concentration of PZA rather than their optimal requirement. The serum half-life of the drug is 9.0 to 10.0 hour in patients of normal renal function [25]. However, in paediatric patients the

half-life was from 11 to 12 hour indicating a delayed elimination pattern. The serum concentration of PZA at 1.0, 2.0, 3.0, 4.0, 8.0 and even 24 hour were consistently higher as observed in respect to the previous studies in adult patients [22]. For effective antimycobacterial efficacy, area under curve constitutes a more comprehensive exposure factor, also denoted as exposure time or exposure factor.

The bioavailability in TBM patients was excellent as reflected by the AUC_{0-24} value of 416.89 μg/ml.h. In TBM paediatric patients the peak serum concentration (35.44 μg/ml) was higher than MIC by 1.8 times. However, at 8.0 h, serum concentration of PZA was nearly equal (19.44 μg/ml) to MIC and significant concentration (8.18 μg/ml) was even found detectable at 24 hours. It implies the adequacy of the oral dose of PZA to paediatric patients of TBM for effective therapeutic bactericidal concentration. Even though it is more meaningful and a direct evidence of the effective therapeutic dose to estimate the concentration of the drug in the CSF of the patients, the fact that PZA has excellent and quick CSF penetration is reflected by the attainment of near equal CSF levels to that achieved in serum [26].

Ellard *et al*. (1987) in a large study on Chinese adult patients of tuberculosis meningitis, found CSF:serum ratios of PZA of 0.74 at 2.0 hours, 1.15 at 5.0 hours and 1.09 at 8.0 hours of oral administration of 34-41 mg/kg of the drug [27]. The concentration of PZA observed in the serum of paediatric patients in present study can be considered as a near mirror image of the concentration of the drug in the CSF. In addition, the time to achieve maximal serum concentration 2.22 hour indicating a rapid absorption state of PZA like other antitubercular drugs isoniazid, 1.0 hour, rifampicin 2.0 hour [28, 29]. However, higher side of half-life signifying a little prolonged drug elimination.

The interpretation of the results of the present study suggests that there appears a scope of further lowering of PZA dosages in paediatric patients especially with tuberculosis meningitis. In addition, results also suggest that special consideration should be given to the need for dose reduction in paediatric patients who may be at higher risk of toxicity. Such approach could results in reduction of cost and therefore better adherence to therapy along with fewer side effects. Further studies in a larger population with different doses of PZA are required to determine the most optimal dose of PZA in paediatric patients for maximum safety and efficacy with least toxicity without compromising therapeutic benefit.

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