Busulfan Disposition in Children

By Louise B. Grochow, William Krivit, Chester B. Whitley, and Bruce Blazar

Children receive busulfan orally as part of myeloablative therapy before bone marrow transplantation for malignant and nonmalignant conditions. Children have been reported to have a low incidence of severe toxicity and significant rates of failure to achieve full engraftment. We evaluated the disposition of busulfan in children between 2 months and 3.6 years of age with lysosomal storage diseases, leukemia, and immunodeficiency disorders receiving oral doses of 1 or 2 mg/kg using a gas chromatographic assay. Peak concentrations were lower than those previously reported for adults, ranging from 1.4 to 5.2 μmol/L. The harmonic mean of the elimination half-life was 92 minutes, which is only slightly faster than that for adults (140 minutes). However, the area under the curve ranged from 400 to 1,000 (715 ± 240) μmol · min/L, substantially lower than in adults receiving 1 mg/kg (range, 710 to 5,100 μmol · min/kg; mean ± SD, 2,180 ± 1,200). The apparent volume of distribution (assumed complete bioavailability) ranged from 0.28 to 3.53 L/kg (1.42 ± 0.86), which is more than twice that reported for adults (0.60 ± 0.42). Busulfan clearance rate normalized to surface area is twice as high in children (200 ± 100 mL/min/m²) as it is in adults (95 ± 54 mL/min/m²). Alterations in bioavailability (absorption or first pass elimination) or in actual volume of distribution may account for these differences in drug disposition. The observed differences suggest the need for separate phase I dose escalation studies in children with accompanying pharmacokinetic assessment.

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BUSULFAN (MYLERAN, Burroughs-Wellcome Co, Research Triangle Park, NC; 1,4-bis [methanesulfonyloxy] butane) is widely used as part of myeloablative chemotherapy for bone marrow transplantation for both malignant and nonmalignant conditions. The busulfan dose of 1 mg/kg every 6 hours for 16 doses was established in phase I trials in adults in combination with cyclophosphamide and subsequently reconfirmed for busulfan alone. In these trials the dose-limiting toxicities for busulfan were mucositis and hepatic veno-occlusive disease.

In children, minimal toxicity directly attributable to busulfan has been reported in most series, and some institutions have reported higher rates of failure to achieve full engraftment in children.

Because it seemed possible that differences in drug disposition in children resulting in decreased drug exposure might result in both decreased toxicity and decreased efficacy, we proposed to evaluate drug disposition in children receiving busulfan as part of a preparative regimen for bone marrow transplantation. After modifying our original assay to make it feasible to measure busulfan concentrations using small blood samples, we measured busulfan concentrations in young children with a variety of malignant and nonmalignant diseases.

METHODS

Patient selection and treatment. Plasma samples were obtained from 21 patients between 2 months and 5 years of age undergoing bone marrow transplantation between August, 1986, and December, 1988. Parents of all patients provided informed consent for participation in this protocol and in the transplantation protocol, which were approved by the institutional review board of the University of Minnesota Medical School, Minneapolis, MN, in accord with institutional, state, and federal guidelines. Busulfan was given either by mouth (as pulverized tablets in applesauce) or by nasogastric tube. Seven patients with Hurler syndrome, one patient with Niemann-Pick disease, and one patient with Krabbe disease received busulfan 1 mg/kg every 6 hours for eight doses, followed by 1.25 mg/kg for eight additional doses. Five patients with acute nonlymphocytic leukemia and one with aplastic anemia received 1 mg/kg every 6 hours for 16 doses as conditioning for a second transplant. Five patients with immunodeficiency disorders (two with Wiskott-Aldrich syndrome, two with severe combined immunodeficiency, and one patient with viral-associated hemophagocytic syndrome) and one patient with chronic myelocytic leukemia received 2 mg/kg every 12 hours for eight doses.

Assay. Busulfan concentrations in plasma were measured using a previously described gas chromatographic assay.

Samples. Heparinized whole blood samples (3 cc) were obtained from indwelling venous catheters before busulfan and at 30, 60, 90, 120, 180, and 360 minutes after the first dose of busulfan. Seven patients had additional samples obtained on the same schedule after one subsequent dose (one patient after his fourth dose of 2 mg/kg, one after his eighth dose of 2 mg/kg, one after his 12th dose of 1 mg/kg, and four after their 16th dose of 1 mg/kg). Plasma was separated, frozen at −20°C, and shipped frozen to Baltimore, MD, for assay between 1 week and 6 months after treatment.

Pharmacokinetics. Individual disposition curves were visually evaluated for suitable nonlinear models. An inadequate number of samples (≤4) was obtained for three patients; one patient had drug concentrations less than the sensitivity of the assay (0.1 μmol/L); and three patients had concentrations measured that had no apparent pattern with respect to time and were not further analyzed. Fourteen patients had data that were adequately fit by a one-compartment model with first order absorption and elimination. Parameter estimation using nonlinear least squares analysis was performed using PCNONLIN (Statistical Consultants, Lexington, KY).

Kinetic parameters estimated by this program are the absorption rate constant Kα, the elimination rate constant Kε, the hybrid volume of distribution V (calculated as though the bioavailability were...
100%), and the absorption lag time. Area under the curve (AUC) contributed by a single dose of busulfan, absorption and elimination half-lives, and total body clearance rate were calculated using the parametric estimates.

Adult patients. We have previously reported the disposition of busulfan in patients receiving oral doses of 1 mg/kg as part of a preparative regimen for allogeneic or autologous bone marrow transplantation as treatment for acute myelogenous leukemia, lymphoma, acute lymphocytic leukemia, chronic myelogenous leukemia, and aplastic anemia. As previously described, blood samples were obtained at 20, 40, 60, 75, 90, 105, 120, 240, 241, 359, and 360 minutes after the first dose of busulfan, concentrations were determined by high-pressure liquid chromatography (HPLC), and the data fit to a one-compartment model with first order absorption and elimination using PCNONLIN. Of the 28 patients previously described, 25 were ≥16 years old, and their pharmacokinetic parameters have been compared with the 14 young children evaluated in this study, using a two-tailed Student's t-test for independent variables (CSS; Statsoft, Tulsa, OK).

RESULTS

Disposition curves were adequately described by a one-compartment pharmacokinetic model with first order absorption and elimination for 14 children between 2 months and 3.6 years of age. Typical elimination curves after the first busulfan dose are presented in Fig 1. There was considerable variability in individuals' patterns of absorption and elimination, with peak concentrations ranging from 1.4 to 5.2 μmol/L for patients receiving 1 mg/kg, and from 2 to 4.8 μmol/L for patients receiving 2 mg/kg. Table 1 presents the absorption and elimination constants, volumes of distribution (assuming complete bioavailability), and lag times estimated from drug concentrations obtained after the first dose of busulfan, as well as the calculated clearance rates. The elimination half-life ranged from 38 to 260 minutes, with a harmonic mean of 92 minutes. The AUC ranged from 400 to 983 μmol·min/L in patients receiving 1 mg/kg, and from 401 to 998 μmol·min/L in patients receiving 2 mg/kg.

In the previously published study of 25 patients ≥16 years old, the elimination half-life ranged from 58 to 433 minutes, with a harmonic mean of 139 minutes. The AUC ranged from 710 to 5,140 μmol/min/L. Table 2 compares the pharmacokinetic parameters for the 14 children reported here with the 25 adults previously reported, with P values for a two-tailed t test for independent variables.

In addition to intrapatient variability in the disposition of busulfan, there may be variability for a single patient receiving multiple doses. Seven children had plasma samples obtained after two busulfan doses. Four of these children had minimal differences in parameter estimates for the two doses. Two children had changes in elimination half-life: one increased from 60 minutes after the first dose to 95 minutes after the fourth dose; the other had a decrease in half-life from 230 minutes to 80 minutes. One child had a very large change in apparent volume of distribution, from 1.34 L/kg to 5.9 L/kg after the sixteenth dose.

DISCUSSION

Children between 2 months and 5 years of age with a variety of neoplastic and non-neoplastic diseases demonstrated differences in busulfan disposition compared with adults previously studied. Clearance rate normalized to body surface area in these children appears to be 2.2-fold that in adults. There is also a 2.4-fold difference in the apparent volume of distribution, and less difference in the elimination rate constant K. Without parenteral drug, the
bioavailability and the actual volume of distribution cannot be assessed. The fact that most of the difference in clearance rate between adults and small children appears as a difference in V suggests that drug absorption and/or first-pass elimination of busulfan may differ substantially in young children compared with adults.

Three of seven children studied after two busulfan doses had significant alterations in apparent clearance rate (one increased and one decreased) and in apparent volume of distribution (one increased) in subsequent doses compared with the first dose. Ehrrson et al18 reported a significant change in K and V in one of five patients receiving multiple doses of 2 to 6 mg of busulfan, and a significant change in the apparent volume of distribution in another patient. Trough levels measured by Hassan et al19 after each of 16 doses of 1 mg/kg were reproducible. In six adults receiving high doses of busulfan,17 minor differences in kinetic parameters were found between the first and fifth or sixteenth doses. The true frequency of intrapatient variability is unknown; we are assessing this further in a randomized trial of dose adjustment based on therapeutic monitoring.

There are a variety of ways in which drug handling in young children may differ from adults.20-22 Altered drug absorption might be due to differences in drug administration (crushed tablets in applesauce versus tablets enclosed in gelatin capsules). Children have higher gastric pH and differences in transit time, alterations in volume of distribution from changes in the proportion of body fat and body water, and alterations in first-pass clearance related to increased metabolic capacity compared with adults.

Busulfan is extensively metabolized in the liver.23 First-pass clearance rate of busulfan may be higher in children than in adults.20,24 Since parenteral formulations are not currently available, this hypothesis cannot be directly tested at present. Significant first-pass clearance rates have been documented for other drugs that are metabolized in the liver, including 6-mercaptopurine and 5-fluorouracil,6,8,20,28 and increased metabolic disposition of anticonvulsants has been identified in infants between 3 months and 3 years of age.20

Busulfan given in high doses for transplantation is associated with severe toxicities in adults in addition to myelosuppression, including veno-occlusive disease,10,18,27 mucositis, pneumonia, diarrhea, seizures, and rash.2,28 These toxicities are reported to be less common in children.3-7,12-14 Hartmann et al15 reported only 1 of 20 patients (ages 1 to 17 years) with severe mucositis, and no patient with drug-induced veno-occlusive disease. Lucarelli et al12 reviewed 30 children aged 6 months to 7 years and reported minimal side effects. Shaw et al16 reviewed 50 children receiving between 8 and 21 mg/kg of busulfan. One child had seizures and symptoms suggestive of veno-occlusive disease (but a nondiagnostic biopsy), and five others developed jaundice (with biopsies documenting other etiologies). None of their 16 children receiving 8 to 11.9 mg/kg of busulfan had interstitial pneumonitis, but 5 of 31 children receiving 12 to 21 mg/kg did develop interstitial pneumonitis. In the present series, minimal toxicity has also been observed.4,14 However, children have been reported to have a lower incidence of hepatic veno-occlusive disease in regimens that do not include busulfan. Jones et al33 have reported a lower incidence of veno-occlusive disease in children under 10 years old (3 of 36; 8%) than in adolescents and adults (49 of 199; 25%). McDonald et al11 have reported a similar trend (3 of 40; 7.5% versus 50 of 215; 23%). Although veno-occlusive disease and other severe transplant-

### Table 1. Busulfan Pharmacokinetic Parameters

<table>
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<tr>
<th>UPN</th>
<th>Age (y)</th>
<th>Dose (mg/kg)</th>
<th>V (L/kg)</th>
<th>K (min⁻¹)</th>
<th>Kₗ (min⁻¹)</th>
<th>Tlag (min)</th>
<th>Clearance (mL/min/kg)</th>
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</table>

Abbreviations: UPN, unique patient number; Tlag, absorption lag time.

### Table 2. Busulfan Disposition in Adults (≥16 Years) and Children (0.2 to 3.6 Years)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adults (n = 25)</th>
<th>Children (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (L/kg)</td>
<td>0.595 ± 0.421</td>
<td>1.42 ± 0.86</td>
<td>&lt;.001</td>
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<tr>
<td>K (min⁻¹)</td>
<td>0.005 ± 0.003</td>
<td>0.0075 ± 0.0044</td>
<td>.03</td>
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<tr>
<td>Kₗ (min⁻¹)</td>
<td>0.017 ± 0.019</td>
<td>0.0412 ± 0.061</td>
<td>.09</td>
</tr>
<tr>
<td>AUC (µmol · min/L)</td>
<td>2.180 ± 1.200</td>
<td>715 ± 241</td>
<td>&lt;.001</td>
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<tr>
<td>Clearance (mL/min/kg)</td>
<td>2.5 ± 1.4</td>
<td>8.44 ± 4.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Clearance (mL/min/m²)</td>
<td>95 ± 54</td>
<td>197 ± 101</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.
associated toxicities may be multifactorial, the results of this study suggest that the decreased toxicity reported may be due to underdosing children based on busulfan regimens established in adults.

Several authors have expressed concern about engraftment failures in children who have received busulfan (8 to 16 mg/kg) and cytoxan as a preparative regimen.\(^{3,4,12,13}\) Hobbs et al\(^{13}\) reported that they changed their regimen to 80 mg/m\(^2\)/d × 4, with a minimum dose of 16 mg/kg and a maximum dose of 20 mg/kg. Lucarelli et al\(^{12}\) found that 5 of 24 children with β-thalassemia failed to engraft after treatment with 14 mg/kg, but only 1 of 16 treated at 16 mg/kg failed to engraft.

The results presented here suggest that achieving busulfan exposure in children equivalent to adults may require doses 50% to 100% greater on a weight basis. Providing pediatric doses on the basis of surface area\(^{20,22,29}\) will more closely approximate adult exposures, but clearance rate normalized to body surface area in the children studied was still twice the clearance rate in adults. Since recurrent leukemia remains a significant problem for patients undergoing bone marrow transplantation,\(^{16}\) achieving increased drug exposure may provide better antileukemic effect. In addition, higher doses may enhance the likelihood of donor engraftment. Since no pediatric phase I study has been done, we are performing a dose escalation study for busulfan in pediatric patients with pharmacokinetic monitoring. Additional information will be needed to provide dosing recommendations for older children and adolescents.

ACKNOWLEDGMENT

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REFERENCES

22. Evans WE, Relling MV, de Graaf S, Rodman JH, Pieper JA, Christensen ML, Crom WR: Hepatic drug clearance in children:


Busulfan disposition in children

LB Grochow, W Krivit, CB Whitley and B Blazar