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Pharmacokinetics of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation*

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Summary. Busulfan is an alkylating agent that is widely used in preparative regimens for bone marrow transplantation (BMT). We developed a high-performance liquid chromatographic (HPLC) assay for the determination of plasma busulfan concentrations in 30 patients who received oral doses of 1 mg/kg. Concentrations were fit by a one-compartment pharmacokinetic model with first-order absorption. The pattern of absorption and elimination varied widely between patients, with peak concentrations ranging from 1.2 to 10.4 μmol/l (mean, 4.25 ± 2.49). The elimination half-life ranged from 58 to 433 min (harmonic mean, 140 min). The AUC contributed by a single oral dose ranged from 606 to 5,144 μmol-min/l (mean, 2,012 ± 1,223). Patients were evaluated for the development of veno-occlusive disease (VOD), a treatment complication that occurs in 20% of patients undergoing BMT and causes 10% of transplantation-related deaths. All six patients who developed VOD had an AUC greater than the mean, and five of them had an AUC that was > 1 SD above the mean. The occurrence of VOD was highly correlated with an increased AUC (> 1 SD above the mean) ($X^2 = 18$; $P < 0.0001$). Using multivariate logistic regression, no other statistically significant pharmacokinetic predictor of VOD was found. The tenfold variability in the busulfan AUC and the statistical association of increased AUC with the development of VOD suggest a possible role for therapeutic monitoring in this setting.

Introduction

Busulfan [Myleran; 1,4-*bis*-(methanesulfonyloxy)butane] is a bifunctional alkylating agent that has been in clinical use since 1959. It is currently included at high doses in preparative regimens for bone marrow transplantation (BMT) and is a mainstay of treatment for chronic myelogenous leukemia [4, 20]. Investigation of the disposition of busulfan has been limited until recently by the lack of sensitive, specific, inexpensive assays [6, 16, 22]. The chemical fate of busulfan is not yet fully characterized [3]; it is extensively metabolized, and its metabolites are ultimately excreted in the urine [8, 18, 21]. In addition, irreversible binding to plasma (32%) and red blood cells (47%) has been documented [5]. Its main metabolite in isolated, perfused rat liver has been identified as the sulfonium ion of glutathione [9]. Three subsequent metabolites have been isolated in rat urine; they are not cytotoxic [8].

Veno-occlusive disease of the liver (VOD) occurs in approximately 20% of patients undergoing BMT for malignancy [1, 12, 14, 19]. As many as 5%–10% of deaths following BMT are due to this clinical syndrome of hepatic dysfunction, making it the third leading cause of death in allogeneic graft recipients and the second leading cause in patients receiving autologous grafts [12, 15]. The pathogenesis of VOD remains obscure. However, damage to hepatic venous endothelium from the pretransplant preparative regimen appears to be important [19]. Although the type of preparative regimens used has not influenced the occurrence of VOD [12, 14], this disease is the dose-limiting toxicity of high-dose busulfan and cyclophosphamide [2], and hepatic toxicity is dose-limiting for busulfan as a single agent [17]. We developed a high-performance liquid chromatographic (HPLC) assay for the determination of busulfan in plasma, analyzed the disposition of busulfan, and correlated pharmacokinetic parameters with the occurrence of VOD in 30 patients undergoing BMT following preparation with busulfan and cyclophosphamide.

Materials and methods

Assay. All chemicals except chromatography solvents were obtained from Aldrich (Milwaukee, Wis). Methanol for chromatography was obtained from Waters Associates (Milford, Mass). Electron impact mass spectrometry (EI-MS) was done in a duPont 491 mass spectrometer.

Synthesis of internal standard and derivatives. As an internal standard, 1,8-*bis*(methanesulfonyloxy)octane (II) was synthesized as follows. A solution of methanesulfonyl chloride (25 g, 0.22 mol) in methylene chloride (40 ml) was added slowly to a solution of 1,8-octanediol (14.6 g, 0.1 mol), pyridine (17.4 g, 0.22 mol), and methylene chloride (40 ml) under stirring at 0° C. The mixture was stirred at 25° C for 1 h, extracted three times with water (40 ml), and evaporated at reduced pressure. Crystallization from ethanol yielded 26.8 g (90%) white crystals with an mp of

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Abbreviations: VOD, veno-occlusive disease of the liver; BMT, bone marrow transplantation; k_a, absorption rate constant; k, elimination rate constant; t_{lag}, absorption lag time; V, volume of distribution

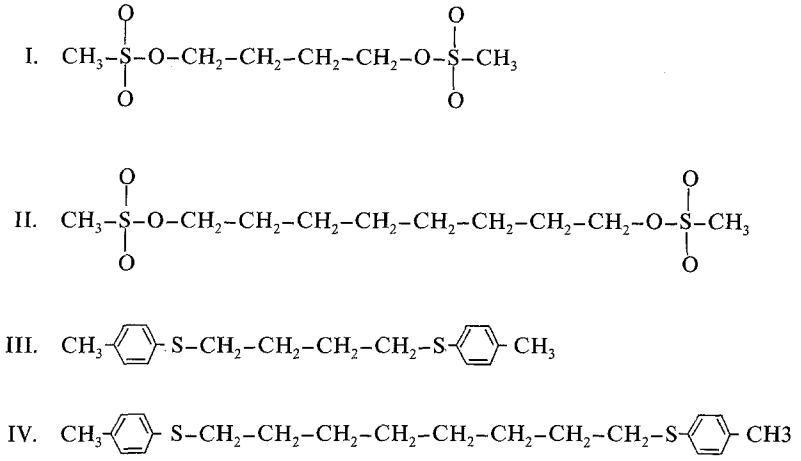

Fig. 1. Structures of busulfan (*I*), the internal standard (*II*), the busulfan derivative for chromatography (*III*), and the internal standard derivative (*IV*)

64° –65° C (literature: mp, 67.5° C [11]). Crystalline derivatives for use in peak identification were synthesized as follows. 1,4-*bis* (*p*-Tolylthio)butane [busulfan derivative (III)]: a mixture of *p*-thiocresol (5 g, 40 mmol), busulfan (5 g, 20 mmol), aqueous sodium hydroxide (40 mmol, 1 *N*) and methanol (40 ml) was refluxed for 2 h. Water (100 ml) and methylene chloride (100 ml) were added and the organic phase was separated and evaporated at reduced pressure. Crystallization from methanol gave 5.4 g (85%) white crystals with an mp of 64° –65° C (literature: mp, 65° C [13]. EI-MS was done on a duPont 491 mass spectrometer; the m/e was 302 for this product. 1,8-*bis*(*p*-Tolylthio)octane (IV), the internal standard derivative, was synthesized using the same method. Crystallization from ethanol gave an 85% yield of white crystals with an mp of 67° –68° C (EI-MS m/e, 358). Figure 1 shows the structures of all substances tested.

Extraction and derivatization of serum samples. A 0.5 g/l (1.95 mmol/l) solution of internal standard (II) in ethyl acetate was prepared. A total of 5 µl (0.01 µmol) was added to 2.5 ml fresh human plasma, 5 ml distilled ethyl acetate was added, and the mixture was vigorously shaken by hand for 1 min and centrifuged at low speed for 5 min. Supernatant (4 ml) was removed to a clean glass test tube and dried under nitrogen. The residue was dissolved in 0.2 ml H_2O, and 20 µl 1 *M* thiocresol in methanol and 20 µl 1 *M* NaOH was added. The solution was heated at 70° C for 2 h. An additional 5 ml 1 *N* NaOH was added and the reaction products were extracted with 5 ml ethyl acetate. The emulsion was centrifuged at low speed for 5 min, the aqueous layer was discarded, and the residual, clear ethyl acetate was dried under N_2 after transfer to a clean test tube. The products were redissolved in 0.5 ml methanol with 0.05 *M* tributylphosphine to reduce disulfides formed by oxidation, as disulfides present in large amounts produce interfering peaks on chromatography. Samples were filtered through a Durapore filter (Millipore Corp., Bedford, Mass) prior to chromatography. Standard curves were produced by adding 0–100 µl 0.5 g busulfan/l ethyl acetate to plasma samples prior to the addition of internal standard.

Extraction efficiency and reaction efficiency were determined with ten samples in duplicate at 4 and 40 µmol/l; reproducibility was determined with ten duplicate samples at 2 and 20 µmol/l. Samples prepared at concentrations of 4 and 40 µmol/l were stored at −20° C for up to 6 months and analyzed monthly for stability.

Chromatography. Using a Waters U6K injector, 50 µl filtered solution were injected onto a C_8 Radial Pak (Waters Associates, Milford, Mass) with a CN precolumn. Solvent A comprised 50:50 methanol:2 m*M* $HKPO_4$, 2 m*M* H_2KPO_4, adjusted to pH 7; solvent B was 100% methanol. Solvents were run at 3 ml/min from 40% to 80% B in a linear gradient over 10 min using a Waters Model 660 solvent programmer and model 6000A pump. Detection was carried out at 254 nm using a Waters model 450 variable wavelength detector. From 15 to 20 samples can be derived and analyzed by hand during an 8-h shift; additional samples can be handled using an autosampler.

Patient selection and treatment. Plasma samples were obtained in 15 patients undergoing BMT between June 1982 and September 1986. All patients provided informed consent for participation in this protocol and in the transplantation protocol, both of which were approved by the Joint Commission of Clinical Investigation (institutional review board) of the Johns Hopkins Medical Institution in accord with institutional, state, and federal guidelines. These patients were reviewed retrospectively for VOD by an investigator blinded to the kinetic results. Exploratory data analysis revealed a possible correlation between busulfan exposure and the occurrence of VOD. In all, 15 additional patients were then evaluated prospectively for busulfan disposition and VOD between September 1986 and January 1987. All patients receiving busulfan were eligible for the study; however, patients whose treatment began when a study phlebotomist was not available were not included. Thus, study entry was biased according to the day of protocol start and included only 30 of 84 patients receiving busulfan over the entire period.

All patients were treated with 1 mg/kg oral busulfan every 6 h for 16 doses, followed by 50 mg/kg intravenous cyclophosphamide/day for 4 days prior to BMT. Supportive measures for these patients have previously been published [20]. There were 16 patients with allogeneic grafts, 13 with autologous grafts, and 1 with a syngeneic graft. Acute myelogenous leukemia had been diagnosed in 18 patients; pediatric sarcomas, in 4; Hodgkins' disease, in 3; and acute lymphocytic leukemia, lymphoblastic lymphoma, chronic myelogenous leukemia, and aplastic ane-

mia, in the remaining 5 patients. Eight patients were treated at the time of their first remission or first relapse.

Pharmacokinetics. A total of 10 cc heparinized whole blood for drug assay was obtained from indwelling venous catheters prior to treatment and at 20, 40, 60, 75, 90, 105, 120, 240, 241, 359, and 360 min after a dose of busulfan. Duplicate samples were obtained at late time points to improve the statistical power of least-squares curve fitting. In all, 25 patients were studied after the first dose of busulfan. Additional plasma samples were obtained for 6 patients after their 5th (3) or 16th (3) busulfan dose. Plasma was separated and frozen at $-20°$ C until assay. Assays were carried out between 1 and 12 weeks of sample acquisition.

Individual disposition curves were visually evaluated for suitable nonlinear models. Of 30 patients, 28 had data that were adequately fit by a one-compartment model with first-order absorption and elimination. Parameter estimation by nonlinear least-squares analysis was carried out using the PCNONLIN program (Statistical Consultants, Lexington, Ky) on an IBM AT. All data points were equally weighted. Data for all patients were fit to

$$C = \frac{Dk_a}{V(k_a - k)} \left[e^{-k(t-t_{lag})} - e^{-k_a(t-t_{lag})} \right] .$$

In 13 patients, this technique yielded estimates of k_a nearly equal to k, and these patients were reanalyzed using

$$C = \frac{Dk}{V}(t - t_{lag})\, e^{-k(t-t_{lag})} .$$

If an absorption lag time of < 1 min was estimated, patients were reanalyzed using

$$C = \frac{Dk_a}{V(k_a - k)}(e^{-kt} - e^{-k_a t}) .$$

One patient had concentrations measured that had no apparent pattern and was not further analyzed; one vomited, was given replacement doses of busulfan, and was not further analyzed. Patients from whom additional samples were drawn after subsequent busulfan doses were evaluated using the appropriate equations for multiple doses [7].

Kinetic parameters estimated by the program included the absorption constant k_a, the elimination constant k, and a hybrid volume of distribution, V, calculated assuming complete bioavailability. The actual bioavailability of oral busulfan in this setting is not known, and it should be noted that it is unlikely to be 100% or to be identical for all patients, even in the absence of clinical abnormalities of gastrointestinal function. The AUC contributed by a single busulfan dose estimated from 0 to infinity, absorption and elimination half lives, clearance, and the estimated maximal concentration and time to maximal concentration were calculated. Pharmacokineticc analysis was carried out by two of the investigators (LBG and TLC) independent of information regarding the clinical course of patients.

VOD. Patients were evaluated for the development of VOD by clinicians blinded to the busulfan pharmacokinetic results, using previously described criteria [12]. VOD was identified by the development of a consistent clinical syndrome of hepatic dysfunction within 3 weeks after BMT, characterized by hyperbilirubinemia peaking at ≥ 34 μmol/l (2 mg/dl) and at least two of three additional findings: painful hepatomegaly, ascites, and a weight gain of $\geq 5\%$. Of the 30 patients studied, 5 had autopsies. The presence of other identified risk factors (elevated aspartate aminotransferase and treatment after a second or subsequent remission) were also noted.

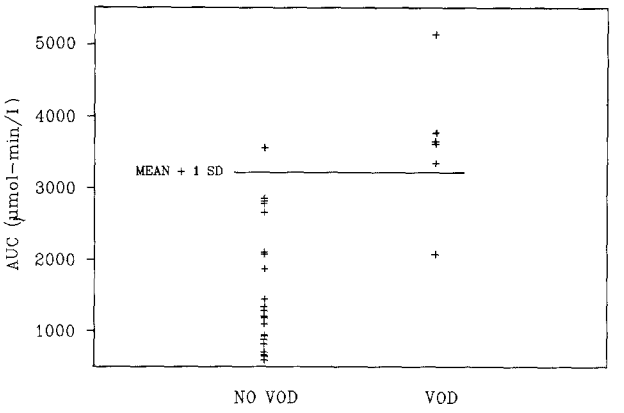

Fig. 2. Busulfan AUC for patients with and without VOD. *Line* at 3,100 μmol-min/l represents 1 SD above the mean for the entire group

Pharmacodynamics. Scatter plots of k, V, and AUC were examined for relationships with the occurrence of VOD; only AUC appeared to be related (Fig. 2). The association between VOD and the pretransplantation risk factors (vide supra) was examined using contingency tables. The chi-square test was used to evaluate the occurrence of VOD in patients with and without elevated AUC. All variables were tested for their association with VOD using multivariate logistic regression; we attempted to predict the binary outcome (occurrence of VOD) using all the covariants. All variables were initially entered into the logistic model, and those factors that were not significant predictors of VOD were removed from the model in a stepwise fashion. Comparison of the group of patients retrospectively reviewed for VOD with the group studied prospectively revealed no significant differences, and the groups were combined for this analysis.

Results

The busulfan derivative (III) had a retention time of 10.8 min and the C8 derivative (IV), 13.8 min. The efficiency of derivatization was 63% and 56% at 4 and 40 μmol/l; reproducibility was $\pm 9.1\%$ at 2 μmol/l and $\pm 5.6\%$ at 20 μmol/l. The lower limit of detection was 0.4 μmol/l. The detector response was linear over the range of 0.4–40 μmol/l. Samples were stable under storage conditions for 6 months. Figure 3 shows chromatograms of pure crystalline busulfan and internal standard derivatives, a patient plasma blank, and a patient sample. The specificity of the derivatization procedure was not assessed by mass spectrometry, and weak nucleophiles may be displaced by *p*-thiocresol. Thus, this assay will potentially measure both parent drug and possible akylating derivatives, in a situation analogous to the derivatization of active platinum after cisplatin administration.

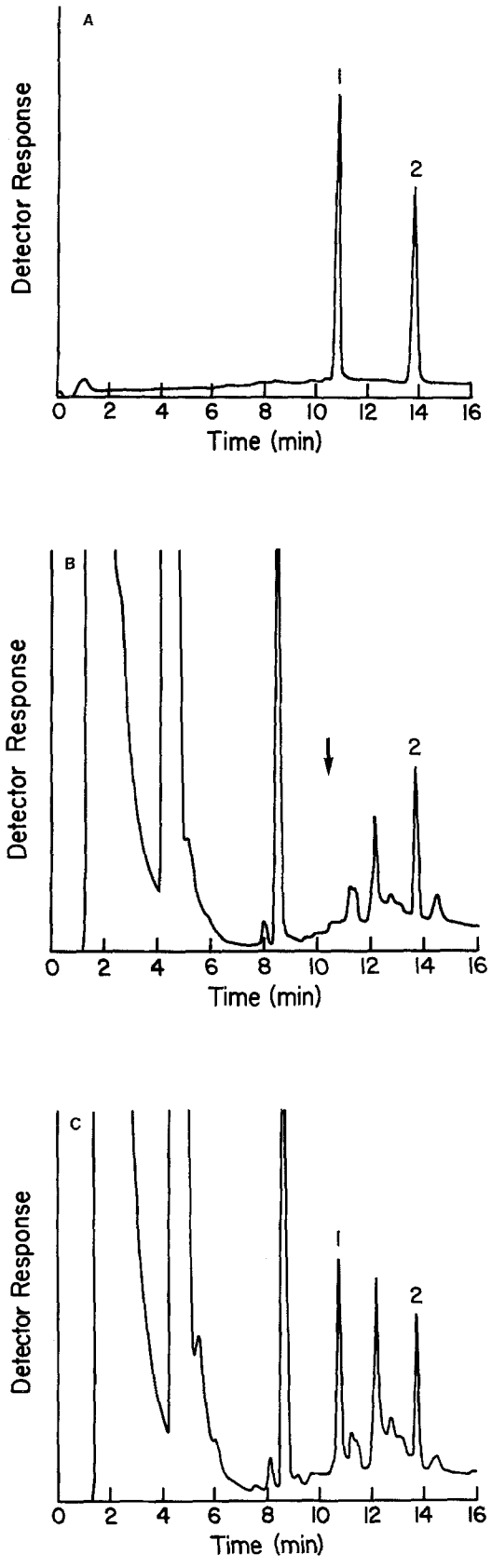

Fig. 3. Sample chromatograms. **A** Crystalline derivatives of busulfan (*1*) and internal standard (*2*). **B** Derivatized blank plasma with internal standard. **C** Derivatized plasma with internal standard added. The busulfan derivative has a retention time of 10.8 min, and the internal standard derivative has a retention time of 13.8 min

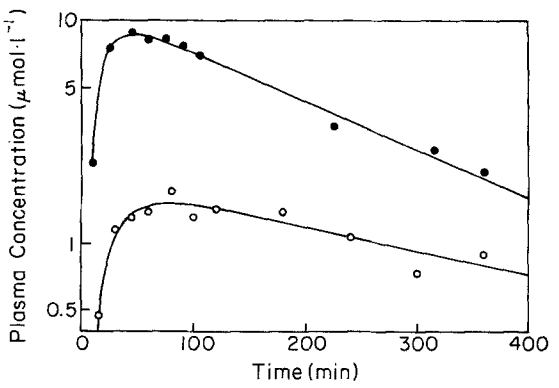

Fig. 4. Busulfan concentrations in two patients receiving an oral dose of 1 mg/kg

The disposition curves in 28 patients were adequately described by a one-compartment pharmacokinetic model with first-order absorption and elimination. Typical elimination curves after the first busulfan dose are presented in Fig. 4. Patterns of absorption and elimination varied greatly, with peak concentrations ranging from 1.2 to 10.4 μmol/l (mean, 4.25 ± 2.49). Table 1 presents the absorption and elimination constants, volumes of distribution (assuming complete bioavailability), and lag times estimated for all patients evaluated. The elimination half-life ranged from 58 to 433 min, with a harmonic mean of 140 min. The samples for 22 patients were obtained for only 6 h after the first busulfan dose, resulting in large

Table 1. Busulfan pharmacokinetic parameters

Patient number	k_a (min^{-1})	k (min^{-1})	V (l/kg)	t_{lag} (min)
358	0.069	0.0016	0.877	0
359	0.0077	0.0077	0.403	0
369	0.012	0.012	0.124	19
375	0.014	0.0039	0.541	0
377	0.0237	0.00458	0.648	26
387	0.0045	0.0075	0.563	0
459	0.039	0.0032	0.44	0
462	0.017	0.0033	0.338	0
473	0.0086	0.0047	0.235	0
487	0.0086	0.0086	0.383	0
490	0.072	0.005	0.377	6
502	0.015	0.0045	0.243	13
506	0.029	0.0037	0.747	25
633	0.00624	0.00624	0.773	0
679	0.00182	0.00182	0.655	0
734	0.00357	0.00357	1.26	0
740	0.002	0.002	0.5	0
751	0.0061	0.0061	0.69	19
771	0.0027	0.0027	0.287	0
773	0.0062	0.0062	0.314	0
781	0.029	0.0046	1.270	0
788	0.00588	0.0059	0.612	0
790	0.014	0.0024	0.578	0
794	0.011	0.011	0.312	35
801	0.006	0.00602	1.095	10
804	0.0148	0.0026	0.741	15
805	0.099	0.0049	1.2	12
832	0.043	0.0025	2.230	8
Mean	0.0204	0.00496	0.658	6.7
SD	0.024	0.00261	0.439	10

standard errors in the estimation of k for 5 of these patients, with longer half-lives. The reliability of the estimation of the AUC was less affected, but a coefficient of variation of >20% was seen in four patients, including three of the six who developed VOD. The AUC from time 0 to infinity for the first dose ranged from 606 to 5,144 μmol-min/l (mean, 2,012 ± 1,223 μmol-min/l). Analysis of the six patients evaluated on more than 1 day of busulfan administration showed no differences in the parameter estimates for each dose assessed separately and for all doses together. Increases in peak and trough concentrations occurred as predicted over the first 2 to 3 doses, after which an apparent steady state was maintained through the 16th dose.

Six patients were diagnosed as having VOD by clinical criteria; three of them died of VOD. Postmortem examinations were carried out on these three patients as well as two others without VOD; the clinical and histologic diagnosis as to the presence or absence of VOD agreed in all five cases. Table 2 lists the occurrence of VOD, the AUC, and the pretransplant risk factors for all 28 evaluable patients. The occurrence of VOD was highly correlated with an increased AUC (>1 SD above the mean) ($X^2 = 18$; $P < 0.0001$). The busulfan AUC was ≥3,200 in 5 of the 6 patients who developed VOD; it was <3,200 in 20 of 21 patients who did not develop VOD. There was no association between the degree of elevation in busulfan exposure and the severity of VOD as measured by outcome in these six patients. Both patients with the highest busulfan exposure recovered from their VOD. The kinetic parameters in five of the six patients who subsequently developed VOD were estimated using plasma samples obtained with their first busulfan dose; one patient's samples were obtained after the first and fifth doses.

Other clinically identified risk factors for VOD [12] were present in 16 patients. Using multivariate logistic regression, all variables except AUC were stepped out of the model. Treatment in a second or subsequent remission was the next most significant factor, but the estimate of its parameter did not achieve statistical significance. Six patients showed pretransplantation increases in aspartate aminotransferase (mean SGOT, 261 IU; range, 114–602); only one developed VOD, and his busulfan AUC was 3,625 μmol-min/l. A total of 15 patients were transplanted while in second or subsequent remission; 6 developed VOD, and only 1 of these had an AUC of <3,200 μmol-min/l. One patient with a lower AUC (2,085 μmol-min/l) who developed VOD was transplanted while in second remission.

Table 2. Occurrence of VOD and possible risk factors

Patient number	VOD	AUC μmol·min·l^{-1}	Pretransplant aminotransferase elevation	Transplant after first remission
358	no	2790	no	no
359	no	1290	no	n/a
369	no	2670	no	no
375	no	1880	yes	yes
377	no	1350	no	no
387	no	940	no	no
459	no	2820	no	yes
462	no	3570	yes	no
473	yes	3630	no	yes
487	no	1210	no	yes
490	no	2110	no	no
502	yes	3660	yes	yes
506	no	1450	yes	no
633	no	830	no	yes
679	yes	3360	no	yes
734	no	890	yes	yes
740	yes	3790	no	yes
751	no	950	no	yes
771	yes	5140	no	no
773	no	2080	no	no
781	no	680	yes	yes
788	no	1110	no	yes
790	no	2870	no	no
794	no	1200	no	no
801	no	600	no	no
804	yes	2090	no	yes
805	no	680	no	no
832	no	710	no	yes

n/a, not applicable

Discussion

Busulfan is only available as an oral preparation. Its absolute absorption has not been defined since the original animal studies with *^{14}C-busulfan. We used a relatively simple, inexpensive assay for busulfan that is suitable for therapeutic monitoring of patients in the transplantation setting. The disposition of busulfan in these patients exhibited wide variability, as would be expected with potentially variable absorption and altered elimination in this clinically varied group of patients. The underlying physiology that accounts for these differences is not apparent. No patient had known absorption abnormalities during the pharmacokinetic study, and all had apparently normal gastrointestinal and renal function by usual clinical measures. Interpatient variability in absorption may be assumed (but cannot be measured, since the drug is not available in parenteral form); there was somewhat more variation in the hybrid volume of distribution (which is affected by absorption) than in the elimination rate constant k (which is affected by metabolism and excretion), as illustrated in Table 1.

The kinetic parameters in this population were similar to those in the small groups previously described [6, 16, 22]. The disposition of busulfan has been studied after the administration of radiolabelled drug [16, 22] and by selected ion monitoring [6]. Ehrsson et al. [6] studied five patients receiving 2–4 mg busulfan and reported an elimination half-life of 2.5 h, compared to 2.3 h in the present study. These half-lives are similar to the initial plasma half-life of radioactive material (1.3–3.3 h) previously reported by Vodopick et al. [22]. Absorption lag times have been reported in the range of 0–2 h [6, 16, 22]. Little information is available regarding the bioavailability of the oral preparation. Nadkarni et al. [16] gave [^{14}C]- and [^{35}S]-labelled busulfan both i.v. and p.o. and found that plasma concentrations were not equivalent until 4 h after a dose. Ehrsson et al. [6] found that absorption kinetics was best described by zero-order kinetics in patients receiving conventional busulfan doses. We achieved a better fit assuming first-order absorption with our data. Maximal concentrations achieved in the present study were linear when compared with those reached in patients previously stud-

ied by Ehrsson et al. [6] (4 μmol/l at 70 mg vs 0.33 μmol/l at 6 mg). However, the AUC reported in the present study is about twofold greater than would be expected based on the series of Ehrsson et al. (88 μmol/min/l at 6 mg vs 2,000 μmol-min/l at 70 mg); the busulfan clearance may be lower at high doses than at conventional doses.

Estimating pharmacokinetic parameters when the sampling period is less than two half-lives can potentially result in large standard errors. Based on the elimination half-lives in the present study, as many as 25% of patients who are monitored over 6 h will have large standard errors in the estimate of k. Therapeutic monitoring for only 6 h after a busulfan dose is currently being evaluated in a randomized, prospective trial in which samples are obtained after the first and fifth doses to assess the reliability of parametric estimates based on data after the first dose.

A persistently elevated SGOT value prior to transplantation has previously been associated with an increased risk of developing VOD [12, 14]; patients in first remission or relapse are associated with a decreased risk [12]. Five of the six patients with pretransplantion increases in SGOT showed a busulfan AUC in the "normal" range and did not develop VOD. All six of these patients (and the other 24 patients) had normal pretransplantation bilirubin, albumin, prothrombin, and partial thromboplastin times. The association of busulfan AUC and the development of VOD appears to be independent of these pretransplant risk factors.

The reasons for the positive association between busulfan AUC and the risk of developing VOD are unknown. Since VOD appears to be a toxicity of BMT preparative regimens [19], the most likely explanation is that VOD is a dose-related drug toxicity. The fact that VOD is generally seen after extremely high-dose therapy with busulfan, nitrosoureas, or mitomycin, whereas it is only rarely seen following conventional doses of these drugs [19], supports this conclusion. However, it is possible that a high busulfan exposure is not etiologic but instead is a marker for patients at high risk for the development of VOD. Specifically, subclinical liver damage may possibly both decrease busulfan clearance and increase the risk of VOD. However, the association between busulfan exposure and the development of VOD appears to be independent of pretransplant risk factors (including a persistently elevated SGOT value) in this cohort of patients. The clinical risk factors for VOD may, in fact, be related to the occurrence of VOD by causing changes in busulfan elimination. Unfortunately, there is no prospective physiologic test or marker substance that can accurately predict such altered drug metabolism [10].

VOD is a potentially fatal complication of BMT. If there is an etiologic association between increased exposure to chemotherapy and the development of VOD, adjusting busulfan or other drug doses may reduce the risk of VOD. The possible correlation of exposure to other cytotoxic agents and the occurrence of VOD in this setting is currently being prospectively evaluated at the Johns Hopkins Oncology Center. A randomized trial of busulfan dose adjustment based on therapeutic monitoring is under way.

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References

1. Berk PO, Popper H, Krueger GRF, Decter J, Herzig G, Graw RG (1979) Veno-occlusive disease of the liver after allogeneic bone marrow transplantation. Possible association with graft-versus-host disease. Ann Intern Med 90: 158
2. Beschorner WE, Pino J, Boitnott JK, Tutschka PJ, Santos GW (1980) Pathology of the liver with bone marrow transplantation. Effects of busulfan, carmustine, acute graft-versus-host disease, and cytomegalovirus infection. Am J Pathol 99: 369
3. Bishop JB, Wassom JS (1986) Toxicologic review of busulfan (Myleran). Mutat Res 168: 15
4. Canellos GP (1977) The treatment of chronic granulocytic leukemia. Clin Haematol 6: 113
5. Ehrsson H, Hassan M (1984) Binding of busulfan to plasma proteins and blood cells. J Pharm Pharmacol 36: 694
6. Ehrsson H, Hassan M, Ehrnebo M, Beran M (1983) Busulfan kinetics. Clin Pharmacol Ther 34: 86
7. Gibaldi M, Perrier D (eds) (1982) Pharmacokinetics. Marcel Dekker, New York, p 132
8. Hassan M, Ehrsson J (1987) Urinary metabolites of busulfan in the rat. Drug Metab Dispos 15: 399
9. Hasson M, Ehrsson H (1987) Metabolism of *^{14}C-busulfan in isolated perfused rat liver. Eur J Drug Metab Pharmacokinet 12: 71
10. Hepner GW, Vesell ES, Lipton A, Harvey HA, Wilkinson GR, Schenker S (1977) Disposition of aminopyrine, antipyrine, diazepam and indocyanine green in patients with liver disease or on anticonvulsant therapy: diazepam breath test and correlations in drug elimination. J Lab Clin Med 90: 440
11. Hudson RF, Timmis GM, Marshall RD (1958) A physicochemical investigation into the biological action of Myleran and related sulphonic acid esters. Biochem Pharmacol 1: 48
12. Jones RJ, Lee KSK, Beschorner WE, Vogel VG, Grochow LB, Braine HG, Vogelsang GB, Sensenbrenner LL, Santos GW, Saral R (1987) Veno-occlusive disease of the liver following bone marrow transplantation. Transplantation 44: 778
13. Kader AT, Stirling CJM (1962) Elimination-addition: I. Displacement of sulphonyl groups from diarylsulphonylalkanes. J Chem Soc 3: 3686
14. McDonald GB, Sharma P, Matthews DE, Shulman HM, Thomas ED (1984) Veno-occlusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. Hepatology 4: 116
15. McDonald GB, Sharma P, Matthews DE, Schulman HM, Thomas ED (1985) The clinical course of 53 patients with veno-occlusive disease of the liver after marrow transplantation. Transplantation 39: 603
16. Nadkarni MV, Trams EG, Smith PK (1959) Preliminary studies on the distribution and fate of TEM, TEPA and myleran in the human. Cancer Res 19: 713
17. Peters WP, Henner WD, Grochow LB, Olsen G, Edwards S, Stanbuck H, Stuart A, Gockerman J, Moore J, Bast RC Jr, Seigler HF, Colvin OM (1987) Clinical and pharmacologic effects of high dose single agent busulfan with autologous bone marrow support in the treatment of solid tumors. Cancer Res 47: 6402
18. Roberts JJ, Warwick GP (1961) The mode of action of alkylating agents: III. The formation of 3-hydroxytetra-hydrothiophene-1:1-dioxide from 1:4-dimethane sulphonyloxybutane (Myleran), *S-B*-alanyl-tetrahydrothiophenium mesylate, tetrahydrothiophene-1:1 dioxide in the rat, rabbit and mouse. Biochem Pharmacol 6: 217
19. Rollins BJ (1986) Hepatic veno-occlusive disease. Am J Med 81: 297
20. Santos GW, Tutschka PJ, Brookmeyer R, Saral R, Beschorner WE, Bias WB, Braine HG, Burns WH, Elfenbein GJ, Kai-

zer H, Mellits D, Sensenbrenner LL, Stuart RK, Yeager AM (1983) Marrow transplantation for acute non-lymphocytic leukemia after treatment with busulfan and cyclophosphamide. New Engl J Med 309: 1347

21. Trams EG, Nadkarni MV, de Quattro V, Maengwyn-Davies GD, Smith PK (1959) Dimethane sulphonoxybutane (myleran): preliminary studies and metabolic fate in the rat. Biochem Pharmacol 2: 7

22. Vodopick H, Hamilton HE, Jackson HL, Peng CT, Sheets RF (1969) Metabolic fate of busulfan in man. J Lab Clin Med 73: 266

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