Pharmacokinetics of pyrazinamide in children with primary progressive disease of lungs

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Background & objectives: As the dosages recommended for children are based on weight, empirical and derived by extrapolation from the studies in adults, pyrazinamide (PZA) pharmacokinetics in children is likely to be different from adults. Limited information exists regarding the pharmacokinetics of PZA in paediatric patients of primary progressive disease (PPD) of lungs. This study aims to look at the changed pharmacokinetics of pyrazinamide in children with PPD of lungs by using reverse phase high-pressure liquid chromatography (HPLC).

Methods: A total of 40 children (age range 5 to 13 yr) of PPD were receiving pyrazinamide (30 mg/kg/day). On 11th day of short course antitubercular therapy, blood samples (two per day from 11th to 13th day) were collected at 0 h (pre-dose), 1, 2, 3, 4, 8 and 24 h after pyrazinamide administration and concentration of pyrazinamide was estimated by reverse phase high-pressure liquid chromatography. The mean peak serum concentration, the time to reach mean peak serum concentration, total clearance, concentration at time zero, volume of distribution, terminal elimination rate constant, elimination half-life, total area under serum concentration-time curve were measured.

Results: The mean serum concentrations of pyrazinamide were found higher than its minimum inhibitory concentration (20 µg/ml) required to inhibit the growth of tubercle bacilli from 1 to 8 h continuously.

Interpretation & conclusions: Our results suggest that a dose of 30 mg/kg/day achieves much higher concentration of pyrazinamide as compared to its minimum inhibitory concentration (20 µg/ml). Therefore, lowering of pyrazinamide dosage is suggested in children for better patient compliance along with reduction in cost, side-effects and toxicity without compromising its efficacy.

Key words Pharmacokinetics - progressive primary disease of lungs - pyrazinamide
drugs for the treatment of tuberculosis. The population pharmacokinetics of pyrazinamide has been studied well in adult healthy humans and patients. The studies showed minimal effects of food and antacids on the absorption of pyrazinamide. Although, major and most common side effects of pyrazinamide are hepatotoxicity and hyperuricemia, its other side effects include arthralgia-itching, skin rash, gastrointestinal-anorexia, nausea, and abdominal pain.

The conventional recommended doses in children based on weight is empirical and derived by extrapolation from the studies in adults, PZA pharmacokinetics in children is likely to be different from adults. Altered absorption or elimination of pyrazinamide could not only compromise the efficacy or increase the toxicity of the treatment regimens but may also lead to treatment failure which ultimately results in severe forms of tuberculosis, including tuberculous meningitis. Additionally, limited information exists regarding the pharmacokinetics of PZA in paediatric patients of PPD in lungs.

Hence, the present investigation was aimed to study the changed pharmacokinetics of pyrazinamide in children being treated for PPD using improved analytical technology reverse phase high-pressure liquid chromatography (HPLC).

Material & Methods

The study was conducted from January 1991 to June 1993, at the Department of Paediatrics, All India Institute of Medical Sciences (AIIMS), New Delhi, India. Written informed consent was obtained from the parents or legal guardians of all patients before any study related procedure was conducted. The study protocol was approved by the Institutional Review Board of AIIMS, New Delhi.

Study subjects: The patients were selected randomly from paediatric tuberculosis clinic of AIIMS Hospital, New Delhi. Forty children (19 male and 21 female) with primary progressive disease of lungs who fulfilled the exclusion and inclusion criteria and underwent a standardized diagnostic procedure participated in the present study. The (mean ± SD), age, weight and height of the patients were 7.8 ± 3.06 yr (range 5 to 13 yr), 19.58 ± 6.27 kg (range 13 to 26 kg) and 117.22 ± 18.17 cm (range 99 to 135 cm) respectively. The exclusion criteria followed were (i) those patients received antitubercular therapy in part or were receiving therapy at the time of registration in the paediatric tuberculosis clinic or in paediatric wards; (ii) patients with doubtful diagnosis of the disease; and (iii) patients with accompanying gastrointestinal, hepatic, renal or any other major organ disorder. During the study, no concomitant medications with propensities to influence the pharmacokinetics of pyrazinamide such as inhibitors/inducers of cytochrome P 450 enzymes were allowed. In addition, restriction was imposed on the intake of methylxanthine containing beverages, as well as on the consumption of grapefruit juice.

Study design: After an overnight fast, pyrazinamide tablet (Lupin Laboratories Limited, Pune, India) was administered orally in a total single dose calculated as 30 mg/kg/day bodyweight along with other antitubercular drugs of the treatment regimen. It was extrapolated from the adult dose. Food intake and water was withheld for the subsequent 2.0 h.

Sample collection: A total of 7 blood samples (not more than two samples per day) of 3.0 ml each were collected. The blood samples were collected before initiation of drug therapy and on 11th and 13th day of initiation of the antitubercular therapy in each patient. The blood samples were collected at 0, 1, 2, 3, 4, 8 and 24 h so as to obtain serial samples. Thereafter, the blood samples were centrifuged (Remi Centrifuge, Mumbai, India) to separate serum. All serum samples were stored at -70°C until analysis.

Sample analysis: A well standardized and validated, reverse phase HPLC procedure was applied to determine the pyrazinamide concentration in serum samples. The instrument used was Waters (Millipore, Billerica, USA) model 501 pump, a 7125 Rhodyne injector, a Micro-Bondapak C18 column and a 481 LC UV detector (Waters, Milford Massachusetts, USA). The reagents used were analytical grade and included sodium hydroxide (NaOH), disodium monohydrogen phosphate (Na2HPO4·2H2O), potassium dihydrogenmonophosphate (KH2PO4), perchloric acid (HClO4), methanol, distilled water and pyrazinamide standard (Sigma Chemical Co., St Louis, Mo). The linear concentration range for pyrazinamide analysis was found to be 0.5 to 1.0 µg/ml with more than 99.9 per cent recovery from serum. Inter- and intra-day precision values for the quality control sample were 2.2 to 3.2 and 2.8 to 3.3 per cent respectively. Pyrazinamide was 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 (peak height) against respective concentration in calibration standards and pyrazinamide concentration from serum samples was calculated. An UV detector at
268 nm was used for detection of pyrazinamide in the serum samples.

**Sample processing:** The buffer (pH 7.4) was prepared with 18.7 ml of 0.02 M KH$_2$PO$_4$ and 80.3 ml of 0.02 M Na$_2$HPO$_4$·2H$_2$O. The mobile phase consists of buffer and methanol (98:2 v/v), at a flow rate of 1.0 ml/min. Mobile phase was thoroughly degassed for 15 min. One ml aliquot of 0.7M perchloric acid was added to 5.0 ml glass tube containing 1.0 ml of serum and then thoroughly mixed for 10 sec. After centrifugation at 1957 x 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 pyrazinamide was plotted with concentrations ranging between 50 and 80 µg/ml. All chromatograms were recorded at a chart speed of 5 mm/min.

**Pharmacokinetic analysis:** Pharmacokinetic constants were calculated using WinNonlin Software (WinNonlin Standard Edition, Version 1.1, Scientific Consulting, Inc., Apex, N.C.). Also, some pharmacokinetic parameters for each subject were calculated from the serum concentration curves, according to one compartment model with first-order elimination kinetics using routine non-parametric equations. They were mathematically 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$_{d}$). Terminal elimination rate constant was also mathematically calculated (K$_{el}$) from serum concentrations fitting a straight line to the last serum concentration measurements (concentration vs. time) using linear regression. Total area under serum concentration time curve [AUC$_{0-24}$] was calculated from concentration at the time zero to the concentration at 24 h by the linear trapezoidal rule. Extrapolated area under serum concentration [AUC$_{24-∞}$] against time was calculated from concentration at the time 24 h to infinite time by dividing the last measurable serum concentration with K$_{el}$. AUC$_{0-∞}$ was calculated as the sum of AUC$_{0-24}$ plus extrapolated AUC$_{24-∞}$. The terminal half-life was calculated by using the formula 0.693/K$_{el}$.

**Statistical analysis:** Data were expressed as mean±SD. To test the significance of the difference in means of different pairs, student Newman’s multiple range test was applied.

**Results & Discussion**

The mean ± SD of age, body weight and height of the patients were 7.8 ± 3.06 yr (range 5 to 13 yr), 19.58 ± 6.27 kg (range 13 to 26 kg) and 117.22 ± 18.17 cm (range 99 to 135 cm) respectively. All 40 paediatric patients completed the study and there was no significant protocol deviation. Safety and tolerance of the drug was evaluated in all patients. Each patient was monitored for adverse events by vital signs and clinical laboratory tests. There were no reports of serious adverse event or treatment related laboratory abnormalities during the study period. Short course chemotherapy was found to be effective during the study. The mean pharmacokinetic parameters after single oral dose of pyrazinamide (30 mg/kg/day) are presented in Table I. Serum concentration at different time points in patients with primary progressive disease and their relationship with minimum inhibitory concentration (MIC) of pyrazinamide are presented in Table II. Administration of pyrazinamide at 30 mg/kg single oral dose produced mean peak plasma concentration 43.43 ± 6.74 µg/ml (at T$_{max}$ 2.25 ± 0.77 h), mean plasma concentration 34.90 ± 1.25 µg/ml (at 1.0 h) and 30.61 ± 1.18 µg/ml (at 8.0 h) is 2, 1.7 and 1.1 times of the minimum inhibitory concentration values (20 µg/ml). Though the peak concentration was achieved at 2.0 h of the drug administration, effective therapeutic concentrations were equally achieved, the concentration even at 1.0 h remained 1.7 times the MIC values signifying the onset of quick and effective activity even before 1.0 h. The peak concentration remained above MIC value up to 8 h of treatment. At peak concentration, there remains

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values (range)</th>
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<tbody>
<tr>
<td>C$_{max}$ (µg/ml)</td>
<td>43.43 ± 6.74 (34.58-56.83)</td>
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<tr>
<td>T$_{max}$ (h)</td>
<td>2.25 ± 0.77 (1-4)</td>
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<tr>
<td>T$_{1/2}$ (h)</td>
<td>7.78 ± 1.30 (4.46-9.84)</td>
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<tr>
<td>C$_{v}$ (µg/ml)</td>
<td>47.99 ± 9.8 (29.7-69.94)</td>
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<tr>
<td>V$_{d}$ (l)</td>
<td>12.94 ± 5.57 (5.58-28.42)</td>
</tr>
<tr>
<td>CL (l/h)</td>
<td>1.16 ± 0.49 (0.61-2.67)</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (µg.h/ml)</td>
<td>496.11 ± 138.15 (256.35-639.76)</td>
</tr>
<tr>
<td>AUC$_{24-∞}$ (µg.h/ml)</td>
<td>68.82 ± 34.17 (24.17-112.69)</td>
</tr>
<tr>
<td>AUC$_{0-∞}$ (µg.h/ml)</td>
<td>520.45 ± 106.74 (397.19-688.19)</td>
</tr>
<tr>
<td>K$_{el}$ (per h)</td>
<td>0.09 ± 0.001 (0.0107-0.155)</td>
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Values are mean ± SD (n=40);
C$_{max}$, maximal plasma concentration after pyrazinamide administration; T$_{max}$, time when C$_{max}$ achieved; T$_{1/2}$, apparent terminal half-life; C$_{v}$, plasma concentration at time zero; V$_{d}$, volume of distribution; CL, clearance; AUC$_{0-24}$, area under plasma concentration vs time curve from the time zero to the concentration at 24 h; AUC$_{24-∞}$, area under plasma concentration vs time curve from the time T$_{max}$ to the concentration at infinite time; K$_{el}$, apparent terminal elimination rate constant.
a static equilibrium of the drug absorption and drug elimination. This concentration was 2 times higher than the MIC of pyrazinamide, which is 20 µg/ml for Mycobacterium tuberculosis strain. A steady rise was seen in the concentrations at 1.0 and 2.0 h but an abrupt rise to the peak concentration from the baseline (0.0 h) to 2.0 h of drug administration was observed.

The study demonstrated that pyrazinamide administration in children at dose of 30 mg/kg produced significantly higher mean peak and mean serum concentration at 1.0 to 8.0 h of its MIC. Pyrazinamide, a synthetic pyrazine analog of nicotinamide, is an important component of short course multiple drug therapy of tuberculosis in both adults and children. It is well absorbed from the gastrointestinal tract and widely distributed throughout the body. The plasma half-life of the drug is 9 to 11 h in patients with normal renal function. It has been demonstrated that simultaneous administration of the antitubercular drugs such as isoniazid, rifampicin and pyrazinamide does not significantly alter the serum levels of one another. Hence, the determination of only pyrazinamide level in serum samples is justified. Under experimental conditions, achievement of the serum level of a drug in the therapeutic range does not necessary mean that in the operational research one will be able to achieve the same by prescribing the same dosages schedule.

In combination with isoniazid (T<sub>max</sub> = 1.0 h) and rifampicin (T<sub>max</sub> = 2.0 h), pyrazinamide was able to exert its effective bactericidal action. The maximum concentration achieved and the course of the concentration with the time is equally important for antimycobacterial efficacy. Carlone et al. in a study on mouse macrophages harbouring tubercle bacilli exposed to 30 µg/ml concentration of pyrazinamide, have shown the highest rates of killing of 93 per cent for pyrazinamide and 92 per cent for pyrazonoic acid as compared to 59 per cent in the controls. It can be suggested that serum concentration of pyrazinamide is in tandem with the microbiological parameter i.e., sterilizing action. Thus, the very high rate of killing of tubercle bacilli due to pyrazinamide justifies the adequacy and efficiency of the orally administered dose of pyrazinamide in the present study. The serum concentrations at 1.0 and 8.0 h observed in the present study were in agreement with studies performed previously in adult patients. Since no published study on pharmacokinetics of pyrazinamide is available in paediatric population with tuberculosis using HPLC, the present results were compared with the studies in adult patients. However, comparisons were made with only those adult studies in which patients received 30 mg/kg/day of the drug. The mean value of T<sub>max</sub> was 2.25 h, which is in conformity with results obtained in adult patients.

In conclusion, our results suggest that there is a scope of lowering of dosage of pyrazinamide in the treatment of primary progressive disease in children. This may benefit those patients who are at a higher risk of toxicity. Further, this would lead to reduction in cost along with fewer side effects and better compliance to therapy. Further studies on a larger sample are required to confirm the optimal therapeutic dose of pyrazinamide in children.

References


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