Busulfan Pharmacokinetics Using a Single Daily High-Dose Regimen in Children With Acute Leukemia

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The pharmacokinetics of busulfan, given as a single daily dose (either 4 mg/kg or 150 mg/m²), was determined in 22 children undergoing bone marrow transplantation for acute leukemia. The single daily dose regimen showed similar pharmacokinetics to previously reported regimens of 4 x 1 mg/kg, except for fourfold higher mean peak plasma levels and negligible trough levels. Daily systemic exposure for single dose regimens based on weight (4 mg/kg) or surface area (150 mg/m²), respectively, were very similar to regimens of (4 x 1 mg/kg) or (4 x 37.5 mg/m²). Dose (milligrams per kilogram), peak plasma level, and area under the curve (AUC) were all higher in 12 children treated with 150 mg/m² busulfan than in 9 children treated with 4 mg/kg. AUC was age dependent for the 4 mg/kg dose but not for the 150 mg/m² dose. The use of a 150 mg/m² dose allows escalation of the dose above 4 mg/kg, eliminating the tendency for younger children to receive lower systemic exposure. Little toxicity was observed in this study. Clearance and distribution volume correlated negatively with age, and AUC correlated positively with dose (milligram per kilogram). Administration of busulfan as crushed rather than whole tablets reduced the delay time for appearance of busulfan in plasma but had no effect on absorption or other pharmacokinetic parameters.

Since the early 1980s, busulfan (Bu) and cyclophosphamide in combination (4 days busulfan followed by 4 days cyclophosphamide) has been widely used as an alternative to cyclophosphamide and total body irradiation as conditioning for patients with acute myeloid leukemia (AML) undergoing bone marrow transplantation (BMT). The combination of Bu and cyclophosphamide conditioning (BuCy) has the advantage of avoiding irradiation for pediatric patients, and there is good evidence for antileukemic activity in AML, particularly with the cyclophosphamide reduced to 2 days. However data on the long-term follow-up of children transplanted with BuCy is limited. Almost all regimens of BuCy use Bu at a dose of 1 mg/kg administered every 6 hours (4 x 1 mg/kg) for 4 days, and pharmacokinetic analysis has been performed and reported in several of these studies. Recent publications from two centers provide pharmacokinetic data on Bu doses of 6-hourly 37.5 mg/m² dose regimens (4 x 37.5 mg/m²) in children.

There is clinical evidence that Bu toxicity, including veno-occlusive disease (VOD) and convulsions, is related to higher doses of Bu and there is some pharmacokinetic evidence that VOD may be related to higher systemic exposure to Bu. Two papers reported the use of BuCy in children transplanted for genetic diseases, where Bu was administered as a single daily dose of 80 mg/m² (equivalent to 2 mg/kg/d in a 70-kg adult of 1.73 m²). This dose, equivalent to 2.5 to 5.25 mg/kg/d in children, was associated with minimal toxicity and a high rate of engraftment. Thus, when our center began using BuCy for AML, a single daily 4 mg/kg dose regimen (1 x 4 mg/kg) was adopted. Because of the low toxicity encountered with this regimen, but still unacceptable relapse rate in the autografted patients, we have further escalated the dose of Bu to 150 mg/m²/d. The main advantage of a single daily dose regimen in children is the simplicity of administration, especially in very young children. However, the single daily dose regimen also allows an accurate and complete pharmacokinetic evaluation without the need to correct for the effect of previous or following doses. Circadian effects and other within-day fluctuations that complicate pharmacokinetic analyses of qid regimens are also avoided.

Materials and Methods

Bu for clinical administration and for use as standards in the assay was obtained from Wellcome Australia Pty Ltd (Sydney). Most patients received initial chemotherapy according to an Australian New Zealand Children's Cancer Study Group (ANZ CSG) protocol for AML. The study, including sampling for pharmacokinetics and dose escalation of Bu, was approved by the Children's Hospital Ethics Committee.

A total of 22 children between 1 and 14 years of age were involved in this study. Nineteen with AML and 3 with ALL. Table 1 gives details of the clinical data and the chemotherapy conditioning regimens for these children. Bu was given as whole or crushed tablets in a single dose each on one of four mornings. A normal diet was offered on each day of Bu administration. Nine patients were given a single daily dose of Bu for 4 days at 4 mg/kg/d. Two of these patients, who were recipients of unrelated transplants, had melphalan at 140 mg/m² after Bu. Thirteen patients were administered a single daily dose for 4 days at 150 mg/m²/d. Bu (5 mg/m²) was followed by 2 days of cyclophosphamide at 60 mg/kg/d. All patients received anti-convulsant prophylaxis. None received phenytoin; two patients already on carbamazepine continued this drug; the others all received clonazepam, 0.05 mg/kg twice daily orally from the day before to the day after Bu administration.

The Bu dose was administered during the day, before 1 pm in 19 of 21 patients with the mean starting time of 11:00 hours (range: 9:00 to 14:30 hours). Thus, the effects of diurnal variation on Bu pharmacokinetics was minimized. Heparinized whole blood samples (1 mL) were collected from central venous lines. The first sample was collected before Bu was administered and the remainder at 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours after the dose. Occasionally an 18-hour sample was also collected. Plasma samples were separated by centrifugation for 10 minutes at 4°C at 3,000 rpm, then frozen and stored at −20°C until analysis.

Bu was determined in plasma samples using a modified version of a previous method by conversion to the 1,4-diiodobutane derivative and measurement by Gas Chromatography with Electron Capture. An aliquot of plasma (0.2 mL) was added to acetone (0.1 mL) and 1 mol/L sodium phosphate buffer, pH 7.0 (0.1 mL), in a screw

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and the mixture was vortexed for 20 seconds then heated at 70°C for 40 minutes with brief vortexing every 10 minutes. The hexane capped 4 mL Wheaton vial with a Teflon liner. Freshly prepared 5 mol/L potassium iodide (1.6 mL) and hexane (0.5 mL) were added and the mixture was vortexed for 20 seconds then heated at 70°C for 40 minutes with brief vortexing every 10 minutes. The hexane layer was transferred to a crimp sealed autosampler vial and 5 μL was injected into the gas chromatograph. A Hewlett Packard 5730A Gas Chromatograph was used, equipped with a 60 Ni, model 18713A linear electron capture detector and a 0.61 mm long, 2 mm internal diameter (i.d.) packed column of 2% OV101 on 100-120 mesh High Performance Chromosorb W. The carrier gas of 5% methane in argon had a flow rate of 27.3 mL/min. The instrument was operated isothermally with oven, detector, and injector port temperatures of 70°C, 250°C, and 250°C, respectively. 1,4-Diiodobutane had a retention time of 6.8 minutes.

Quantitation of plasma Bu involved the construction of a standard curve using concentrations of 2.5, 5.0, 7.5, and 10 μg/mL Bu. The standards were added in acetone (0.1 mL) to water (0.2 mL) and 1 mL sodium phosphate buffer pH 7.0 (0.1 mL). They were then extracted, derivatized, and analyzed by GC as described for the plasma samples. The peak height (millimeters) was plotted against Bu concentration (micrograms per milliliter) and unknown levels of Bu from plasma extracts were determined from the graph. Concentration was linear with peak height from 0.025 to at least 20 μg/mL (81.2 μmol/L) Bu. The limit of detection of the assay was 0.1 μmol/L Bu. The within-day coefficients of variation were 2.6% for a Bu concentration of 14.7 μmol/L (n = 9) and 4.8% for a Bu concentration of 6.8 μmol/L (n = 10). The between-day coefficients of variation were 5.1% for a Bu concentration of 14.7 μmol/L (n = 7) and 13.4% for a Bu concentration of 6.8 μmol/L (n = 7).

A simple one-compartment computer model was developed for pharmacokinetic analysis with simple exponential terms used for absorption and elimination. Kinetic parameters were varied until the computer-generated theoretical curve conformed on visual inspection with the plotted plasma drug measurements. The model gave a good fit of the actual data points in all cases except one patient with trisomy 21 (patient 113), whose data was excluded from the analysis. The profile of plasma Bu levels found in this patient was quite different from all other patients, suggestive of a biphasic absorption mechanism. Another patient (126) also had trisomy 21, but did not have atypical pharmacokinetics, so the cause of the atypical pharmacokinetics in patient 113 remains unknown.

Means and standard deviations were calculated for the various pharmacokinetic parameters and compared by the Wilcoxon Rank Sum test using the Statistical Package for Interactive Data Analysis (SPIDA) version 6.04 (The Statistical Computing Laboratory, Macquarie University, NSW, Australia). Pharmacokinetic parameters from literature sources were taken from the published figures and, if necessary, converted to micromoles per liter or converted to a different time unit, in order to allow direct comparisons. In some cases published data tables were used to calculate means and standard deviations, elimination and absorption constants being first converted to half-lives.

RESULTS

A semi-logarithmic plot of the disposition of Bu from a single daily oral dose of Bu administered at 4 mg/kg or 150 mg/m² is shown in Fig 1. Mean plasma Bu levels and 95% confidence intervals for the two groups of patients are shown for a 24-hour period. There was a short delay time after the dose was administered before Bu appeared in the plasma. Bu reached a peak in plasma after about 2 hours and was almost completely eliminated by 24 hours as 17 of 21 patients had 24-hour trough-levels below the 0.1 μmol/L limit of detection and the remaining 4 were below 0.4 μmol/L.

None of the patients vomited on the day that Bu pharmacokinetics was performed.

In 9 of 21 patients a 4 mg/kg single dose per day regimen was used and the remaining 12 patients had a 150 mg/m²
Fig 1. Semi-logarithmic plot of the mean disposition of busulfan in children on the 4 mg/kg and 150 mg/m² dosing regimens. The pharmacokinetics of these two groups are compared in Table 2. There were no significant differences between the two groups in age, absorption, elimination, delay time, clearance, volume of distribution, or time when the plasma peak occurred. For the 150 mg/m² dose regimen mean values for dose (milligrams per kilogram), area under the curve (AUC) and peak Bu concentration were significantly higher, being, respectively, 35%, 63%, and 49% higher than the 4 mg/kg dose regimen. The systemic exposure to Bu was higher for the 150 mg/m² single dose regimen than the 4 mg/kg regimen (Fig 1).

We found a positive correlation between AUC and age in the group of 9 children taking a single daily Bu dose of 4 mg/kg (Fig 2A). This shows that younger children have a lower systemic exposure to Bu than older children on the same weight-based dose regimen. There was no correlation between AUC and age for the 150 mg/m² regimen (Fig 2B), demonstrating that younger children had equivalent exposure to Bu as the older children when the surface area based dose was used. Bu pharmacokinetics in more children less than 5 years of age will confirm this finding.

We have observed a very clear correlation between AUC and dose (milligrams per kilogram) on the whole group of
and the remainder four times per day. We found a negative correlation between clearance and age in the whole group of 21 patients (Fig 3B), showing that younger children had higher Bu clearance. A previous study also showed that clearance was significantly higher in younger children. There was also a negative correlation between volume of distribution and age (Fig 3C), indicating that younger children in our study had higher distribution volumes than older children.

Younger children in the study were given crushed tablets of Bu whereas the older children were given whole tablets. Patients taking crushed tablets had a significantly shorter delay time (Table 3), but there was no significant difference in absorption, elimination, AUC, or dose (milligram per kilogram). The mean delay time was 40 minutes for whole tablets compared with 8 minutes for crushed tablets. The possibility was previously raised that the form of drug administration (crushed tablets or capsule) may affect absorption, but we have shown that the form of drug administered affects delay time rather than absorption.

Toxicity and outcome are summarized in Table 1. One patient suffered a brief convolution after the third dose of Bu. She was on carbamazepine and after loading with oral clonazepam the fourth dose of Bu was given uneventfully. Definite VOD occurred in one patient at the 4 mg/kg dose and another had possible VOD with the 150 mg/m² dose (124), but this is uncertain as she also had skin, gut, and liver graft-versus-host disease. Interstitial pneumonitis of unknown etiology requiring ventilator support also occurred in this patient. The pharmacokinetic profiles of the patients who developed VOD or pneumonitis did not differ from the rest of the group, but the numbers are small.

**DISCUSSION**

High-dose therapy with Bu has customarily been based on body weight as a dose of 1 mg/kg four times daily. We have used a single daily dose of 4 mg/kg and found the

![Graph A](image)

**Fig 3.** Correlations obtained for various pharmacokinetic parameters in the whole group of 21 children. (A) Positive correlation of AUC and dose (milligrams per kilogram). (B) Negative correlation of clearance and age. (C) Negative correlation of distribution volume and age.

21 patients with a dose range from 3.9 to 7.25 mg/kg (Fig 3A). A recent report demonstrated a good correlation between AUC and dose (mg/kg) with a dose range from 0.9 to 2.6 mg/kg where 26/40 patients received Bu twice daily

![Graph B](image)

![Graph C](image)

**Table 3. Crushed or Whole-Tablet Dose Effects**

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Whole Tablets (n = 11)</th>
<th>Crushed Tablets (n = 10)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>109</td>
<td>34.6</td>
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<tr>
<td>Range</td>
<td>67.5-162</td>
<td>15.5-64</td>
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</tr>
<tr>
<td>Delay (min)</td>
<td>Mean ± SD</td>
<td>40 ± 24</td>
<td>8 ± 13</td>
</tr>
<tr>
<td>Time peak Bu (min)</td>
<td>Mean ± SD</td>
<td>138 ± 5</td>
<td>108 ± 31</td>
</tr>
<tr>
<td>K (abs) (h⁻¹)</td>
<td>Mean ± SD</td>
<td>1.12 ± 0.49</td>
<td>1.09 ± 0.46</td>
</tr>
<tr>
<td>T/2 (abs) (min)</td>
<td>Mean ± SD</td>
<td>42 ± 33</td>
<td>44 ± 16</td>
</tr>
<tr>
<td>K (elim) (h⁻¹)</td>
<td>Mean ± SD</td>
<td>0.30 ± 0.05</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>T/2 (elim) (min)</td>
<td>Mean ± SD</td>
<td>145 ± 24</td>
<td>144 ± 30</td>
</tr>
<tr>
<td>AUC (µmol/L, h)</td>
<td>Mean ± SD</td>
<td>100 ± 25</td>
<td>107 ± 48</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>Mean ± SD</td>
<td>4.8 ± 0.8</td>
<td>5.6 ± 1.2</td>
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</table>
regimen well tolerated, with little toxicity. As our regimen of 4 mg/kg/d was well tolerated but associated with an appreciable relapse rate following autologous BMT, it was logical to consider dose escalation. One approach was to use a dosing regimen based on body surface area. Pharmacokinetic studies showed that a surface-area based single dose of 150 mg/m² Bu in children was on average 35% higher than the weight-based 4 mg/kg dose and produced a 63% increase in the AUC indicating considerably higher systemic exposure was achieved. This higher dose was also well tolerated with little toxicity.

A comparison between our results for the 4 mg/kg and 150 mg/m² regimens with previously reported Bu pharmacokinetics is shown in Table 4. The values we obtained for mean delay time, absorption half-life, elimination half-life, clearance, distribution volume, and time to peak Bu compared well with the results obtained by other centers. The mean peak plasma level for our 4 mg/kg dose was found to be 14.0 μmol/L, which was 4.2-fold higher than the value reported by Vassal et al.10 using a 1 mg/kg dose. For our 150 mg/m² group we found a 4.1-fold higher mean AUC than that found by Vassal et al.10 using a 37.5 mg/m² dose and a 6.7-fold higher mean AUC than that found by Yeager et al.11 in children on a 38.9 mg/m² dose. The comparisons show that systemic exposure on a single daily dose weight-based regimen (1 X 4 mg/kg) is equivalent to a qid regimen (4 X 1 mg/kg) and that the systemic exposure for a single daily dose surface-area based regimen (1 X 150 mg/m²) is the same or greater than qid (4 X 37.5 mg/m² or 4 X 38.9 mg/m²) regimens.

We observed that AUC increased with age for a weight-based regimen confirming that younger children have a relatively reduced systemic exposure to Bu on this commonly used regimen. Younger children received equivalent systemic exposure to the older children when the dose was based on body weight. It has been proposed that higher clearances and larger volumes of distribution may lower systemic exposure in younger children. The higher clearance and larger volume of distribution higher than that found by Vassal et al using a 1 mg/kg dose. In contrast to the mean peak level above, the mean AUC was 4.3-fold higher than Hassan et al.2 for a 1 mg/kg dose. For our 150 mg/m² group we found a 4.8-fold higher mean AUC than that found by Vassal et al.10 using a 37.5 mg/m² dose and a 6.7-fold higher mean AUC than that found by Yeager et al.11 in children on a 38.9 mg/m² dose. The comparisons show that systemic exposure on a single daily dose weight-based regimen (1 X 4 mg/kg) is equivalent to a qid regimen (4 X 1 mg/kg) and that the systemic exposure for a single daily dose surface-area based regimen (1 X 150 mg/m²) is the same or greater than qid (4 X 37.5 mg/m² or 4 X 38.9 mg/m²) regimens.


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