The apparent oral clearance of busulfan expressed relative to body surface area is 2–3-fold higher in children 1–4 years old than it is in adults. The first step in busulfan elimination is the formation of a tetrahydrothiophenium ion (THT+) in a glutathione S-transferase-catalyzed reaction. We present computer simulations that demonstrate that the ratio of the AUC of THT+ to that of busulfan over 6 h \([\text{AUCTHT+}/\text{AUCbus}0]\) is highly correlated \(r^2 = 0.805\) with the determinants of THT+ formation and is virtually independent of the determinants of its elimination \(r^2 = 0.0201\). We compared \([\text{AUCTHT+}/\text{AUCbus}0]\) determined in 14 children (0.5–4 years) to that of 11 adults (12–54 years) and found a 1.5-fold elevation in the area ratio \(P = 0.0098\) and a similarly significant increase in busulfan apparent oral clearance expressed relative to body surface area \(P = 0.042\). The only common explanation for the elevated busulfan apparent oral clearance and \([\text{AUCTHT+}/\text{AUCbus}0]\) is an enhanced ability of children to metabolize busulfan through glutathione conjugation.

**INTRODUCTION**

High-dose busulfan with allogeneic bone marrow transplantation is a potentially curative treatment for hematopoietic cancers and certain genetic diseases (1). Busulfan is a bifunctional electrophilic cytotoxin that acts putatively through DNA alkylation. In the bone marrow transplantation setting, excessively high busulfan plasma concentrations are associated with hepatic veno-occlusive disease, whereas low levels allow relapse of chronic myelogenous leukemia, and even lower levels allow rejection of grafted marrow (2–5).

In pharmacokinetic studies, the CL/F of busulfan expressed relative to BSA (in m²) is 2–3-fold higher in children (1–4 years old) than it is in adults (4, 6, 7). CL/F is a pharmacokinetic term defined as the ratio of clearance (a specific measure of efficiency of elimination):the case, the gastrointestinal tract) to the systemic circulation (8). Because CL/F is a function of both drug absorption and metabolism, busulfan apparently is either absorbed less extensively in children or metabolized more efficiently by them. Currently, there is no approved i.v. formulation of busulfan available to distinguish between these possibilities.

The primary elimination pathway for busulfan involves GSH conjugation, resulting in the formation of THT+. The THT+ species that is formed directly on conjugation of busulfan with GSH is \(\gamma\)-glutamyl-\(\beta\)-(S-THT+)-alanyl-glycine, which has been identified in rat bile after i.v. infusion (9) and in the rat liver perfusion model (10). THT+ accounted for 38% of busulfan supplied to the liver after a 4-h isolated rat liver perfusion experiment. Ethylnitro acid coadministration reduced THT+ formation to 12% of the dose after 4 h, presumably by inhibiting GST or depleting GSH (10). In humans, the busulfan-GSH conjugate has never been measured. However, THT has been detected after base treatment of urine (11). Recently, we demonstrated that busulfan conjugation with GSH is catalyzed by human liver cytosolic GST (12), and that GSTA1–1 is the predominant isoenzyme responsible for busulfan conjugation (13).

We have now characterized the formation of THT+ as reflected in the plasma concentrations of the ion and parent drug after oral busulfan administration to patients being prepared for hematopoietic stem cell transplantation. We hypothesized that if children have an enhanced ability to metabolize busulfan, then they should have elevated \([\text{AUCTHT+}/\text{AUCbus}0]\) relative to busulfan AUC \([\text{AUCbus}0]\) in comparison to adults. We present mass balance equations to show that the ratio of \([\text{AUCTHT+}/\text{AUCbus}0]\) is independent of the fraction of the dose absorbed and computer simulations to support interpretation of these data when the AUCs are measured for 6 h after the first dose of busulfan \([\text{AUCbus}0]\). The computer simulations show that for these case, \(\text{AUCTHT+}/\text{AUCbus}0\) yields selective insight into the ability to form THT+. The data indicate that young children have an enhanced ability to form the GSH conjugate of busulfan.

**PATIENTS AND METHODS**

**Reagents and Chemicals.** GSH, monobromobimane, THT, 1-bromopentane, and busulfan were purchased from Sigma Chemical Co. (St. Louis, MO). GC-MS-grade hexane was purchased from Burdick and Jackson (Muskegon, MI). All other solvents were analytical grade or higher.

**Patients.** Blood for busulfan and THT+ analysis was obtained from 25 patients receiving busulfan in preparation for allogeneic bone marrow transplantation. All patients received a phenytoin loading dose of 5 mg/kg administered three times and a 300-mg daily maintenance dose for seizure prophylaxis. Patients received busulfan tablets at a dose of 1–2 mg/kg body weight with the exception of three children who received a 0.5-mg/kg dose (Table 1) as required to achieve steady-state busulfan concentrations required for therapy. Because the pharmacokinetics of busulfan are independent of dose (14), differences in busulfan dose did not influence the results of the study. For young children incapable of swallowing tablets, a suspension was administered, otherwise busulfan was administered as 3-mg tablets (Glaxo-Wellcome, Research Triangle Park, NC). Plasma GSH concentration was determined in a second group of patients in whom THT+ levels were not measured.

**For measurement of busulfan and THT+ concentrations in plasma, serial blood samples (2–3 ml) were collected in heparinized tubes after the administration of the first dose of busulfan. For adult patients, samples were collected 30, 60, 90, 120, 180, 240, 300, and 360 min after the dose. For children (age < 5 years), an additional sample at 15 min was taken to better characterize the absorption phase, which is more rapid in children receiving the suspension than in adults receiving tablets. For patients at the Fred Hutchinson Cancer Research Center, plasma was separated within 1 h of collection and stored at −20°C until analysis. For patients at other institutions, plasma samples were frozen and shipped on dry ice to the Fred Hutchinson Cancer Research Center on the day of collection and were kept frozen until analysis. All samples were assayed within 24 h of blood collection. Blood samples drawn for GSH analysis (Fred Hutchinson Cancer Research Center patients only) were ob-
Table 1: Characteristics of busulfan/THT<sup>+</sup> patients

<table>
<thead>
<tr>
<th>UPN</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>BSA (m&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Busulfan dose</th>
<th>mg</th>
<th>mg/kg</th>
<th>mg/m&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10018</td>
<td>M</td>
<td>12</td>
<td>34</td>
<td>1.18</td>
<td>34</td>
<td>1.01</td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td>10371</td>
<td>F</td>
<td>17</td>
<td>56</td>
<td>1.65</td>
<td>58</td>
<td>1.04</td>
<td>35.2</td>
<td></td>
</tr>
<tr>
<td>10450</td>
<td>M</td>
<td>25</td>
<td>72</td>
<td>1.92</td>
<td>72</td>
<td>1.01</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>10160</td>
<td>F</td>
<td>34</td>
<td>72</td>
<td>1.88</td>
<td>72</td>
<td>1.00</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>10101</td>
<td>M</td>
<td>36</td>
<td>70</td>
<td>1.82</td>
<td>70</td>
<td>1.00</td>
<td>38.5</td>
<td></td>
</tr>
<tr>
<td>9595</td>
<td>F</td>
<td>36</td>
<td>61</td>
<td>1.67</td>
<td>61</td>
<td>0.99</td>
<td>35.9</td>
<td></td>
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<tr>
<td>9711</td>
<td>F</td>
<td>38</td>
<td>59</td>
<td>1.63</td>
<td>58</td>
<td>0.99</td>
<td>35.6</td>
<td></td>
</tr>
<tr>
<td>9824</td>
<td>M</td>
<td>45</td>
<td>72</td>
<td>1.75</td>
<td>72</td>
<td>1.00</td>
<td>41.1</td>
<td></td>
</tr>
<tr>
<td>9933</td>
<td>M</td>
<td>46</td>
<td>68</td>
<td>1.74</td>
<td>60</td>
<td>0.88</td>
<td>40.9</td>
<td></td>
</tr>
<tr>
<td>9936</td>
<td>M</td>
<td>53</td>
<td>95</td>
<td>2.02</td>
<td>84</td>
<td>0.88</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>6000</td>
<td>M</td>
<td>54</td>
<td>95</td>
<td>2.13</td>
<td>84</td>
<td>0.88</td>
<td>39.4</td>
<td></td>
</tr>
</tbody>
</table>

Note: The table presents characteristics of busulfan/THT<sup>+</sup> patients, including sex, age, weight, body surface area (BSA), and busulfan dose. The data are presented as mean ±SD for the specified number of patients.

...plastic conical tubes, and 30 μl of 50% trichloroacetic acid were added. The tubes were vortexed and centrifuged for 2 min. The supernatant was transferred to a clean 1.5-ml conical tube for storage at −20°C until high-performance liquid chromatography analysis. The high-performance liquid chromatography system consisted of a Hewlett-Packard 1050 series system with a quaternary pump, an autosampler, and a 1046A programmable fluorescence detector. The excitation wavelength was 394 nm, and the emission wavelength was 484 nm. Mobile phase A was 87.5 mm acetate buffer with 6% acetonitrile (pH 7.8), whereas mobile phase B was water with 40% acetonitrile (v/v). Initially, the mobile phase was set at 92.2% A and 2.8% B. As soon as the sample was injected, B was linearly increased to 4.8% for the first 7.26 min and then abruptly increased to 100% B to wash the column for 3 min, at which time the mobile phase was returned to the initial condition, and the column was reequilibrated for 11 min before the injection of the next sample. The flow rate was 0.8 ml/min. Analyses were separated using a 4.6 mm × 10 -cm, 3-μm Ramin microsorb C18 column. The injection volume was 10 μl, and the run time was 21 min. A time-averaged GSH concentration was calculated for each dosing interval by calculating the AUC and dividing by 6 h.

Liver Size. A direct measurement of liver size was performed in the course of autopsy of cadavers at Harborview Medical Center (Seattle, WA). All specimens were obtained from individuals who had died within 2 days of head trauma. Organ weights were measured in 38 subjects between the ages of 1 and 4 years (1.91 ± 1.42 years) and 49 subjects between the ages of 14 and 36 years (27 ± 6 years). Liver weight was expressed relative to either body weight (in kg) or BSA (in m<sup>2</sup>). BSA was calculated as $\text{BSA} = \text{log}^2$.
The ratio of AUC_{11}/AUC_{|} over both time intervals was evaluated by randomly constructing 685 individuals from Gaussian distributions of pharmacokinetic parameter values corresponding to measured or estimated values for busulfan and THT^+ using the random number generator in Excel. The AUCs of parent drug and metabolite were calculated according to the time-integrated forms of equations B and C are shown in Fig. 2 for AUC_{\infty}. The numerical identity of the area ratio (AUC_{m}/AUC_{p})_{0-\infty} and the determinants of the ratio as given on the right side of equation A, F_{m}(CL_{m}/Cl_{m} + (CL/Cl_{m})(1 - F_{m}/F_{m})), validates the simu-

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RESULTS

Plasma busulfan and THT^+ concentrations over the first dosing interval in a representative child (UPN 11300) and adult (UPN 10450) are shown in Fig. 1. In both adults and children, THT^+ concentrations rose over the 6-h sampling period to a broad peak from which a decline was not evident during the dosing interval. A second dose of busulfan administered at 6 h as required for therapy prevented estimation of an elimination rate constant for THT^+ from the decline of THT^+ concentration and, therefore, AUC_{0-\infty} of the metabolite. 

To estimate the half-life of THT^+ for computer simulations conducted to assess the utility of AUC_{0-\infty}, the accumulation of THT^+ to steady state was evaluated by assaying THT^+ concentration over the 6-h dosing interval after doses 1, 5, and 9 of a 16-dose regimen given over 4 days (every 6 h). These measurements were carried out in two adult patients with a constant busulfan dose. Mean (AUC_{THT}^+)_{0-\infty} was 27.2, 104, and 102 pmol x min/ml after the first, fifth, and ninth doses of busulfan. There was a 3.8-fold increase in (AUC_{THT}^+)_{0-\infty} between the first and fifth dose of busulfan (P < 0.001), and there was no change between the fifth and ninth dose (P = 0.31). Thus, THT^+ attained steady-state levels after the first 24 h of busulfan administration, from which it was evident that the apparent elimination half-life for THT^+ is ~5 h.

The results of the computer simulations in which plasma concentration of parent drug and metabolite were calculated according to the time-integrated forms of equations B and C are shown in Fig. 2 for AUC_{0-\infty}. The numerical identity of the area ratio (AUC_{m}/AUC_{p})_{0-\infty} and the determinants of the ratio as given on the right side of equation A, F_{m}(CL_{m}/Cl_{m} + (CL/Cl_{m})(1 - F_{m}/F_{m})), validates the simu-

![Graph](Fig. 1. Representative time course of plasma busulfan and THT^+ concentrations in a child (upper panel; UPN 11300) and an adult (lower panel; UPN 10450).)
Fig. 2. The relationship between \( \frac{\text{AUC}_m}{\text{AUC}_p} \) and selected parameters from equations A–C as determined by computer simulation.

The lack of correlation with either \( V \) or \( V_m \) also validates the simulations, because \( \text{AUC}_p \) and \( \text{AUC}_m \) are theoretically independent of volumes of distribution. Because \( \text{AUC}_p = \frac{\text{CL}_f}{\text{CL}_m} \) for a drug administered i.v. and eliminated only in the liver, the correlation between \( \text{AUC}_m/\text{AUC}_p \) was evaluated, and \( r^2 \) was found to be 0.692. This result is expected, because in the busulfan-THT model, \( \text{CL}_f \) does not completely account for the formation of the metabolite (the terms \( \text{F}_H \) and \( \text{F}_G \) describe loss of drug to formation of metabolite on first pass). \( \text{CL}_f/\text{CL}_m \) were weakly related to \( \text{AUC}_m/\text{AUC}_p \), with \( r^2 \) values of 0.287 and 0.339, respectively. To evaluate the correlation between all parameters that account for formation of the busulfan-GSH conjugate and its elimination as they appear in the model, correlations between \( \text{AUC}_m/\text{AUC}_p \) and \( \text{F}_H(\text{CL}_f + \text{CL}(1 - \text{F}_H)) \) and \( 1/\text{CL}_m \), respectively, were evaluated and found to be 0.644 and 0.303.

The analogous simulations for \( \text{AUC}_m/\text{AUC}_p \) are shown in Fig. 3. In this case, the relationships between the area ratio and the determinants of the formation of metabolite on the right side of equation A are no longer numerically identical \( (r^2 = 0.655) \), because the areas are incomplete. When the determinants of the formation and the elimination of the busulfan-GSH conjugate were evaluated as determinants of \( \text{AUC}_m/\text{AUC}_p \), \( \text{F}_H(\text{CL}_f + \text{CL}(1 - \text{F}_H)) \) was found to be a very strong determinant \( (r^2 = 0.805) \), whereas \( 1/\text{CL}_m \) was found to be weak \( (r^2 = 0.0201) \). This result indicates that \( \text{AUC}_m/\text{AUC}_p \) is a function of the formation of the metabolite and has little dependence on the parameter describing its elimination, \( 1/\text{CL}_m \). Fig. 3 also shows that the area ratio is weakly related to \( V_m \) \( (r^2 = 0.127) \) but is essentially independent of \( V \) \( (r^2 = 0.006) \). This result is expected, because in theory, \( \text{AUC}_p \) is independent of volume of distribution, and \( 1/\text{CL}_m \) is a weak determinant of \( \text{AUC}_m/\text{AUC}_p \), whereas \( \text{AUC}_m \) is only a fraction of \( \text{AUC}_p \). \( \text{CL}_f/\text{CL}_m \) and \( \text{CL}_f \) are all relatively weak determinants of \( \text{AUC}_m/\text{AUC}_p \), with \( r^2 \) of 0.311, 0.363, and 0.396, respectively.

The ratios of \( \text{AUC}_m/\text{AUC}_p \) for children and adults are shown in Table 3. \( \text{AUC}_m/\text{AUC}_p \) was 1.5-fold higher in children \( (P = 0.0098) \). Busulfan \( \text{CL}/\text{F} \) declined with age in a manner consistent with previous reports. Children had a busulfan \( \text{CL}/\text{F} \) that was 2.3-fold higher than that of adults when \( \text{CL}/\text{F} \) was expressed relative to body weight \( (P = 0.0012) \) and 1.5-fold higher when expressed rela-
tive to BSA (P = 0.0423). Although busulfan CL/F was significantly elevated in children, there was no difference in the terminal half-life of busulfan elimination between children and adults. Correlations between (AUC_{THF}/AUC_{BU})_{0→∞} and busulfan CL/F expressed relative to body weight or BSA were observed (r² = 0.738 and P < 0.001 and r² = 0.711 and P < 0.001, respectively; Fig. 4). Excluding the one outlier in these relationships, a child with a busulfan CL/F of 18 ml/min/kg (430 ml/min/m²) had no effect on the estimated slope and intercept; however, the r² values were 0.480 (P < 0.001) and 0.405 (P < 0.001). These results suggest that children are more proficient in forming the GSH conjugate of busulfan than are adults.

To gain some insight into the apparent difference between children and adults in the ability to form GSH conjugates, we measured plasma time-averaged GSH levels in young children and in older children and adults. Serial samples (n = 6) were obtained from patients in the 14348.00.1380.04210.01200.0098 first dose of busulfan. A plot of time-averaged plasma GSH concentration versus age showed no relationship (Fig. 5, upper panel). The mean ± SD GSH concentration was 3.20 ± 1.23 µM in children <4 years old and 4.19 ± 2.06 µM in older children and adults (P = 0.27). In addition, we examined time-averaged plasma GSH concentrations in 3 patients <4 years old and 5 patients >4 years old after doses 1, 5, 9, and 13 of busulfan (Fig. 5, lower panel). Time-averaged plasma GSH concentrations did not change over the course of busulfan therapy.

Because the GSH conjugate of busulfan is formed most actively by GSTA1-1, which is the predominant GST in liver, we examined the relationship between liver size and body size in children and adults at autopsy (Fig. 6). Liver weight expressed relative to body weight was approximately 1.7-fold higher in children ages 1 week to 4 years (0.0345 ± 0.0083 g/kg) than it was in older children and adults (0.0205 ± 0.0050 g/kg; P < 0.001). The ratio of liver weight expressed relative to BSA was not different between young children (0.792 ± 0.222 g/m²) and adolescents and adults (0.851 ± 0.208 g/m²; P = 0.21).

Table 3 Busulfan disposition in children and adults after a single oral dose

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>CL/F (ml/min/kg)</th>
<th>CL/F (ml/min/m²)</th>
<th>t_{1/2} (min)</th>
<th>(AUC_{THF})<em>{0→∞} / (AUC</em>{BU})_{0→∞}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3</td>
<td>7.32</td>
<td>176</td>
<td>118</td>
<td>0.0631</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>3.71</td>
<td>89</td>
<td>24.5</td>
<td>0.0237</td>
</tr>
<tr>
<td>Adults and adolescents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>36</td>
<td>3.18</td>
<td>120</td>
<td>143</td>
<td>0.0421</td>
</tr>
<tr>
<td>SD</td>
<td>14</td>
<td>0.75</td>
<td>27</td>
<td>48.0</td>
<td>0.0120</td>
</tr>
<tr>
<td>P</td>
<td>0.0002</td>
<td>0.0423</td>
<td>0.138</td>
<td>0.0098</td>
<td></td>
</tr>
</tbody>
</table>

The (AUC_{THF})_{0→∞} divided by (AUC_{BU})_{0→∞} after the first dose of busulfan.
greater than that of adults, which agrees with conclusions made by others using indirect measures of liver size or small numbers at autopsy (23–25), whereas the liver:BSA ratio is not different between children and adults. Thus, if children and adults had the same intrinsic clearance per gram of liver weight, children would seem to have a higher clearance when clearance was expressed per kilogram of body weight, but the difference would vanish when clearance was expressed relative to BSA. The same would be true for liver blood flow, assuming it is proportional to liver mass. The observed age-dependent clearances for theophylline, caffeine, carbamazepine, and valproic acid seem to reflect liver size to body weight differences rather than differences in intrinsic clearance per gram of liver weight. This does not seem to be the case with busulfan.

There are no examples of drugs that have age dependence in the extent of the dose absorbed, although there are age-dependent changes in the physiology of the gastrointestinal tract (20). Changes in gastric pH and gastric acid secretion occur during development. Lowering of the gastric pH and increased gastric acid secretion would not alter busulfan absorption, because busulfan is very lipophilic and has no ionizable groups. The motility of the gut and the gastric emptying time are diminished in newborns; however, these changes tend to alter the time course of drug absorption rather than the extent of absorption.

Busulfan seems to be quite different from other drugs with regard to age-dependent clearance. Previous pharmacokinetic studies in young children (1–4 years old) have demonstrated an elevated busulfan CL/F relative to adults whether busulfan CL/F is expressed

![Diagram](image)

**Fig. 4.** The relationship between (AUC\(_{\text{THT}}\)/AUC\(_{\text{BU}}\))\(_{0-\infty}\) and busulfan CL/F in patients undergoing stem cell transplantation. The **solid line** excludes the outlier, a child with a busulfan CL/F of 18 mL/min/kg or 430 mL/min/m². **Upper panel,** clearance relative to body weight; \(r^2 = 0.738\) with the outlier, and \(r^2 = 0.405\) without the outlier, \(P < 0.001\). **Lower panel,** clearance relative to BSA; \(r^2 = 0.711\) with the outlier, and \(r^2 = 0.405\) without the outlier, \(P < 0.001\).

**DISCUSSION**

We have shown that: (a) children <4 years of age have an elevated ratio of \((\text{AUC}_{\text{THT}}/\text{AUC}_{\text{BU}})_{0-\infty}\) in comparison to older children and adults; (b) the increase in this area ratio indicates that children are able to form the busulfan-GSH conjugate more efficiently than adults; (c) children do not have elevated GSH levels in plasma in comparison to older children and adults; and (d) the expression of CL/F relative to BSA appropriately compensates for differences in liver size relative to body size when making comparisons of clearance across age. The only common factor between the increase \((\text{AUC}_{\text{THT}}/\text{AUC}_{\text{BU}})_{0-\infty}\) and the increase in CL/F relative to surface area is an enhanced ability to form the GSH conjugate of busulfan in young children.

Age dependencies in drug clearance have been reported for a number of drugs that are primarily eliminated by hepatic metabolism including theophylline, caffeine, carbamazepine, and valproic acid (20). In all of these examples, clearance expressed relative to body weight is about 2-fold higher when comparing the youngest children (1–3 years old) to adolescents (>15 years old). Generally, a steady decline in clearance is reported until adult clearance values are attained around puberty. Because these drugs have a low hepatic extraction ratio, clearance is a function of protein binding and intrinsic clearance (according to the well-stirred model of hepatic drug clearance). For both valproic acid and carbamazepine, the unbound drug concentration does not vary with age (21, 22). Intrinsic clearance is a measure of drug-metabolizing enzyme activity. We found that the liver:body weight ratio in young children (0–4 years old) is 1.7-fold greater than that of adults, which agrees with conclusions made by

![Diagram](image)

**Fig. 5.** Upper panel, relationship between plasma time-averaged GSH levels and age in patients administered busulfan (1 mg/kg) p.o. Points and error bars represent the mean ± SD for 3 or 4 determinations measured after doses 1, 5, 9, and 13 of busulfan. Only the mean is shown for those individuals in whom only one or two time-averaged plasma GSH determinations were made. Lower panel, plasma time-averaged GSH levels plotted as a function of busulfan dose number. Busulfan was given every 6 h for a total of 16 doses. Plasma time-averaged GSH levels were measured after doses 1, 5, 9, and 13 of busulfan. △, children <4 years old; ●, older children and adults.
relative to body weight or BSA, an observation confirmed in this study. A single study has examined busulfan bioavailability after oral and i.v. dosing in children and adults and found no difference (17). However, in this study, no elevation in busulfan CL/F was observed in the children (5.2 ± 2.1 and 3.6 ± 1.3 ml/min/kg, children versus adults; P > 0.10), which was probably due to the age of the children studied. Only five of eight children were less than 4 years old; the oldest was 6 years old.

In the present study, we have measured plasma THT+ levels in children and adults after the first dose of busulfan. We were not able to completely characterize AUC_{THT+} due to the short busulfan dosing interval. Using computer simulations, we found that (AUC_{THT+}/AUC_{BU})_{0–∞} is strongly determined by parameters describing the formation of the busulfan-GSH conjugate and is essentially independent of CL_m, which describes the elimination of the conjugate. This result is consistent with intuition in that (AUC_{THT+}/AUC_{BU})_{0–∞} is comprised of a nearly complete (AUC_{BU})_{0–∞} but contains only a fraction of the (AUC_{THT+})_{0–∞}, because the half-life of THT+ is considerably longer than that of busulfan.

We found that young children have a (AUC_{THT+}/AUC_{BU})_{0–∞} that is 50% higher than that of adults. The magnitude of this elevation corresponds to the increase in busulfan CL/F observed in young children. It is important to note that (AUC_{THT+}/AUC_{BU})_{0–∞} is not a function of the fraction of the dose absorbed. As indicated by the simulations, (AUC_{THT+}/AUC_{BU})_{0–∞} is solely dependent on metabolic factors and displays age dependence similar to busulfan CL/F when expressed relative to BSA. Furthermore, the only common factor between CL/F and (AUC_{THT+}/AUC_{BU})_{0–∞} is the formation of the busulfan-GSH conjugate. Taken together, these data strongly suggest that the elevated busulfan CL/F in children is a consequence of enhanced ability to form the busulfan-GSH conjugate.

To further investigate the mechanistic basis for enhanced busulfan metabolism in children, we measured plasma time-averaged GSH levels in children and adults receiving high-dose busulfan. The plasma GSH values we report are comparable to those observed by others (26, 27). In rats, plasma GSH concentrations were reflective of hepatic GSH depletion after oxidative stress induced by acetaminophen metabolism (28). There was not a significant difference in plasma GSH levels comparing young children and adults. In addition, there have been no reports of age dependency in GSH levels in young children. It seems unlikely that the apparently enhanced ability of young children to form the busulfan-GSH conjugate is due to enhanced availability of GSH.

In conclusion, our findings indicate that young children are more efficient metabolizers of busulfan than adults. Because busulfan elimination is solely mediated by GST, it seems likely that children have a higher level of GST expression than do adults. The elimination half-life of busulfan does not seem to be different between children and adults, which implicates the gut in a first-pass effect. GST-α is expressed to an appreciable extent in the small intestinal mucosa (29) and could participate in first-pass busulfan metabolism. Future studies must focus on the potential metabolic differences in tissues collected from children and adults.

### References


Fig. 6. The relationship between age and liver weight expressed relative to body weight (upper panel) and BSA (lower panel).

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John P. Gibbs, Georgia Murray, Linda Risler, et al.


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