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Busulfan disposition and hepatic veno-occlusive disease in children undergoing bone marrow transplantation

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Abstract Hepatic veno-occlusive disease (HVOD) is a frequent life-threatening toxicity in patients undergoing bone marrow transplantation (BMT) after the administration of a high-dose busulfan-containing regimen. Recent studies have shown that the morbidity and mortality of HVOD may be reduced in adults by pharmacologically guided dose adjustment of busulfan. We analyzed the pharmacodynamic relationship between busulfan disposition and HVOD in 61 children (median age, 5.9 years) with malignant disease. Busulfan, given at a dose ranging from 16 mg/kg to 600 mg/m^2, was combined with one or two other alkylating agents (cyclophosphamide, melphalan, thiotepa). Only 3 patients received the standard busulfan/cyclophosphamide (BUCY) regimen. A total of 24 patients (40%) developed HVOD, which resolved in all cases. A pharmacokinetics study confirmed the previously reported wide interpatient variability in busulfan disposition but did not reveal any significant alteration in children with HVOD. The mean area under the concentration-time curve (AUC) after the first dose of busulfan was higher in patients with HVOD (6,811 $\pm$ 2,943 ng h ml^{-1}) than in patients without HVOD (5,760 $\pm$ 1,891 ng h ml^{-1}; $P = 0.10$). This difference reflects the higher dose of busulfan given to patients with HVOD. No toxic level could be defined and, moreover, none of the toxic levels identified in adults were relevant. The high incidence of HVOD in children given 600 mg/m^2 busulfan may be linked to the use of more intensive than usual high-dose chemotherapy regimens and/or drug interactions. Before the prospective evaluation of busulfan dose adjustment in children, further studies are required to demonstrate firmly the existence of a pharmacodynamic relationship in terms of toxicity and allogeneic engraftment, especially when busulfan is combined with cyclophosphamide. The maximal tolerated and minimal effective AUCs in children undergoing BMT are likely to depend mainly upon the disease, the nature of the combined high-dose regimen, and the type of bone marrow transplant.

Key words Busulfan · Pharmacodynamics · Hepatic veno-occlusive disease

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Introduction

In bone marrow transplantation (BMT) settings, hepatic veno-occlusive disease (HVOD) is the most frequent life-threatening complication among high-dose chemotherapy-related toxicities. After the administration of a busulfan-containing regimen, the incidence of HVOD ranges from 0 in children with a genetic disease [17] up to 52% in adults with hematological malignancies [11, 16]. No HVOD was observed in patients receiving single-agent high-dose busulfan [12, 15]. However, busulfan seems to be involved in HVOD, since the incidence of the latter was found to be 4% after high-dose chemotherapy without busulfan versus 22% after treatment with a busulfan-containing regimen before BMT in children [26].

Busulfan pharmacokinetics display wide interpatient variability in adults and children [5–7, 8, 24, 27, 28]. With the standard dose of busulfan (16 mg/kg), the incidence of HVOD proved to be lower in children than in adults [2, 17, 19]. This difference could be due to the age-dependent pharmacokinetics of the drug: busulfan clearance is higher in children than in adults [6, 8, 28]. We investigated a new busulfan dose level

(600 mg/m^2) that eliminates the differences in systemic exposure between adults and children [27]. This new dose was expected to enhance the drug's antitumor and antileukemic activity. However, it has given rise to an increased incidence of HVOD [25]. We have recently demonstrated that the major independent risk factors for the occurrence of HVOD in children undergoing autologous BMT for malignant disease after the administration of a busulfan-containing regimen were the dose of busulfan, the intensity of the high-dose chemotherapy regimen, the timing of busulfan dosing within three-drug regimens, and the use of ketoconazole [13].

Grochow et al. [5] demonstrated that the occurrence of HVOD was significantly correlated with high systemic exposure after the first dose of busulfan in adults receiving busulfan and cyclophosphamide (BUCY regimen) before BMT. HVOD might be linked to an altered busulfan disposition of an unknown origin. The definition of a toxic level led these authors to propose dose adjustment as a means of decreasing the incidence of lethal HVOD in adults [4]. Since HVOD is dose-dependent in children, we studied the relationship between busulfan disposition and HVOD in 61 children undergoing BMT for malignant disease.

Patients and methods

Patients

From 1987 to 1991, plasma busulfan pharmacokinetics were studied in 61 children with a median age of 5.9 years (range, 1–15 years) in a single institution. There were 30 boys and 31 girls. In all, 59 patients were treated for a malignant solid tumor (28 neuroblastomas, 13 brain tumors, 5 non-Hodgkin's lymphomas, and 3 Ewing's sarcomas) and 2 patients were treated for acute leukemia. At the time of BMT, 40 patients had measurable refractory disease or a relapse and 21 were in complete remission. Altogether, 53 patients had received conventional multidrug chemotherapy and 8 patients with a brain tumor had received only CNS radiation therapy. In all, 58 and 3 patients underwent autologous and allogeneic BMT, respectively. All the patients had normal liver and kidney functions before receiving high-dose chemotherapy.

Treatment

Busulfan (Misulban, Techni-Pharma Laboratory, Principality of Monaco) was given orally every 6 h over 4 consecutive days for a total of 16 doses. Three different doses were used: 16 mg/kg in 11 patients, 480 mg/m^2 in 3 patients, and 600 mg/m^2 in 47 patients. The mean dose of busulfan was 22.8 ± 4.3 mg/kg (range, 15.2–29.2 mg/kg) or 554 ± 82 mg/m^2 (range, 331–669 mg/m^2). Busulfan was given in tablet and in capsule form to 11 and 50 patients, respectively. Capsules containing the prescribed dose were composed of pure busulfan and lactose. They were either swallowed whole or opened and mixed with preserves for the very young children. The treatment was started at 12 a.m. and 2 p.m. for 50 and 11 patients, respectively. Patients received nothing per os (except water) for 2 h before and 30 min after ingestion.

Busulfan was combined with 1 and 2 other alkylating agents in 22 and 39 patients, respectively (Table 1). In 54 patients, busulfan was the first drug given. High-dose chemotherapy was delivered along with hyperhydration (3 l/m^2 daily). In addition, all patients except those receiving a busulfan dose of 16 mg/kg were given continuous intravenous infusion of clonazepam to prevent busulfan-induced seizures [26]. No prophylaxis was used against HVOD. Methotrexate was used as prophylaxis against graft-versus-host disease in patients who underwent allogeneic BMT. Children were treated under isolated reverse-barrier and laminar air-flow conditions.

Table 1 High-dose chemotherapy regimens used in the 61 patients evaluated

Treatment	Number of patients
Busulfan, cyclophosphamide (200 mg/kg)	3
Busulfan, melphalan (140 mg/m^2)	6
Busulfan, thiotepa (900 mg/m^2)	13
Melphalan (140 mg/m^2), busulfan, cyclophosphamide (200 mg/kg)	7
Busulfan, cyclophosphamide (4.4 g/m^2), melphalan (140 mg/m^2)	30
Busulfan, cyclophosphamide (4.4 g/m^2), thiotepa (900 mg/m^2)	2

Evaluation of liver toxicity

The diagnosis of veno-occlusive disease of the liver was based on MacDonald's criteria [10] as follows: (1) hepatomegaly, (2) jaundice or hyperbilirubinemia of $\geq 25\ \mu M$, and (3) ascites and/or a weight gain of $\geq 5\%$. Other biological symptoms [elevated levels of aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), reversible renal dysfunction, changes in coagulation factors, platelet consumption] were often present but were not considered for the diagnosis. Abdominal ultrasonography confirmed the presence of ascites and frequently showed obscuring of major hepatic veins. No liver biopsy was performed. At least two of the three MacDonald's criteria had to be fulfilled for the diagnosis of HVOD. The day of onset was not taken into account since all but three patients received an autologous bone marrow transplant. For this retrospective study the clinical records of all the patients were reviewed by a clinician blinded for the pharmacokinetic findings. A total of 60 patients were evaluable for liver toxicity. One patient died on day 2 post-BMT of septicemia due to *Candida albicans* and was not included in the pharmacodynamics study.

Blood sampling and busulfan assay

Heparinized whole-blood samples (2 ml) were drawn through the central line after the first dose of busulfan. Samples were obtained from 11 patients before busulfan administration and at 20 and 40 min as well as 1, 1.5, 2, 3, 4, and 6 h. Samples were obtained from 50 patients before busulfan dosing and at 30 min as well as 1, 2, 3, 4, and 6 h. Plasma was separated and frozen at − 80°C until analysis. The study was designed in accordance with the requirements and recommendations of the ethics committee and parental consent was obtained. Busulfan plasma levels were measured in a gas chromatography-mass spectrometry assay using a deuterated analog as an internal standard, as previously described [23].

Pharmacokinetic and statistical analysis

The decrease in the plasma concentration-time curve after the first dose was mono- and biexponential in 53 and 5 patients, respectively. The time required for maximal plasma concentration was 6 h, i.e.,

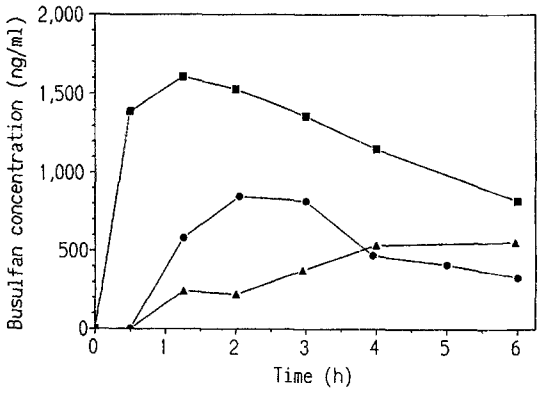

Fig. 1 Busulfan disposition in three patients with HVOD (*Filled squares* Patient 7–AUC_{0-inf} = 13,129 ng h ml^{-1}, *filled triangles* patient 10–AUC_{0-inf} not available, *filled circles* patient 13–(AUC_{0-inf} = 4,261 ng h ml^{-1})

just before the next dose, in 3 patients (Fig. 1). Since no elimination phase was defined, no pharmacokinetic parameter could be calculated for these 3 patients. Pharmacokinetic parameters were evaluated after the first dose (n = 58) by model-independent estimation using the PKCalc program as previously described [24]. The area under the concentration-time curve (AUC) was calculated with the trapezoidal rule from time 0 to 6 h and extrapolated to infinity (AUC_{0-inf}) according to the log-linear regression of the elimination phase. For the 5 patients with a biexponential decay, the second elimination half-life was considered. The pharmacokinetic results obtained in 38 of these children have been published elsewhere [24, 27]. Proportions were compared using the Chi-square test. Values were compared by nonparametric tests (Wilcoxon).

Results

According to MacDonald's criteria, 24 children (40%) developed HVOD [95% confidence interval ($CI_{95\%}$), 28–52%]. Altogether, 22 patients exhibited the 3 standard criteria and 2 patients fulfilled 2 criteria (Table 2). The median day of onset was day 18 (range, days 6–42) and the median duration was 14 days (range, 5–46 days). In all, 6 had severe HVOD with pleural effusion, abundant ascites requiring repeated evacuation, and/or encephalopathy. No patient died of HVOD. Of the 36 patients without HVOD, 19 had an abnormal liver function that did not fulfil HVOD criteria.

Most of the patients (50/61, 82%) were given a dose of busulfan according to body surface area (BSA). Patients with HVOD received a significantly higher dose of busulfan (corrected for BSA) than did patients without HVOD (Table 3). In the population studied, the incidence of HVOD was higher in girls (15/29, 52%) than in boys (9/31, 29%; Chi-square test, $P = 0.07$). The occurrence of HVOD was related neither to age nor to the number of alkylating agents included in the high-dose chemotherapy regimen.

Table 2 Characteristics of the 24 patients with HVOD (*HPM* Hepatomegaly, + + + abundant ascites requiring repeated evacuation, *NA* not applicable since pharmacokinetic parameters could not be calculated)

Patient number	Sex	Busulfan AUC_{0-inf} (ng h ml^{-1})	Day of onset	Duration (days)	HPM	Weight gain	Ascites	Bilirubin (μM)	Other signs
1	M	3,623	23	8	+	6%	–	31	–
2	F	3,875	21	12	+	6%	+	43	–
3	F	3,692	11	20	+	6%	+	57	–
4	F	7,280	27	5	+	11%	+	54	–
5	F	9,203	18	18	+	19%	+ + +	200	Pleural effusion, hepatic insufficiency
6	M	6,108	10	21	+	8.5%	+ + +	45	–
7	M	13,129	9	11	+	8.5%	+	28	–
8	F	7,684	23	11	+	–	–	30	–
9	M	3,567	18	12	+	5%	+	46	–
10	M	NA	14	13	+	–	+	27	–
11	M	4,751	30	7	+	6%	+	25	–
12	F	6,755	6	34	+	16%	+ + +	53	Pleural effusion
13	F	4,261	15	15	+	6%	+	65	–
14	M	4,413	24	19	+	7%	+	124	–
15	F	NA	6	13	+	22%	+ + +	85	–
16	F	11,559	42	15	+	7%	+	48	–
17	F	6,489	28	13	+	9%	+	26	–
18	F	8,194	23	10	+	7%	+	54	–
19	F	5,491	12	22	+	12%	+	26	–
20	F	6,563	20	6	+	4%	+	13	–
21	F	10,957	18	21	+	18%	+	33	–
22	M	12,088	14	18	+	9%	+	57	–
23	F	4,112	19	20	+	8%	+	158	–
24	M	6,059	17	46	+	17%	+ + +	283	Encephalopathy Pleural effusion, hepatic insufficiency, Encephalopathy

Table 3 Comparison of patients with and without HVOD. Data are given as mean values ± SD (C_{max} maximal plasma concentration, C_{min} minimal plasma concentration, Cl/F clearance rate uncorrected for bioavailability, Vd/F volume of distribution uncorrected for bioavailability, $t_{1/2}$ elimination half-life, AUC_{0-inf} area under the plasma concentration-time curve extrapolated to infinity, *NS* not significant)

	HVOD	No HVOD	*P*
Number of patients evaluable	24	36	
Age (years)	6.5 ± 4.2	5.5 ± 3.3	NS
Sex ratio	1.62 ± 0.50	1.39 ± 0.50	0.07
High-dose chemotherapy:			
Number of patients evaluable	24	36	
Number of drugs	2.6 ± 0.5	2.7 ± 0.5	NS
Dose of busulfan (mg)	470 ± 179	407 ± 160	NS
Dose of busulfan (mg/kg)	23.4 ± 3.9	22.4 ± 4.7	NS
Dose of busulfan (mg/m^2)	580 ± 52	537 ± 95	< 0.05
Pharmacokinetics:			
Number of patients evaluable	22	35	
C_{max} (ng/ml)	1,363 ± 778	1,246 ± 524	NS
C_{min} (ng/ml)	425 ± 192	379 ± 145	NS
Cl/F (ml min^{-1} kg^{-1})	4.05 ± 1.35	4.5 ± 1.85	NS
Cl/F (ml min^{-1} m^{-2})	103 ± 37	109 ± 46	NS
Vd/F (l/kg)	1.0 ± 0.4	1.0 ± 0.7	NS
Vd/F (l/m^2)	2.5 ± 1.2	2.4 ± 1.5	NS
$t_{1/2}$ (h)	3.05 ± 1.50	2.65 ± 0.90	NS
AUC_{0-inf} (ng h ml^{-1})	6,811 ± 2.943	5,760 ± 1.891	0.10

Pharmacokinetic parameters were available for 58 patients after the first dose of busulfan. Wide interpatient variability was observed with a 7-fold variation in clearance uncorrected for bioavailability (Cl/F; mean ± SD, 4.3 ± 1.7 ml min^{-1} kg^{-1}; range, 1.4–10.6 ml min^{-1} kg^{-1}), a 9-fold variation in volume of distribution uncorrected for bioavailability (Vd/F; mean ± SD, 1.0 ± 0.6 l/kg; range, 0.5–4.4 l/kg), and a 6-fold variation in elimination half-life (mean ± SD, 2.8 ± 1.2 h; range, 1.3–8.5 h). The AUC_{0-inf} ranged from 1,649 to 13,129 ng h ml^{-1} and the mean value (± SD) was 6,212 ± 2,369 ng h ml^{-1}.

The correlation between busulfan disposition and HVOD could be studied in 57 patients. Univariate analysis showed no difference in terms of pharmacokinetic parameters between patients with and those without HVOD after the first dose of busulfan (Table 3). However, the mean AUC tended to be higher in patients with HVOD ($P = 0.10$; Fig. 2). In the 6 patients with severe HVOD the AUC ranged from 4,112 to 9,203 ng h ml^{-1}. Of the 3 unevaluable patients with slow absorption, 2 developed HVOD (patients 10 and 15). Only 1 child (patient 7) had an AUC value close to the toxic level (3,210 μ*M* min = 13,180 ng h ml^{-1}) defined in adults by Grochow et al. [5] and developed HVOD. If we consider the toxic level used by Grochow [4] in his prospective controlled study (1,500 μ*M* min = 6,160 ng h ml^{-1}), 28 and 29 patients had an AUC value below and above this limit, respectively. The incidence of HVOD in these 2 groups of children was 36% and 41%, respectively.

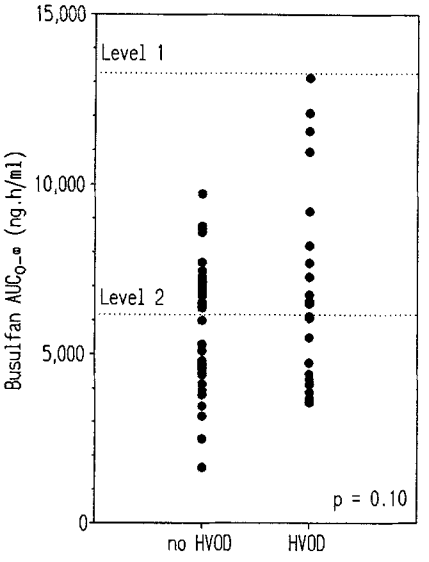

Fig. 2 Systemic exposure in patients with and without HVOD (*Level 1* Toxic level defined in adults by Grochow et al. [5], *Level 2* toxic level used for dose adjustment in adults by Grochow [4])

Discussion

In this study we observed a high incidence of HVOD (40%) in children undergoing BMT as compared with other studies in patients of the same age [2, 17, 19]. We do not think that HVOD was overdiagnosed in our study. No histologic analysis could be performed in these children with hemostasis alterations, but no other explanation was found for the hepatotoxicity observed. The predictive value of MacDonald's criteria, which are commonly used, is good (88%) [10]. In addition, the early use of symptom treatment may have attenuated the clinical symptoms such as weight gain and ascites and their duration. The incidence of HVOD was the same as that observed in a larger series of 136 children treated with busulfan-containing high-dose chemotherapy in the same institution [13]. We used a higher than usual high dose of busulfan and more

intensive regimens containing three alkylating agents. A multivariate analysis showed that the occurrence of HVOD was significantly associated with a higher dose of busulfan (600 mg/m^2 > 16 mg/kg) and a more intensive regimen (3 drugs > 2 drugs) [13]. Recently, MacDonald et al. [11] reported an incidence of 54% in a cohort of 355 patients prospectively studied for the development of HVOD. Again, in this study the intensity and nature of the cytoreductive regimen was an independent risk factor for severe HVOD.

Grochow et al. [5] found a significant correlation between a high systemic exposure after the first dose of busulfan and the occurrence of HVOD in 28 adults undergoing BMT after the standard BUCY regimen (busulfan, 16 mg/kg; cyclophosphamide, 200 mg/kg). As all the patients received the same dose of busulfan, this suggests that HVOD is correlated with an altered busulfan disposition. These authors then embarked upon a prospective controlled study of pharmacologically guided busulfan dose adjustment [4]. A decrease was observed in morbidity and mortality due to HVOD when doses were reduced in patients with an AUC that exceeded 1,500 μM min after the first dose of busulfan. We have to point out that the definition of HVOD in the adult pharmacodynamics study by Grochow et al. [5] was somewhat different from that used in the present study. HVOD was identified by the development of a consistent clinical syndrome of hepatic dysfunction characterized by hyperbilirubinemia peaking at $\geq 34\ \mu M$ and by at least two of three additional findings: painful hepatomegaly, ascites, and a weight gain of $\geq 5\%$. With such a definition, HVOD would have been diagnosed in 15 of the 57 children with available pharmacokinetic data. Even by this more stringent definition, the AUC value recorded for patients with HVOD (mean $\pm$ SD, 6,808 $\pm$ 2,986 ng h ml^{-1}) was not significantly different from that noted for patients without HVOD (5,926 $\pm$ 2,125 ng h ml^{-1}).

In our study, the dose of busulfan was prescribed in milligrams per square meter of BSA for 82% of the patients, and there was a weak but significant correlation between the dose of busulfan expressed according to BSA and the occurrence of HVOD. This confirms the dose dependency of busulfan-induced hepatotoxicity reported previously [13, 25]. No alteration was observed in busulfan disposition after the first dose in children with HVOD. These patients had a higher, albeit nonsignificant, AUC value, probably merely reflecting the higher dose they had received. Factors other than the dose (or systemic exposure) of busulfan may be involved in the high incidence of HVOD observed. No significant toxic level could be established, and only one patient had an AUC value close to the toxic level initially defined in adults [5]. If we consider the toxic level used by Grochow [4] in her prospective study, the incidence of HVOD is 36% and 41% in the two groups of children with an AUC value below and above this level, respectively. Thus, dose adjustment to achieve an AUC value below 1,500 μM min may not significantly reduce the incidence of HVOD in these children, and, even worse, 30% of the patients with malignant disease would be receiving pointless dose reduction. In conclusion, HVOD proved to be unrelated to altered busulfan disposition after the first dose in this pediatric population. Moreover, none of the toxic levels used in adults was relevant in these children.

All the pharmacodynamic data thus far reported have been obtained in adults receiving a standard BUCY regimen [4, 5]. In the present study, busulfan was combined with one or two of the following alkylating agents: thiotepa, melphalan, and cyclophosphamide. Only three patients received the BUCY regimen. The number of alkylating agents did not correlate with the occurrence of HVOD. However, in larger series the intensity of the high-dose chemotherapy regimen was clearly an independent risk factor for the development of HVOD [11, 13]. The high incidence of HVOD might be related to an altered disposition of the other alkylating agents and/or drug interactions. Interindividual variability in the disposition of high-dose melphalan [3, 22], high-dose thiotepa [9, 14], and high-dose cyclophosphamide [1] is well documented. Recently, Ayash et al. [1] found a correlation between a fast cyclophosphamide clearance and the development of acute cardiotoxicity. Many alkylating agents are conjugated to reduced glutathione (GSH) by glutathione-S-transferases. This conjugation will eventually induce intracellular GSH depletion and render the cells more sensitive to the effects of another alkylating agent. Such a process was observed by Teicher et al. [21] during a study on the synergistic effect between cyclophosphamide and thiotepa. In animal models, GSH depletion enhances the hepatotoxicity of anticancer drugs. Shulman et al. [20] have shown that melphalan induces HVOD in dogs only when combined with a GSH-depleting agent, buthionine sulfoximide. The use of GSH-depleting alkylating agents before high-dose melphalan treatment may increase the toxic lesions caused by melphalan in the liver. The high incidence of HVOD observed in our study may therefore be related to the use of more intensive than usual high-dose chemotherapy regimens, the altered disposition of alkylating agents other than busulfan, and/or drug interactions through the GSH system or other detoxifying processes.

The present study shows that the high incidence of HVOD observed with a 600-mg/m^2 dose of busulfan is not related to altered busulfan disposition but might be due to the particularly heavy regimens we are using. Since the dose of busulfan may be critical in terms of its antileukemic effect, it is important to evaluate the dose of 600 mg/m^2 busulfan in combination with cyclophosphamide in terms of liver toxicity and survival in children with acute leukemia. Interestingly, two recent studies have reported the use of higher than standard

high-dose busulfan (600 and 650 mg/m^2, respectively) in combination with cyclophosphamide in children [18, 29]. No dramatic increase in the incidence of HVOD was observed in these small series.

As far as dose adjustment is concerned, none of the toxic levels defined in adults proved to be relevant in children. The prospective evaluation of dose adjustment cannot be performed until a pharmacodynamic relationship is clearly established in a pediatric population. In our opinion, the negative results of our pharmacodynamics study cast no doubt on the eventual relevance of busulfan dose adjustment in children. They do, however, emphasize the need for pharmacodynamics studies according to a given busulfan-containing regimen, especially the BUCY regimen. Finally, with the prospect of dose adjustment, in the light of the results of the present study we are now aware that certain pitfalls will inevitably be encountered, as has previously been pointed out by Grochow [4]. For example, due to slow absorption it will be impossible to estimate reliably the AUC in some patients and, thus, to apply accurate dose adjustment. In the present study, three patients exhibited slow absorption and two of them developed HVOD.

In conclusion, further pharmacodynamics studies are required in children. These studies should focus not only on liver toxicity but also on allogeneic bone marrow graft rejection as a clinical endpoint. Indeed, definition of the minimal AUC required to achieve allogeneic engraftment may help to improve the outcome, especially in children with nonmalignant disease.

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