The Impact of Obesity and Disease on Busulfan Oral Clearance in Adults

By John P. Gibbs, Ted Gooley, Bruce Corneau, Georgia Murray, Patricia Stewart, Frederick R. Appelbaum, and John T. Slattery

The apparent oral clearance (CL/F, mL/min) of busulfan was measured in 279 adolescent and adult patients. Significant (P < .05) determinants of CL/F by linear regression were: actual body weight (BW; r² = 0.300), body surface area (BSA; r² = 0.277), adjusted ideal body weight (AIBW; r² = 0.265), and ideal body weight (IBW; r² = 0.173); whereas body mass index (BMI), height, age, gender, and disease were less important predictors. CL/F (mL/min) for normal weight patients (BMI, 18 to 27 kg/m²) was 16.2% lower (P < .001) than for obese patients (BMI, 27 to 35 kg/m²). Thus, expressing CL/F relative to BW did not eliminate statistically significant differences between normal and obese patients. However, busulfan CL/F expressed relative to BSA (110 ± 24 v 110 ± 24 mL/min/m², P = 1.0) or AIBW (3.04 ± 0.65 v 3.19 ± 0.67 mL/min/kg, P = .597) were similar in normal and obese patients. Non-Hodgkin’s lymphoma patients (n = 10) had an approximately 32% lower mean busulfan CL/F expressed relative to BW, BSA, or AIBW compared with patients with chronic myelogenous leukemia (n = 73). Routine dosing on the basis of BSA or AIBW in adults and adolescents does not require a specific accommodation for the obese. However, dosing based on BSA may be improved by considering CL/F differences in certain diseases. Adjusting dose for body size or disease does not diminish interpatient variability sufficiently to obviate plasma level monitoring in many indications.

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BUSULFAN IS A BIFUNCTIONAL alkylating agent commonly used in preparative regimens before hematopoietic stem cell transplantation for treatment of various malignancies and inherited disorders. In hematopoietic stem cell transplantation, outcome at a fixed mg/kg dose has been related to area under the plasma concentration-time curve (AUC) and average steady-state concentration (CSS). Excessively high busulfan under the plasma concentration-time curve (AUC) and average concentration, outcome at a fixed mg/kg dose has been related to area under the plasma concentration-time curve (AUC) and average steady-state concentration (CSS).

It is choosing the appropriate measure of body size with which to calculate dose, because actual body weight is intuitively the most appropriate for the calculation of dose because it will minimize variability in the resultant AUC or CSS in comparison to alternative measures of body size.

Disease has also been linked to alterations in busulfan pharmacokinetics. Previous studies have shown that the disposition of busulfan is altered in children with inherited disorders. Children with lysosomal storage diseases have been reported to have lower elimination half-lives and a trend towards lower CL/F compared with children with immune deficiencies, acute leukemias, and malignant lymphohistiocytosis.6 Children with inherited genetic disorders have been reported to have enhanced elimination half-lives after the first dose of busulfan and elevated busulfan CL/F compared with children with leukemias,7 although patient numbers were small.

The only known pathway for the elimination of busulfan involves glutathione (GSH) conjugation to form γ-glutamyl-β-(S-tetrahydropyrimidinum ion) alanyl-glycine (THTγ). Busulfan is uncommon in this respect, because there are few other drugs that are primarily eliminated by GSH conjugation. We have found that human cytosolic glutathione S-transferase (GST) catalyzes THTγ formation,8 and that GSTA1-1, the major liver GST, is the predominant GST isofrom in busulfan conjugation.9 Less than 2% of an oral busulfan dose is excreted unchanged.10 The effect of obesity on GSTA1-1 activity is unstudied.

The purpose of this report is to compare busulfan CL/F in obese and normal patients treated for various diseases to provide a pharmacokinetic rationale for the appropriate dosing of busulfan, and to gain insight on the activity of GSTA1-1 in obesity and disease. We used a base of 279 adult and adolescent patients undergoing hematopoietic stem cell transplantation at the Fred Hutchinson Cancer Research Center (Seattle, WA) between January 1992 and December 1996, in whom busulfan CL/F had been measured on at least two occasions during conditioning for hematopoietic stem cell transplantation.

MATERIALS AND METHODS

Patients. Records collected as part of routine clinical busulfan monitoring between January 1992 and December 1996 at the Fred Hutchinson Cancer Research Center (Seattle, WA) were analyzed.

The apparent oral clearance (CL/F, mL/min) of busulfan was measured in 279 adolescent and adult patients. Significant (P < .05) determinants of CL/F by linear regression were:

- Actual body weight (BW; r² = 0.300)
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Whereas body mass index (BMI), height, age, gender, and disease were less important predictors.

Busulfan CL/F expressed relative to BSA (110 ± 24 mL/min/m², P = 1.0) or AIBW (3.04 ± 0.65 v 3.19 ± 0.67 mL/min/kg, P = .597) were similar in normal and obese patients.

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Hutchinson Cancer Research Center were examined retrospectively. Inclusion required that the patients were in treatment protocols that stipulated busulfan monitoring. These patients represent about 32% of all patients treated with a busulfan-based conditioning regimen during that time period. The patients provided informed consent for the pharmacokinetic analysis. All patients with complete information (consisting of age, dose, height, weight, dose five CL/F, and dose nine CL/F) were included in the analysis, and individuals less than 12-years old (due to age-dependence in busulfan CL/F) were excluded. The final database contained 279 patients who received 0.44 to 1.8 mg/kg oral busulfan tablets every 6 hours for 4 days as part of their transplant preparative regimen. No other cytotoxic agents or irradiation were administered immediately before or concomitantly with busulfan. Patients received phenytoin for seizure prophylaxis. The diseases treated included acute myelogenous leukemia (AML; n = 60), breast cancer (BrCa; n = 55), (CML; n = 73), myelodysplastic syndrome (n = 49), multiple myeloma (MM; n = 25), non-Hodgkin’s lymphoma (NHL; n = 10), and ovarian cancer (n = 7).

**Determination of CL/F.** Blood samples were collected just before and 60, 120, 180, 240, and 360 minutes after the administration of busulfan. Plasma busulfan concentrations were determined by gas chromatography with mass selective detection, and mean CL/F was calculated for the 5th and 9th doses of busulfan as previously described. Body size estimates were calculated using the following equations in which height is measured in cm and weight in kg.

\[
\text{BSA} (m^2) = \sqrt{\frac{\text{height} \times \text{BW}}{3600}}
\]

\[
\text{IBW} (kg; \text{men}) = 50 + 0.91 \times (\text{height} - 152)
\]

\[
\text{IBW} (kg; \text{women}) = 45 + 0.91 \times (\text{height} - 152)
\]

\[
\text{AIBW} (kg) = \text{IBW} + 0.25 \times (\text{BW} - \text{IBW})
\]

\[
\text{BMI} = \frac{\text{BW}}{(\text{height} \times 10^{-5})^2}
\]

Patients were classified by BMI: underweight, BMI < 18 kg/m²; normal, BMI 18 to 26.9 kg/m²; obese, BMI 27 to 35 kg/m², and severely obese, BMI > 35 kg/m². BMI and the percentage of BW relative to IBW are correlated (BMI = 0.2 (%IBW) + 2.10, \( r^2 = 0.907 \)), such that a BMI of 27 corresponds to 125% BW/IBW.

**Statistical analysis.** All statistical comparisons were performed using SPSS version 7.5 (SPSS Inc, Chicago, IL). In univariable linear regression analysis, the relationship between CL/F (mL/min) and each of the following individual patient variables was assessed: age, AIBW, BMI, BSA, height, IBW, gender, disease, and BW. One-way analysis of variance (ANOVA) was used to compare differences in body size-normalized CL/F among different disease categories with the Bonferroni correction for multiple comparisons. The Levene statistic was used to test for homogeneity of variance.

**RESULTS**

In univariable linear regression analysis, the regression coefficients for absolute busulfan CL/F and each of the following variables were: BW (\( r^2 = 0.300 \)), BSA (\( r^2 = 0.277 \)), AIBW (\( r^2 = 0.265 \)), IBW (\( r^2 = 0.173 \)), height (\( r^2 = 0.164 \)), BMI (\( r^2 = 0.176 \)), and age (\( r^2 = 0.019 \)). Figure 1 shows the relationship between absolute busulfan CL/F and BW, BSA, AIBW, and IBW. All correlations were statistically significant (\( P < .001 \) except for age, \( P = .022 \)). This analysis suggests that BW, BSA, and AIBW are comparable single predictors of CL/F.

Busulfan CL/F is listed in Table 1 for patients in four BMI categories: underweight, normal, obese, and severely obese. The ratio of BW:IBW for each respective BMI category was: 78.4% ± 7.4% (range, 65.6% to 88.1%), underweight; 106% ± 12% (range, 76.4% to 138%), normal; 136% ± 13% (range, 117% to 166%), obese; and 177% ± 23% (range, 151% to 215%), severely obese. Statistically significant differences in absolute (mL/min) CL/F between genders were noted within normal and obese categories. (We did not test for gender differences in the underweight and severely obese patients due to the small sample size of these BMI categories (n = 7 and n = 10, respectively.) Males had a higher absolute (mL/min) CL/F than females in both normal and obese patients.

We compared the mean CL/F among underweight, normal, obese, and severely obese patients using one-way ANOVA (Table 2). Absolute (mL/min) CL/F was elevated in obese (17%) and severely obese (32%) patients compared with normal patients, \( P < .05 \), but there was no statistical difference in CL/F for normal and underweight patients. CL/F relative to BW
(mL/min/kg) was 12% and 21% lower in obese and severely obese patients compared with normal patients, respectively, and 32% higher in underweight patients compared with normal patients. There was not a statistically significant difference in CL/F expressed relative to BW, AIBW, or BSA among underweight patients. We compared busulfan CL/F as a function of age by decade (Table 3). There was no age-dependence in busulfan CL/F when expressed relative to BW, AIBW, or BSA.

Busulfan CL/F was compared among disease categories after expressing busulfan CL/F relative to BW, AIBW, and BSA (Table 4). There was a statistical difference when comparing the mean CL/F expressed relative to BW in patients with NHL to those with AML (2.15 ± 0.22 v 2.82 ± 0.66 mL/min/kg, *P < .045*, respectively), and NHL v CML (2.15 ± 0.22 v 2.92 ± 0.70 mL/min/kg, *P < .007*, respectively). Significant differences in mean busulfan CL/F expressed relative to BSA were found in patients with NHL in comparison to those with CML (87.9 ± 12.3 v 116 ± 25 mL/min/m², *P < .006*, respectively). The mean CL/F expressed relative to AIBW was statistically significantly different in patients with NHL compared with those with BrCa (2.41 ± 0.42 v 3.15 ± 0.62 mL/min/kg, *P < .017*, respectively), NHL v CML (2.41 ± 0.42 v 3.20 ± 0.70 mL/min/kg, *P < .006*, respectively), and NHL v MM (2.41 ± 0.42 v 3.24 ± 0.61 mL/min/kg, *P < .012*, respectively).

We sought an effect of elevations in liver function tests (ie, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase) and kidney function tests (ie, blood urea nitrogen and creatinine) on busulfan CL/F expressed relative to BSA. The normal range for each test was: alkaline phosphatase, 50 to 120 U/L; alanine aminotransferase, 10 to 35 U/L; aspartate aminotransferase, 20 to 48 U/L; blood urea nitrogen, 5 to 20 mg/dL; and creatinine, 0.1 to 1.1 mg/dL. We found that elevations in liver and kidney function tests were not significant predictors of changes in CL/F relative to BSA (*P > .14*). Also, 15 patients included in this analysis were undergoing a second transplant (at least 3 months after the first transplant). There was no difference in the mean CL/F relative to BSA for patients undergoing first versus second transplant (109 ± 23 v 107 ± 22 mL/min/m², *P = .732*, respectively).

### Table 1. Busulfan CL/F* in Underweight (BMI < 18 kg/m²), Normal (BMI = 18 to 26.9 kg/m²), Obese (BMI = 27 to 35 kg/m²), and Severely Obese Patients (BMI > 35 kg/m²)

<table>
<thead>
<tr>
<th>Category (1)</th>
<th>Underweight</th>
<th>Normal</th>
<th>Obese</th>
<th>Severely Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>23.2 ± 12.7</td>
<td>39.3 ± 12.2</td>
<td>41.7 ± 11.8</td>
<td>40.7 ± 12.0</td>
</tr>
<tr>
<td>CL/F, mL/min/kg†</td>
<td>169 ± 34</td>
<td>209 ± 48</td>
<td>175 ± 36</td>
<td>190 ± 45</td>
</tr>
<tr>
<td>mL/min/kg BWT</td>
<td>3.84 ± 0.87</td>
<td>2.88 ± 0.62</td>
<td>2.91 ± 0.62</td>
<td>2.90 ± 0.62</td>
</tr>
<tr>
<td>mL/min/m² ‡</td>
<td>119 ± 22</td>
<td>111 ± 23</td>
<td>106 ± 23</td>
<td>108 ± 23</td>
</tr>
<tr>
<td>mL/min/kg AIBW‡</td>
<td>3.11 ± 0.54</td>
<td>2.91 ± 0.63</td>
<td>3.08 ± 0.63</td>
<td>3.01 ± 0.63</td>
</tr>
</tbody>
</table>

*Data are mean ± SD.
†Statistical comparisons of CL/F among different BMI categories are made in Table 2.
‡There were no statistically significant differences in the mean CL/F expressed relative to BSA or AIBW among underweight, normal, obese, and severely obese patients.
§Denotes a statistically significant difference (*P < .05*) for the mean CL/F in males and females within a BMI category.

### Table 2. Statistical Comparisons of Table 1 Mean Busulfan CL/F Among Underweight, Normal, Obese, and Severely Obese Patients

| BMI Category (1) | % Difference in Absolute CL/F* (mL/min) | % Difference in CL/F/BW* (mL/min/kg) | *P*
|-----------------|----------------------------------------|---------------------------------------|-----
| Underweight     | -12.4                                  | 24.5                                  | .000
| Obese           | -32.0                                  | 33.3                                  | .000
| Severely obese  | -47.9                                  | 40.1                                  | .000
| Normal          | 11.1                                   | -32.4                                 | .000
| Obese           | -17.4                                  | 11.7                                  | .000
| Severely obese  | -31.6                                  | 20.7                                  | .014
| Obese           | 24.2                                   | -50.0                                 | .000
| Normal          | 14.8                                   | -13.3                                 | .000
| Severely obese  | -12.1                                  | 10.2                                  | 1.000

*The percent difference in CL/F = (BMI category (1) – BMI category (2)/BMI category (1)) × 100.
with a variety of diseases undergoing hematopoietic stem cell transplantation.\textsuperscript{3} Severe-grade 3-4 RRT was only observed in patients with $\text{CSS} > 900\text{ ng/mL}$. The incidence of graft rejection for those receiving HL A partially matched related or unrelated donor grafts with a busulfan $\text{CSS} < 600\text{ ng/mL}$ was 7 in 9, whereas graft rejection in patients with $\text{CSS} > 600\text{ ng/mL}$ was 1 in 7. Based on these findings, the therapeutic window for busulfan is $\text{CSS}$ of 600 to 900 ng/mL (which corresponds to mean AUC of 900 to 1,350 µmol/L × minute) over 16 doses for these patients.\textsuperscript{3} More recent findings suggest that tolerance to busulfan may vary by disease. CML patients tolerate busulfan $\text{CSS}$ of 600 to 900 ng/mL without severe-grade 3-4 RRT.\textsuperscript{4} In addition, the incidence of relapse was significantly higher in CML patients with busulfan $\text{CSS}$ levels below the median for the entire group of 45 patients (917 ng/mL).

Interpatient variability in busulfan CL/F is large and is an important determinant of transplantation outcome. In the studies just cited (in which patients received a constant mg/kg dose), inappropriate busulfan AUC was the single most important determinant of toxicity, rejection, or relapse. The coefficients of variation for busulfan CL/F expressed relative to AIBW and BSA, relative to disease CL/F are shown in Table 3. Previous studies have identified the threshold for severe toxicity to be $\text{CSS}$ of 900 to 1,000 µmol/L.\textsuperscript{1-3} Thus, the current data suggest that the apparent reduction in busulfan CL/F in NHL patients could result in enhanced toxicity after a fixed 1 mg/kg dose. However, the small number ($n = 10$) of NHL patients included in this analysis suggests that this issue should be investigated further.

In conclusion, absolute busulfan CL/F is elevated in obesity. Expressing CL/F relative to AIBW or BSA eliminated mean differences in CL/F among underweight, normal, obese, and severely obese patients. There appears to be a potentially important difference between NHL patients and those with CML in busulfan CL/F expressed relative to BW, BSA, or AIBW. Even when expressed relative to BSA or AIBW, interpatient variability in busulfan CL/F expressed relative to any measure of body size is large relative to the therapeutic window in certain indications.\textsuperscript{3,4} The need for adjusting busulfan dose based on AUC or $\text{CSS}$ measured in the individual patient remains in certain settings regardless of body size measure.

| Table 3. Body Mass Index and Busulfan CL/F (Mean ± SD) as Functions of Age |
|------------------|------------------|------------------|------------------|------------------|
| Busulfan CL/F     | Age, years (n)   | 12-20 (16)       | 21-30 (26)       | 31-40 (63)       | 41-50 (104)      | 51-60 (70)       |
| mL/min/m² BSA     | 116 ± 23         | 108 ± 24         | 108 ± 25         | 109 ± 23         | 110 ± 24         |
| mL/min/kg AIBW    | 3.19 ± 0.58      | 3.01 ± 0.72      | 2.99 ± 0.61      | 3.11 ± 0.65      | 3.13 ± 0.71      |

| Table 4. Disease-Dependent Differences in Busulfan CL/F Expressed Relative to BW, BSA, or AIBW |
|------------------|------------------|------------------|------------------|------------------|
| Busulfan CL/F Expressed Relative to: | Disease (1) | Disease (2) | % Difference* | $P$ |
| BW (mL/min/kg)   | NH L AML       | NH L AML       | 31.2           | .045 |
|                  | NH L CML       | NH L CML       | 36.0           | .007 |
| BSA (mL/min/m²)  | NH L CML       | NH L CML       | 32.0           | .006 |
| AIBW (mL/min/kg) | NH L BrCa      | NH L CML       | 31.1           | .017 |
|                  | NH L MM        | NH L CM        | 33.1           | .006 |
|                  | NH L MM        | NH L CM        | 34.8           | .012 |

*The percent difference in CL/F = $\frac{\text{Disease (1)} - \text{Disease (2)}}{\text{Disease (1)}}$ × 100.
REFERENCES


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