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Conditioning regimens

Intravenous busulfan in children prior to stem cell transplantation: study of pharmacokinetics in association with early clinical outcome and toxicity

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Summary:

We studied the pharmacokinetics of intravenous busulfan (Bu) in children in order to further optimize intravenous Bu dosing in relation to toxicity and survival. A total of 31 children undergoing Bu-based conditioning for allogeneic SCT were enrolled in a study. The starting dose was 1.0 mg/kg (age <4 years) and 0.8 mg/kg (age ⩾4 years), four doses per day during 4 days. Dose adjustment was allowed up to a maximum dose of 1.0 mg/kg per dose if the target area under the serum concentration–time curve (AUC) was not reached. Pharmacokinetic studies were performed after the first dose. Donor engraftment was established in 28 out of 31 patients. The average AUC after the first dose was the same in children <4 years as in children ⩾4 years. Mean clearance was higher in children <4 years than in children ⩾4 years. In 35% of all patients, total AUC was within the target AUC. The other children's AUCs were below the target range. No relationships were found between systemic exposure to Bu and toxicity or clinical outcome. We concluded that, in accordance with previous data, within the observed AUCs no clear relationship was observed between Bu AUC and outcome with respect to toxicity, engraftment and relapse.
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Busulfan (Bu) is an alkylating agent, which is used in high doses in conditioning regimens for hematopoietic stem cell transplantation (HSCT) for various malignant and non-malignant diseases. Until recently, Bu was only available as an oral formulation. The administration of oral Bu results in variable bioavailability and unpredictable systemic exposure. In patients receiving the oral formulation, the area under the serum concentration–time curve (AUC) correlates with transplant-related toxicity and particularly with hepatic complications, such as veno-occlusive disease (VOD).[1] Low levels were found to be associated with an increased incidence of graft rejection or relapse.[2,3] These observations suggest that optimization and individualization of Bu dosing is recommended. In order to reduce both intra- and interindividual variability of Bu pharmacokinetics, intravenous formulations of Bu have recently been developed. Pharmacokinetics, toxicity and outcome of intravenous Bu have mainly been studied in adults.[1,4–6]

In a previous pilot study, we found that systemic exposure of Bu in children during intravenous administration can be estimated adequately with limited sampling and a Bayesian fitting procedure from a one-compartment model.[7] In the present study, we further characterize the pharmacokinetic parameters of intravenous Bu in children in order to optimize intravenous Bu dosing. Furthermore, transplant-related toxicity, engraftment and disease-free survival in relation to systemic Bu exposure are retrospectively analyzed.

Methods

Patients

Between June 2000 and December 2002, all 31 consecutive children undergoing Bu-based myeloablative conditioning for allogeneic HSCT in the pediatric bone marrow transplantation unit of the Leiden University Medical Center, The Netherlands, were enrolled in the study. The parents of the children gave written informed consent for participation in the transplantation protocol, which was approved by the ethics committee of the hospital. The patient population consisted of 12 females and 19 males (median age 5.0 years (3 months–14 years) at the time of transplant). Six children underwent HSCT from matched family donors (MFDs), four from mismatched family donors (MMFDs), while 21 children received transplants from matched unrelated donors (MUDs). Further patient characteristics, diagnoses and stem cell sources are given in Table 1.

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Table 1 Patient characteristics

Age (years), median, range	5.0 (0.22–14)
Weight (kg), median, range	17.8 (4.4–80)
Sex, male/female	19/12
Diagnosis	
Acute leukemias	4
Other malignancies[a]	14
Nonmalignant hematologic diseases[b]	5
Immunodeficiencies[c]	4
Other inborn errors[d]	4
Donor type	
Matched family	6
Mismatched related family	4
Matched unrelated	21
Conditioning[e]	
Bu, Cy	11
Bu, Mel, Cy	15
Bu, Cy, Eto	2
Bu, Cy, Flu	3

[a]Myelodysplastic syndrome ($n=10$), Ph' chronic myeloid leukemia ($n=1$), juvenile myelomonocytic leukemia ($n=3$).
[b]Thalassemia ($n=2$), idiopathic aplastic anemia ($n=1$), amegakaryocytosis ($n=1$), Shwachmann syndrome ($n=1$).
[c]Severe combined immunodeficiency ($n=1$), hemophagocytic disorders ($n=3$).
[d]Osteopetrosis ($n=2$), leukodystrophies ($n=2$).
[e]Bu = intravenous busulfan; Cy = cyclophosphamide; Mel = melphalan; Eto = etoposide; Flu = fludarabine (for dosages, see text).

Preparative regimen

Bu (Busulfex®, Orphan Medical Inc., USA) was administered on 4 consecutive days in four daily divided doses as intravenous infusions (concentration 0.6 mg/ml) in 2 h (16 doses in total). Busulfex® contains a solution of Bu in dimethylacetamide, polyethylene glycol 400 and water. The starting dose was 1.0 mg/kg for patients <4 years and 0.8 mg/kg every 6 h for patients ⩾4 years according to the manufacturer's initial recommendations (personal communication). Dose adjustment was allowed to a maximum dose of 1.0 mg/kg per 6 h if the target AUC (4925 μg/l·h or 1200 μmol·min; range 4200–5650 μg/l·h or 1000–1400 μmol·min) was not reached. The maximum dose as advised by the manufacturer was based on previous results in children with oral Bu. Three children <4 years were given 0.8 mg/kg Bu, since they were in a poor clinical condition (ie colitis, malnutrition and severe hepatosplenomegaly), but without apparent signs of hepatitis or hepatic functional abnormalities.

Patients received various other cytostatic drugs in combination with Bu: cyclophosphamide (Cy), melphalan (Mel), fludarabine (Flu) and etoposide (Eto) (Table 1). Cy was given to all patients (total dose 200 mg/kg i.v. in 4 days or 120 mg/kg i.v. in 2 days, either when given alone or in combination with Mel, respectively) with a 24-h interval after the last dose of Bu. Mercapto ethane sulfonic acid (MESNA®), cystitis prophylaxis, was given to all patients. Mel (total dose 140 mg/m^2 i.v.) was included in 15, Flu (total dose 150 mg/m^2 i.v. in 5 days) in three and Eto (total dose 700 mg/m^2 i.v. in 2 days) in two together with the Bu/Cy-based conditioning regimens. A total of 22 patients additionally received rabbit anti-thymocyte globulin (ATG) (total dose 10 or 20 mg/kg i.v. in 4 days, from Sangstat IMTIX®) and three children received alemtuzumab (Campath-1H, total dose 1.0 mg/kg i.v. in 5 days).

Supportive care

All patients were cared for in high-efficiency, particle-free air (HEPA)-filtered positive-pressure isolation rooms with total gut decontamination using nonabsorbable antimicrobials. Some patients received itraconazole as antifungal prophylaxis. Prophylaxis against graft-versus-host disease (GvHD) was given with cyclosporin A (2 mg/kg/day i.v. or 6 mg/kg/day orally) with trough level monitoring and short-course methotrexate (10 mg/m^2 i.v.) on days +1, 3 and 6. Patients with increased risk for seizures, such as a history of seizures or neurological abnormalities, were given prophylaxis with clonazepam (0.025 mg/kg/day i.v.) during Bu administration. None of the patients were given hepatic enzyme-inducing anticonvulsants. Routinely, ondansetron or granisetron was given as an antiemesis drug. In case of failure of emetic control, chlorpromazine or dexamethason was added. Some patients received acetaminophen as analgesic treatment.

No VOD prophylaxis was given, although all regular intravenous saline solutions contained 1 IE/ml heparin. Low-dose prostaglandin (10 μg/kg/day i.v.) was given in case of suspected or proven VOD, in addition to fluid restriction and diuretics.[8]

End point for evaluation of therapy

The clinical end points of this retrospective comparison were transplant-related toxicity, incidence and severity of VOD, liver toxicity, mortality, engraftment, acute GvHD and disease recurrence. VOD was diagnosed according to the modified Baltimore criteria: hyperbilirubinemia (bilirubin ⩾34 μmol/l) and at least two of three symptoms (hepatomegaly, ascites or unexplained weight gain of ⩾5% from baseline) present before day 21 after HSCT when other possible causes of these clinical manifestations had been excluded. Severity of VOD was graded according to Bearman *et al*.[9] Mild VOD was defined as clinical disease not needing intervention, moderate disease was defined as VOD needing intensive support and severe disease was defined as VOD leading to multiple organ failure and/or death. Liver transaminase elevation was scored from grade 1 to 4 according to WHO criteria. Acute GvHD was graded according to the scale defined by Przepiorka *et al*.[10] Engraftment was defined as neutrophil recovery to more than 0.5×10^9/l, thrombocyte recovery to more than 50×10^9/l and reticulocyte counts to more than 20×10^9/l. Donor–recipient white blood cell chimerism was determined by XY-FISH or VNTR polymorphism, as previously described.[11]

Pharmacokinetics and statistical analysis

Bu was administered through a double-lumen central venous catheter. Blood samples were collected through the lumen that was not used for the Bu infusion at 2.5 and

4.0 h after the end of the first infusion on day 1 of treatment. Blood was collected in a plain tube and was centrifuged for 5 min (speed 4000 rounds/min). Samples were analyzed immediately or frozen (−20°C) until analysis. Bu was analyzed in plasma by a validated high-performance liquid chromatographic (HPLC) assay, involving precolumn derivatization, liquid/liquid extraction and ultraviolet detection according to Chow *et al*.[12] The assay was linear from 30 to 8000 μg/l. The limit of quantification was 30 μg/l. Precision at 200 and 1500 μg/l was 3.5 and 0.8%, respectively.

Pharmacokinetic parameters for each patient were calculated using concentrations measured in samples at 2.5 and 4 h after start of the first infusion of Bu. A validated limited sampling model was used in order to minimize the number of blood samples necessary to calculate the AUC.[7] Empirical Bayes pharmacokinetic parameter estimates at steady state (clearance and volume of distribution) were generated for all individual children using the pharmacokinetic software package MwPharm.[13] The AUC was calculated from the expression Dose/Cl. Total AUCs were estimated from clearance calculated for the first dose and the total dosage, since in previous studies it was found that the AUC on day 4 was equal to AUC on day 1.[7,14] The total dosage was calculated for each individual patient taking all dose adjustments into consideration. We were able to determine the AUC again after dose adjustment (on the second day of Bu infusion) in nine patients. The median of this second AUC was 103% (95% confidence interval: 92–124%) of the value predicted from the first blood sampling. This indicates that there is no substantial change in pharmacokinetics of the drug during treatment.

Differences between groups were assessed using an unpaired nonparametric two-tailed *t*-test. The hypothesis that a slope was not equal to zero was tested using the nonparametric Kruskal–Wallis test. Results are presented as mean ± s.d. and only *P*-values <0.05 were considered significant.

Results

Patient characteristics and clinical outcome

From August 2000 to December 2002, 31 patients were included in the study (Table 1). All patients became neutropenic following the administration of myeloablative conditioning, containing at least Bu and Cy. Donor engraftment, based on peripheral blood counts and chimerism studies, was ascertained in 28 out of 31 patients. All but one patient had complete donor chimerism. The patient with mixed chimerism (60% donor) received donor lymphocyte infusions 10 months after HSCT to prevent late graft rejection. The transplant was rejected in two patients; one died after second transplantation from adenovirus infection and the other died of disease progression after rejection of a second graft. Another patient with secondary leukemia, 2 years after autologous transplantation, died on day 17 after Bu–Cy–Mel-based conditioning with severe toxic complications with pulmonary bleeding, VOD and multiple organ failure. Although early leukocyte recovery was documented, full trilineage engraftment could not be verified. Leukemic relapses occurred in two patients, both with juvenile myelomonocytic leukemia (JMML). Both patients died from their leukemia despite donor lymphocyte infusions or retransplantation. Three additional children died: one due to systemic adenovirus infection, one from Epstein–Barr virus lymphoproliferative disease and one from grade III GvHD and systemic adenovirus infection.

Bu pharmacokinetics

The results of the pharmacokinetic evaluation are shown in Table 2. AUC and clearance of Bu were calculated for all 31 patients after the first dose. The average AUC was 3624 μg/l/h and was the same in children <4 years as in children ⩾4 years. Mean clearance of Bu was higher in children <4 years than in children ⩾4 years ($P=0.02$). In addition, clearance of Bu decreases with weight ($P=0.002$, data not shown). Table 3 shows that after the first dose of Bu, target AUC is reached in only 40 and 10% of the children aged <4 and ⩾4 years, respectively (Figure 1). For children (⩾4 years) in which the target AUC was not reached, the dose of Bu was adjusted to maximally 1 mg/kg on days 2, 3 and 4. Despite dose adjustment, only in 35% of all patients could the total target AUCs, as advised by the manufacturer, be reached (Figure 1, Table 3).

Table 2 Pharmacokinetic parameters in children receiving intravenous Bu[a]

	Mean ± s.d.		*P-value*
	Age <4 years	*Age ⩾4 years*	
Clearance (l/h/kg)	0.30 ± 0.08	0.24 ± 0.06	0.02
Distribution volume (l/kg)	0.73 ± 0.12	0.77 ± 0.22	0.53
Elimination $T_{1/2}$	2.1 ± 0.4	2.3 ± 0.7	0.39
AUC first dose (μg/l·h)	3622 ± 1125	3627 ± 1138	NS
Estimated total AUC (μg/l·h)	58 175 ± 14 110	61 579 ± 15 457	NS

NS: not significant.
[a]Dose regimen: age <4 years 1.0 mg/kg ($n=13$) and age ⩾4 years 0.8 mg/kg ($n=18$) with dose adjustment to maximally 1.0 mg/kg.

Table 3 Target AUC after the initial dose and after dose adjustment

Starting dose	*AUC (μg/l·h) after starting dose*		
	<4200	*4200–5650*[a]	*>5650*
0.8 mg/kg ($n=21$)	18 (78)[b]	2 (10)	1 (5)
1.0 mg/kg ($n=10$)	6 (60)	4 (40)	0
	AUC (μg/l·h) after total dose[c]		
Total dose	*<67 200*	*67 200–90 400*	*>90 400*
12.8–16.0 mg/kg ($n=31$)	20 (65)	11 (35)	0 (0)

[a]AUC target as given by the manufacturer.[20]
[b]Number of patients (percentage).
[c]Calculated from initial AUC (taking all individual dose adjustments into consideration, see text).

Association of busulfan levels with clinical outcome, major Bu-related toxicities and other toxicities

Table 4 presents total exposure of Bu in relation with clinical outcome and major Bu-related toxicity. Failure of donor engraftment as observed in two patients was not associated with lower exposure to Bu. Leukemic relapse occurred in two patients (with JMML). The total Bu exposure of these patients was within the range of exposures as observed in the present study. At day 100 after transplantation, 24 patients were still alive and three died of relapse or disease progression at a later date. Bu exposure was the same in survivors as in nonsurvivors (Table 4).

Early Bu reactions were seen in only one child who experienced severe hypotension after dose adjustment from 0.8 to 1.0 mg/kg. An epileptic insult occurred in one child shortly after the Bu infusion was finished. This child was not prophylactically treated with antiepileptics. In the same patient, hypomagnesemia was observed, despite total parenteral nutrition. VOD (mild, moderate or severe) was observed in eight children. The average of total AUCs of Bu in children in whom VOD was observed was equal to the average of total AUCs of Bu in children who did not develop VOD (Table 4), and severity of VOD was not related to Bu exposure (Figure 2). Seventeen patients experienced mild (WHO grade 1) to severe (WHO grade 4) transaminase elevations. Total AUCs of all patients *vs* liver toxicity are plotted in Figure 3 and show no relationship between Bu exposure and subsequent hepatic dysfunction. Minor acute GvHD (grade I or II) was observed in five patients, whereas two patients developed severe GvHD (grade III or IV). Development of GvHD was not related to the total AUCs ($P = 0.15$). With a median follow-up of 24 months (range 12–40), no chronic GvHD was seen.

Discussion

Various intravenous Bu formulations have been developed in order to overcome interindividual variability in exposure after oral administration of Bu as a part of the

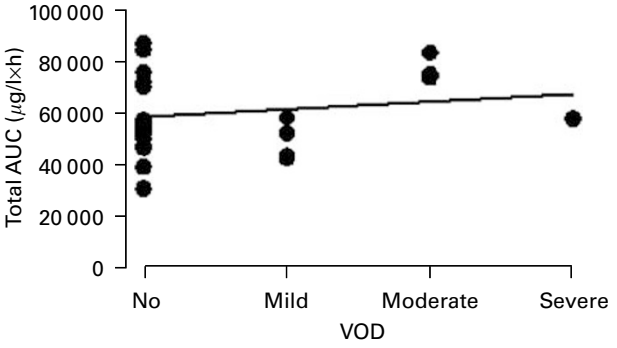

Figure 2 Correlation of VOD with total Bu exposure (AUC total). The solid line is the regression line. The correlation between total AUC and VOD was: total AUC = 58 168 + 3108 × VOD, $r = 0.03$, $P = 0.25$.

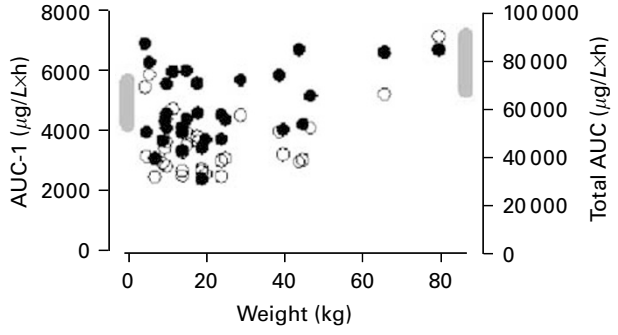

Figure 1 Overview of AUC-1 after the first dose (left axis, open circles) *vs* body weight and total AUC (right axis, solid circles) *vs* body weight. On both axes, the target ranges for the AUCs are indicated. Target AUC-1 was 4925 μg/l·h or 1200 μmol·min; range 4200–5650 μg/l·h or 1000–1400 μmol·min. Target for total AUC was 78 800 μg/l·h or 19 200 μmol·min; range 67 200–90 400 μg/l·h or 16 000–22 400 μmol·min.

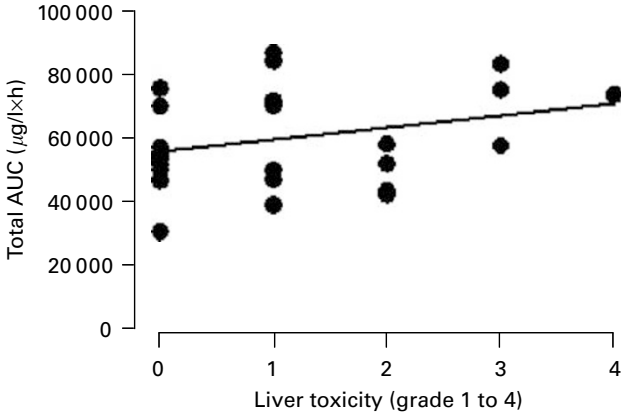

Figure 3 Correlation of liver toxicity with total Bu exposure (AUC total). The solid line is the regression line. The correlation between total AUC and liver toxicity was: total AUC = 54 032 + 4422 × liver toxicity, $r = 0.10$, $P = 0.19$.

Table 4 Total Bu exposure and outcome

Event	*Busulfan AUC (μg/l·h) of total course (mean±s.d. (n))*		
	Yes	*No*	*P-value*
VOD	60 759 ± 15 193 ($n = 8$)	59 941 ± 15 375 ($n = 23$)	0.90
Alive at day 100	59 684 ± 14 383 ($n = 24$)	61 755 ± 18 436 ($n = 7$)	0.76
Engraftment	60 521 ± 15 128 ($n = 28$)	56 706 ± 17 345 ($n = 3$)	0.68
Leukemic relapse	48 304 ± 2399 ($n = 2$)	60 969 ± 15 253 ($n = 29$)	0.26

myeloablative-conditioning regimen prior to HSCT. In a previous study, a limited sampling model was developed in order to estimate systemic exposure to intravenously administered Bu in children.[7] We used this one-compartment model in an extended patient group of 31 children, to study the pharmacokinetic parameters of Busulfex® in relation to patient outcome. Sampling at 2.5 and 4.0 h after the start of a 2-h infusion and a Bayesian fitting procedure did not unnecessarily increase the burden upon these children.

Total exposure, expressed as total AUC, was estimated from initial clearance and total dose. Even after adjustment of the dosage up to a maximum of 1 mg/kg, in only 35% of the children total exposure was as high as the target AUC as given by the manufacturer[15] and literature,[16,17] which was based on studies of oral Bu in adults. Dosages higher than 1 mg/kg were not allowed, even when the reported target range was not reached, as we believed insufficient information on toxicity profile of intravenous Bu was available for the pediatric population.

In this study we report, for the first time, data concerning pharmacokinetic parameters, toxicity and the clinical outcome obtained from a large group of children undergoing a conditioning regimen containing intravenous Bu. In 90% of the children, engraftment was established, which is in accordance with previous reports.[14,18] The pharmacokinetic parameters reported in this study are in the same range as previously seen in studies with both oral[19,20] and intravenous dosing of Bu in children.[7,15,16] Based on the present and previously reported data, it is clear that the excretion of Bu is age and weight dependent. Clearances in our patient group for intravenous Bu were higher than previously reported in adults.[14,16] It has been postulated that higher glutathione *S*-transferases in children as compared to adults may play a role in this. We calculated an average AUC of 3630 μg/l·h after the first Bu dose on the basis of empirical Bayesian estimation using a one-compartment model. In another study performed in children, a mean AUC of 4690 μg/l·h after the first liposomal Bu dose (corrected to 1 mg/kg) was found,[16] which is in accordance with the results from the manufacturer of Busulfex®. These authors found that after a Bu dose equal to that administered in our study, in 73% of the children a target AUC of 3690–5535 μg/l·h was reached. These differences are most probably explained by differences in pharmacokinetic behavior of liposomal Bu *vs* Busulfex®. Another important factor, which may influence the elimination of Bu and thereby the AUC after the first dose, is the etiology of the underlying disease of the children, that is, inherited or malignant.[16,19] Toxicity of previous chemotherapy regimes in children with malignant diseases may impair liver function and thereby influence Bu metabolism. In addition, from previous studies, it cannot be excluded that disease itself influences the metabolism of Bu.[21] The children in our study demonstrated a wide range of diagnoses, which made a clear analysis of their influence on Bu pharmacokinetics difficult.

Furthermore, other variables such as drug interactions may have influenced pharmacokinetic parameters of Bu. A substantial number of patients in our study used acetaminophen or itraconazole concurrently with Bu. Acetaminophen is known to decrease glutathione levels, and thereby possibly may decrease Bu metabolism. Itraconazol is a strong hepatic enzyme inhibitor. However, in our patients, we were unable to demonstrate any correlation between the use of these drugs and the AUC after the first dose. None of the patients were using hepatic enzyme-inducing anticonvulsants or any other known enzyme-inducing drugs.

Overall, the toxicity profile was in the same range as previously reported in intravenous Bu trials.[14,16,22,23] No new or unexpected toxicities were noted in our patients. Eight of the 31 children (26%) developed VOD, which is higher as compared with certain studies in adults,[18,24] but in accordance with other reports.[1,15,25] We could not establish any correlation between AUC and the occurrence of VOD, acute GvHD or engraftment. Previous studies showed that excessive exposure to Bu has been associated with higher morbidity and mortality.[2,18] However, in our study, none of the patients demonstrated Bu levels above the maximal safety level of the AUC, which may explain the current findings. It should be realized that toxicity in our patients is also dependent on a number of other variables, such as health of the child at the start of conditioning, conditioning regimen, previous chemotherapy and type of donor. We analyzed whether patients who developed VOD had received chemotherapy previously. Indeed, 4/8 (50%) of the patients who developed VOD received chemotherapy previously *vs* 2/23 (9%) of the patients who did not develop VOD. This finding suggests that patients, who received chemotherapy previously to Bu conditioning, are more vulnerable to develop VOD. In addition, recently Barker *et al*[25] showed that many other risk factors can be determined for the development of VOD.

After the first dose, target AUC was reached in a minority of the children. Since, at the beginning of this study, no data were known regarding children receiving a dosage higher than 1 mg/kg, we did not increase the dosage above 1 mg/kg even when the target AUC was not reached. For only one patient, the Bu dose was decreased after day 1, as the measured AUC was higher than the target AUC. This patient was over 80 kg body weight with severe cushingoid features due to long-term corticosteroid treatment, which made it difficult to calculate the most optimal lean body weight with respect to Bu dosing. Target AUC in our hospital was initially set between 4200 and 5650 μg/l·h, as generally accepted in adults and children. Total exposure to Bu in this group of children was rather low compared to that reported in adults. No relationship between Bu exposure and any of the outcome parameters could be established. Since clinical outcome and Bu-related toxicity were in accordance with previous data, we suggest that in the future, the target AUC may be set lower for this patient population (ie to the average of our measured AUC: 3600 ± 1100 μg/l·h). If this target AUC for Bu is not reached after the first dose, dose adjustment is indicated in order to reach the target AUC, except when disturbances of the child's liver function tests prohibit further dose increments. We realize that our study does not have enough power to discriminate between different patient groups. On the other hand, since very few data obtained in children are present addressing this issue, children should be treated based on the information currently available.

Obviously, it is important to monitor future patients carefully, in order to be able to make more specific recommendations for different subgroups. Hereby, type of conditioning regimen should also be considered, as concomitant cytostatic drugs might influence the required target AUC of Bu. Our pharmacokinetic model indicates that a weight-based regimen is probably more accurate than an age-based regimen for Bu. Other studies even suggest that a dosage corrected for body surface might be a better approach.[19,23,26] Recent reports, in both adults and children, have shown that a quadruple dose of Bu administered once daily possibly reduces toxicity and results in similar clinical outcome parameters as the currently used regimen of four infusions of Bu a day.[22,23] Thus, it would be feasible to consider a once-daily dosing scheme.

The present study shows that the pharmacokinetics of intravenous Bu in children can be adequately estimated using a previously described limited sampling model with Bayesian forecasting. In our group of children, the target AUC was not reached in 65% of the patients, whereas outcome and toxicity were not related to total exposure, but were in accordance with previous data. This made us conclude that target AUC for intravenous Bu in children may be set lower than in adults. Further studies incorporating larger patient groups are necessary to study this hypothesis.

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