Para-Aminosalicylic Acid Plasma Concentrations in Children in Comparison with Adults after Receiving a Granular Slow-Release Preparation

by A. C. Liwa, H. S. Schaaf, B. Rosenkranz, H. I. Seifart, A. H. Diacon, and P. R. Donald

1Division of Pharmacology, Department of Medicine, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa
2Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa
3MRC Centre for Molecular and Cellular Biology and DST/NRF Centre of Excellence for Biomedical TB Research, Department of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa

Correspondence: P. R. Donald, Desmond Tutu TB Centre, Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg 7505, South Africa. Tel: +27 21 9389592; Fax: +27 21 9389138; E-mail <prd@sun.ac.za>.

Summary

There are no paediatric data regarding slow-release para-aminosalicylic acid (PAS). We studied PAS plasma concentrations in 10 children receiving a single 150 mg/kg dose daily or 75 mg/kg twice daily and 12 adults receiving 4 g twice daily. Blood specimens pre-dose and 2, 4, 6, 8 and 12 h post-dose from the children and 2, 3, 4, 5, 6, 8 and 12 h post-dose from the adults were analysed by high performance liquid chromatography MS/MS. The mean Cmax in children receiving PAS 75 mg/kg and 150 mg/kg and adults receiving 4 g was 45.40, 56.49 and 51.3 µg/ml, respectively (p = 0.614); the AUC0–12 was 233.3, 277.9 and 368.0 µg/h/ml (p = 0.587). No parameters differed significantly between children and adults nor between the two doses in the same children. A 150 mg/kg PAS dosage given as one or two daily doses leads to plasma concentrations in children similar to those of adults receiving 4 g PAS twice daily.

Key words: para-aminosalicylic acid, pharmacokinetics, children.

Introduction

Para-aminosalicylic acid (PAS) was the first effective chemotherapeutic agent used to treat tuberculosis (TB) in a child [1] and remained an important component of anti-TB regimens until the introduction of rifampicin (RMP) and pyrazinamide (PZA). None the less, little is known about PAS pharmacokinetics in children, and only one English language report of PAS pharmacokinetics in children could be found [2]. The worldwide spread of human immunodeficiency virus (HIV) infection, and its association with drug resistance, has again necessitated the use of PAS for management of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB [3]. The daily dosage of the currently available PAS granular formulation recommended by World Health Organization for children is 150 mg/kg [4], but there are no studies supporting this recommendation. Age-related variations in the absorption, apparent volume of distribution, clearance and speed of biotransformation may all contribute to differences between adults and children in their exposure to different drugs [5]. In the case of the ‘first-line’ anti-TB agents isoniazid (INH) [6] and RMP [7], it is clear that younger children require higher INH and RMP doses to achieve drug exposure equivalent to that of adults receiving...
the same doses, but this may not be the case with PZA [8]. Given high protein binding, the influence of gastric pH on dissolution and absorption and importance of the N-acetyltransferase 1 enzyme system in the metabolism of PAS, there is good reason to expect children to require higher PAS doses to reach concentrations similar to adults, but there are no data to support such an assumption [9]. We describe PAS plasma concentrations in a small group of children managed for XDR and MDR TB and compare their findings with those of adults also treated for XDR and MDR TB.

Patients and Methods

Patients with MDR or XDR TB admitted to Brooklyn Hospital for Chest Diseases, Cape Town, South Africa, were enrolled prospectively. Adult participants, aged ≥18 years, provided written informed consent; for children, a parent or legal guardian gave written informed consent; children >7 years of age provided assent. The study was conducted according to the ethical guidelines and principles of the International Declaration of Helsinki and South African Guidelines for Good Clinical Practice and was approved by the Committee for Human Research at Stellenbosch University (No 9/08/212). Exclusion criteria were severe anaemia, diarrhoea or dehydration. Patient characteristics recorded were age, sex, weight and the PAS dose given. The children also received different combinations of PZA, ethionamide, ethambutol, terizidone, capreomycin and linezolid, varied according to susceptibility patterns of each patient’s isolate or, in some children, the susceptibility pattern of the index case’s isolate. In addition, all but one child also received INH, as there is evidence of a response to INH at higher dosages, even in the presence of INH resistance [10, 11], and it may be possible to exceed the INH mutant prevention concentration in some children [12].

Drug administration

All patients were prescribed oral PAS (PASER\textsuperscript{R} granules, Para-aminosalicylic Acid Delayed-Release Granules, Jacobus Pharmaceutical Company, Inc, Princeton, NJ, USA). Children were studied on two occasions; before study days, children received PAS at 75 mg/kg twice daily (i.e. a total daily PAS dosage of 150 mg/kg as recommended by World Health Organization) [4] and adults 4 g twice daily; patients were established on PAS for at least 1 month before study, and there was at least a week between the two study occasions. HIV-infected patients received stavudine, lamivudine and efavirenz, with the exception of one patient receiving zidovudine. On the first study occasion, children received their usual dose of 75 mg/kg body weight PAS and on the second occasion, a 150 mg/kg dose; adults received a dose of 4 g. To prevent early PAS release in the stomach, all doses were taken with acidic food or beverage, orange juice for children and yoghurt for adults. Breakfast was given ~1 h after dosing. All study doses were observed and patients watched for vomiting following PAS administration.

Pharmacokinetic sampling schedule and analysis

Doses, dosing and blood sampling times were recorded. For adults, 1 ml blood specimens were collected through a 20 gauge angiocatheter inserted into a forearm vein, pre-dose and 2, 3, 4, 5, 6, 8 and 12 h post-dose. For children, smaller angiocatheters were used and specimens of 1 ml blood drawn pre-dose and at 2, 4, 6, 8 and 12 h after dosing. Catheters were maintained patent with a dilute heparin solution (10–15 units per ml); 1 ml blood was withdrawn to clear the heparin trap before sampling. Samples were centrifuged at 3000–4000 g for 10 min, and plasma was harvested and frozen at −80°C until assay. All samples were assayed in duplicate by validated high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) assay using binary HPLC (Agilent Series 1100 HPLC, Agilent Technologies, Waldbronn, Germany) equipped with an Agilent Zorbax analytical column (150 mm × 2.1 mm, 3.5 μm particle size); the mobile phase consisted of a gradient between water and methanol (E. Merck, Darmstadt, Germany), both containing 0.1% formic acid (Fluka Chemie GmbH, Buchs, Switzerland). PAS concentrations were determined by API 2000 tandem mass spectrometer (MS/MS) (Applied Biosystems, MDS Sciax, Foster City, Canada) equipped with an atmospheric turbulon ionization chamber. A single transition range was used for the internal standard (thiacetazone) and PAS precursor and product ion of m/z 237.12/119.96 and 154.20/136.20. Retention times were 5.42 min (PAS) and 7.0 min (thiacetazone). To each sample batch, at least eight calibrators of varying concentration were included as quality control samples. For all compounds, a variation of <±5% was found for the entire analysis duration. Duplicates of samples did not vary >2.5% from each other.

The pharmacokinetic model was a one compartmental model with first-order absorption and first-order elimination, assuming all subjects were at steady state. The observed maximal plasma concentration (C\text{max}) and time of occurrence (t\text{max}) were determined by inspection of individual serum concentrations vs. time graphs. Area under the plasma concentration-time curve from time 0 to t (AUC\text{0–t}), where t is the dosing interval, was calculated using the linear trapezoidal method.

Statistical analysis

The independent variables were age, body weight and dose. The mean, standard deviation (SD), median
and range were calculated for each parameter. A repeated measures analysis of variance was performed using the MIXED procedure in SAS version 9. The comparison between groups (adults vs. children and children receiving 75 mg/kg or 150 mg/kg) was treated as a between-subject effect, whereas time after study drug intake was regarded as a within-subject effect.

**Results**

Ten children and 12 adults were studied, and their demographic characteristics appear in Table 1. In both groups, approximately a third of patients were male and a third HIV-infected. The pharmacokinetic results are summarized in Table 2 and illustrated in Fig. 1; the mean $C_{\text{max}}$ and $\text{AUC}_{0-12}$ in children were higher following a dose of 150 mg/kg than after 75 mg/kg, but the difference was not significant. The mean 12 h concentration following the 75 mg/kg dose in the children was 6.8 $\mu$g/ml compared with 21.3 $\mu$g/ml following a dose of 150 mg/kg, and this difference too is not significant ($p = 0.993$). Regarding the adult values, none of the parameters differed significantly from those following either PAS dose in children. A wide coefficient of variation was found in both the children’s and adult’s results. It should be noted that the adult patients median weight was 66 kg, implying that the mean PAS dosage received by these adult patients was 60 mg/kg body weight and thus somewhat lower than the children’s. No differences were found between male and female children or adults, and no problems were encountered with vomiting. Only a minority of adults and children were HIV-infected; although the mean concentrations at all time points were lower in HIV-infected patients, the differences between HIV-infected and non-infected subjects were not significant in either children or adults.

**Table 1**

Demographic characteristics of the children and adults studied

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children ($N = 10$)</th>
<th>Adults ($N = 12$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Median 4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Range 1–12</td>
<td>18–53</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Median 14.15</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Range 5.4–26.6</td>
<td>37–84</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Median 92.8</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>Range 60–130</td>
<td>163–183</td>
</tr>
<tr>
<td>Males (%)</td>
<td>3 (30)</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>HIV-infected (%)</td>
<td>4 (40)</td>
<td>4 (33.3)</td>
</tr>
</tbody>
</table>

**Discussion**

The PAS concentrations found in the adults receiving a PAS dosage of 4 g (60.6 mg/kg for our adult patients) were within the range previously reported for adults receiving the same slow-release formulation of PAS [13, 14]; the relatively late $t_{\text{max}}$ of close to 5 h found in both adults and children is not unexpected. Our results in children do not differ significantly from those in adults (Table 2). From this limited data set, it might be concluded that, in children in the age group studied, receiving a similar mg/kg body weight PAS dose either once or twice daily will achieve a similar exposure to PAS as adults and reach mean and median concentrations well in excess of the minimal inhibitory concentration (MIC) throughout the 12 h interval. The higher PAS dose of 150 mg given to the children on the second study occasion, unexpectedly, did not lead to a significantly greater exposure to PAS, although inspection of the figure suggests that a PAS dose of 150 mg/kg will result in plasma concentrations much closer to those of adults receiving a 4 g dose than a 75 mg/kg dose. Despite the higher dose, the vigorous acetylation of slowly released PAS might be responsible for this finding. The mean 12 h PAS concentration in children following the higher dose was higher than after the 75 mg/kg dose, but, again, the difference was not significant; how long this is maintained and concentrations remain above MIC requires further study. In adult studies, a rapid fall in concentrations occurs as soon as absorption ceases [13]. However, if supra-MIC concentrations were maintained throughout a once daily dosing interval, or for the majority of the period, after a dose of 150 mg/kg, it could be advantageous for treatment supervision to consider once daily dosing.

Only one English language report describes PAS blood concentrations in four children with meningeal and spinal TB; sodium PAS was given five times daily at 4 hourly intervals to a total dosage of 300 mg/kg [2]. The results are presented as figures, and $C_{\text{max}}$ following doses of 50–60 mg/kg is between 5–10 $\mu$g/ml; $C_{\text{max}}$ was at ~60 min, then declining rapidly. In an Italian publication, Maggioni and Assensio studied PAS concentrations in blood, urine and cerebrospinal fluid of young children after intermittent oral, intra-venous and rectal administration of sodium PAS and considered a total daily dosage of 300–400 mg/kg necessary [15].

PAS is most valued for preventing resistance in companion drugs; it is usually considered bacteriostatic and administration recommended in several daily doses to maintain blood concentrations consistently above MIC. Intermittent dosing, particularly with a slow-release preparation, is also said to cause less gastro-intestinal intolerance. None the less several early studies comparing once daily and intermittent dosing found adult patients...
tolerated once daily dosing better [16–18]. Furthermore, single daily dosing was clinically as effective as divided daily doses, both in TB patients [16] and experimental animals [19]. In a small group of pulmonary TB patients, the early bactericidal activity of PAS in a single daily dose of 15 g for the first two treatment days was second only to that of INH, suggesting significant early bactericidal activity at

**Table 2**

PAS plasma concentrations in children receiving a PAS dose of 75 or 150 mg/kg, and adults receiving a PAS dose of 4 g, being treated for XDR and MDR TB

<table>
<thead>
<tr>
<th>Pharmacokinetic values</th>
<th>Children</th>
<th>Adults</th>
<th>P^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75 mg/kg twice daily</td>
<td>150 mg/kg once daily</td>
<td>4 g twice daily</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</td>
<td>Mean (SD)</td>
<td>45.4 (22.7)</td>
<td>56.5 (32.4)</td>
</tr>
<tr>
<td>CV%</td>
<td>49.9</td>
<td>57.4</td>
<td>39.1</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>Mean (SD)</td>
<td>4.8 (2.5)</td>
<td>4.8 (3.6)</td>
</tr>
<tr>
<td>CV%</td>
<td>52.7</td>
<td>74.0</td>
<td>39.4</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–12&lt;/sub&gt; (µg/h/ml)</td>
<td>Mean (SD)</td>
<td>233.3 (135.1)</td>
<td>277.9 (221.3)</td>
</tr>
<tr>
<td>CV%</td>
<td>57.87</td>
<td>79.7</td>
<td>52.7</td>
</tr>
</tbody>
</table>

^aComparison using mixed models analysis of value by the mixed procedure and differences of least square means. AUC, area under the curve; C<sub>max</sub>, maximum concentration; CV, coefficient of variation; t<sub>max</sub>, time to maximum concentration.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Mean (SD) plasma PAS concentrations in children receiving a dose of 75 mg/kg 12 hourly or 150 mg/kg PAS once daily and adults receiving 4 g twice daily.
higher concentrations that would be valuable for prevention of resistance in companion drugs [20]. When daily PAS dosages of 5, 10 or 20 g, in four divided doses, were evaluated for prevention of resistance in the companion drug streptomycin, the proportion of streptomycin resistance after 4 months of treatment was 47 and 43%, respectively, in patients receiving 5 and 10 g PAS daily, but only 15% in those receiving 20 g PAS, suggesting, again, that higher doses of PAS (and a higher Cmax) may be necessary for PAS to achieve its maximum effect [21].

Our study is limited by the wide intra-group variance found in both children and adults. This, together with the small number of patients studied, has also limited our ability to show significant differences between groups. A previous study in adults using the same preparation similarly found a wide coefficient of variation for Cmax of 43.5% and for AUC0–24 of 45.6% [12, 13]. Larger numbers of patients will be needed to demonstrate significant differences between groups receiving different doses of this PAS preparation [15]. An uncertain duration of gastric emptying, feeding of patients 1 h after dosing and a prolonged period of absorption that overlaps excretion and is probably subject to genetic differences might also contribute to this variance.

For the first time, we provide data regarding PAS plasma concentrations following the use of slow-release formulation in children; a dosage of 150 mg/kg, either as a once or twice daily dose, led to PAS concentrations not significantly different from those in adults receiving a dose of 4 g. Given the current perceived necessity of maintaining concentrations above MIC for an entire dosing interval, but also the possibility that PAS at higher concentrations may have a significant bactericidal effect, the slow-release PAS preparation might offer the best of both worlds if given in a single daily dose, thus combining sustained concentrations above MIC and a higher Cmax than is attainable with PAS in split doses. Our conclusions are limited by the small number of patients studied, and further study of the pharmacokinetics of single daily doses of granular PAS in children (and adults) is warranted given the paucity of options available to MDR and XDR TB patients and the need to optimize treatment, and treatment supervision, of these vulnerable patients.

References