Less veno-occlusive disease after intravenous versus oral busulfan for autologous haematopoietic stem cell transplantation: the Belgian paediatric experience

S. Huybrechts, Y. Beguin, V. Bordon, MF. Dresse, S. Dupont, A. Ferster, G. Laureys, I. Meyts M. Renard, C. Vermylen

Busulfan is commonly used in preparative conditioning regimens prior to haematopoietic stem cell transplantation in children and young adults for malignant and non-malignant disorders. For many years busulfan was only available in oral form, resulting in large inter- and intra-patients variability in plasma exposure, associated with higher graft failure rate as well as higher toxicity such as veno-occlusive disease. With the development of an intravenous formulation of busulfan, a more accurate control of both the inter- and intra-patient variability has been provided. The goal of this study was to evaluate the use and efficacy of intravenous busulfan in comparison with the oral formulation in children undergoing an autologous transplantation after conditioning with busulfan. Despite the small number of patients, this study confirmed the apparent benefit of intravenous busulfan in children undergoing an autologous HSCT. The use of a five-level dose schedule defined by body weight resulted in an efficient engraftment with marked reduction in the incidence of veno-occlusive disease compared with oral busulfan. In terms of disease-free outcome, survival and event-free survival, similar results have been obtained in both groups. The choice of this formulation of busulfan should therefore be considered.

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Introduction

Busulfan (Bu) has been widely used as a chemotherapeutic agent in high-dose preparative regimens in children undergoing both allogeneic and autologous haematopoietic stem cell transplantation (HSCT) for malignant and non-malignant disorders.[1-9] For

Authors: S. Huybrechts[1], Y. Beguin[2], V. Bordon[3], MF. Dresse[2], S. Dupont[5], A. Ferster[1], G. Laureys[3], I. Meyts[4], M. Renard[4], C. Vermylen[5].
[1]Department of Paediatric Haematology-Oncology, Hôpital Universitaire Des Enfants Reine Fabiola (HUDERF-UKZKF), Brussels, Belgium; [2]Department of Medicine, Division of Haematology, Liège, Belgium; [3]Department of Paediatric Haematology-Oncology, Ghent University Hospital, Ghent, Belgium; [4]Department of Paediatric Haematology-Oncology, University Hospitals Gasthuisberg, Leuven, Belgium; [5]Department of Paediatric Haematology-Oncology, Cliniques Universitaires Saint Luc, UCL, Brussels, Belgium.
Please send all correspondence to: S. Huybrechts MD, Hôpital Universitaire Des Enfants Reine Fabiola, Department of Paediatric Haematology-Oncology, 15, Av. J.J. Crocq, B-1020 Brussels, Belgium. tel: 0032 2 477 26 78, email: sophie.huybrechts@huderf.be.

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many years Bu was only available in an oral form. Oral Busulfan (BuPO) presents large inter- and intra-patients variability in plasma exposure, especially in young patients, because of age- and weight-dependent differences in drug absorption, metabolism and clearance. This results in important clinical consequences such as increased risk of hepatic veno-occlusive disease (HVOD) in case of overexposure whereas low drug exposure has been associated with a higher risk of disease recurrence and graft rejection.[5-9] In order to reduce the variability of busulfan exposure, an intravenous form of Bu (BuIV) (Busilvex®, Pierre Fabre Medicament, Boulogne, France) has been recently developed and is available in Belgium since 2009.

To evaluate the use and efficacy of BuIV, we compared the intravenous formulation with the oral form of Bu in children undergoing an autologous HSCT, receiving a myeloablative preparative regimen consisting of busulfan and melphalan. This article resumes the experience of different paediatric Belgian transplantation centres.

Patients and methods

Patient and tumour characteristics

Paediatric patients who underwent autologous HSCT between January 2008 and December 2010 in Belgium and received intravenous or oral busulfan as part of their conditioning regimen were retrospectively enrolled in this study. Five institutions participated in the study (University Hospital Gasthuisberg, Cliniques Universitaires Saint Luc, HUDERF-UKZKF, CHU of Liège and Ghent University Hospital). The diagnosis included Ewing Sarcoma, Neuroblastoma and Burkitt Lymphoma. Collection of the epidemiologic and transplantation data was based on the EBMT form A.

Treatment regimens and supportive care

All patients received high-dose chemotherapy consisting in the majority of cases of a combination of Bu and melphalan (Mel). Other conditioning regimens included Bu, Mel and Aracytin or Bu alone. Bu was administered orally or intravenously.

Standard supportive care and prophylaxis were provided according to the local practice. In order to reduce the risk of veno-occlusive disease (VOD), all patients received either a combination of low dose continuous heparin infusion and ursodeoxycholic acid or ursodeoxycholic acid alone, either low dose continuous heparin infusion or twice weekly fresh frozen plasma. No changes were noted in the VOD prophylaxis in all five centres since the introduction of BuIV.

Evaluation of toxicity

Neutrophil recovery was defined as a blood neutrophil count above 0,5 x 10^9/l and platelet reconstitution when platelet count was above 20 x 10^9/l without transfusion.

VOD was defined according to the modified Seattle criteria with development of at least two of the three following clinical features within 20 days after transplantation: hyperbilirubinemia with serum bilirubine > 2 mg/dl, hepatomegaly with right upper quadrant pain, ascites and/or unexplained weight gain > 2%. A subsequent classification system for the severity of VOD was based on the criteria of Seattle.

Statistical methods

We compared the efficacy and differences between the BuIV and BuPO group in terms of underlying disease, transplant characteristics by using a Fischer exact test. VOD, engraftment and platelet reconstitution were also compared in the two groups (Mann Whitney test). The outcome was defined by Overall Survival (OS) and Event-free Survival (EFS). Events were defined as relapse after complete remission (CR) or death from any cause. Survival curves were calculated by the Kaplan-Meier method. Results were considered significant if $p < 0,05$.

Results

Patient and tumour characteristics

Twenty-seven patients, aged between eight months and seventeen years were enrolled in this study. Among the twenty-seven patients, twenty were diagnosed with neuroblastoma (74%), six with Ewing sarcoma (23%) and one with Burkitt lymphoma (3%). All patients received conventional chemotherapy at diagnosis according to the ongoing protocols. Bu was administered in all children as part of their conditioning regimens before transplantation. Twelve patients received oral Bu and fifteen patients intravenous Bu.

All except two patients received standard Bu-Mel

Table 1. Demographics and disease characteristics.

Age at HSCT (years)	Diagnosis	Conditioning regimen	Route of Bu	Stem cell source	VOD	VOD prohylaxis	Engraftment: neutrophils ≥ 0,5 x109/l (days)	Platelet reconstitution ≥ 20x10^9/l (days)	Relapse (days) after HSCT	Outcome
17	EWING SARCOMA	BU-Mel	IV	PB	NO	Ursodeoxy/Heparine	11	10	NO	Alive
16,9	EWING SARCOMA	BU-Mel	IV	PB	NO	FFP	13	64	NO	Alive
12,7	EWING SARCOMA	BU-Mel	IV	PB	NO	FFP	21	32	NE	Dead
4	NEUROBLASTOMA	BU-Mel	IV	PB	NO	Ursodeoxy	11	258	NO	Alive
3	NEUROBLASTOMA	BU-Mel	IV	PB	NO	Ursodeoxy	12	35	NO	Alive
1	NEUROBLASTOMA	BU-Mel	IV	PB	NO	Ursodeoxy	12	15	NE	Alive
2,5	NEUROBLASTOMA	BU-Mel	IV	PB	NO	Heparine	15	14	NO	Alive
2,1	NEUROBLASTOMA	BU-Mel	IV	PB	NO	Heparine	16	20	NO	Alive
11	NEUROBLASTOMA	BU-Mel	IV	PB	NO	Heparine	15	11	NO	Alive
0,8	NEUROBLASTOMA	BU-Mel	IV	PB	NO	FFP	12	82	504	Alive
1,2	NEUROBLASTOMA	BU-Mel	IV	PB	NO	FFP	13	33	427	Alive
8,5	NEUROBLASTOMA	BU-Mel	IV	PB	NO	FFP	12	34	NO	Alive
5	NEUROBLASTOMA	BU-Mel	IV	PB	NO	Ursodeoxy/Heparine	11	37	NO	Alive
2,6	NEUROBLASTOMA	BU-Mel	IV	PB	NO	FFP	20	38	115	Alive -progression
14,4	BURKITT LYMPHOMA	BU-Mel-Arac	IV	PB	NO	FFP	24	42	NO	Alive
13	EWING SARCOMA	BU-Mel	PO	PB	NO	Ursodeoxy/Heparine	12	16	NE	Alive-progression
16	EWING SARCOMA	BU-Mel	PO	PB	NO	Ursodeoxy/Heparine	11	76	NO	Alive
11	EWING SARCOMA	BU-Mel	PO	PB	NO	Ursodeoxy/Heparine	11	22	NO	Alive
6	NEUROBLASTOMA	BU	PO	PB	NO	Ursodeoxy	12	14	336	Dead
1	NEUROBLASTOMA	BU-Mel	PO	PB	Severe	Ursodeoxy	11	88	NO	Alive
3	NEUROBLASTOMA	BU-Mel	PO	PB	moderate	Ursodeoxy	16	36	NE	Dead
4	NEUROBLASTOMA	BU-Mel	PO	BM	moderate	Ursodeoxy	11	43	NO	Alive
14	NEUROBLASTOMA	BU-Mel	PO	PB	NO	Ursodeoxy/Heparine	12	26	NE	Dead
1,2	NEUROBLASTOMA	BU-Mel	PO	PB	NO	Ursodeoxy/Heparine	11	29	NO	Alive
2	NEUROBLASTOMA	BU-Mel	PO	PB	NO	Ursodeoxy/Heparine	11	11	NO	Alive
5	NEUROBLASTOMA	BU-Mel	PO	PB	NO	Ursodeoxy/Heparine	14	76	NE	Alive
3	NEUROBLASTOMA	BU-Mel	PO	PB	Mild	Ursodeoxy/Heparine	15	38	NO	Alive

PB: peripheral blood cells, BM: bone marrow, Bu: Busulfan, Mel: Melphalan, Arac: Aracytin, IV: intravenous, PO: per oral, NE: not evaluable because of no complete remission at the time of transplantation, Ursodeoxy: Ursodeoxycholic acid, FFP: Fresh Frozen Plasma

regimen followed by autologous HSCT as consolidation therapy. One patient received additional doses of Aracytine and one patient Bu alone. Oral Bu was given at a dose of 1mg/kg 4-times daily for four days (total dose of 16 mg/kg). The IV Bu was infused over two-hours every six hours for sixteen doses, according to the body weight (0,8 mg/kg – 1,2 mg/kg). Following Bu, patients received Mel at a dose of 140mg/m^2 for one day. None of the patients underwent total body irradiation.

Median age at transplantation was four years in the group BuIV and four and a half years in the BuPO group. Peripheral blood stem cells (n=26) were used in all patients, except one who received stem cells from bone marrow.

Demographics and disease characteristics are summarized in *Table 1*.

Treatment-related toxicity

All patients experienced profound myelosuppression. Engraftment was observed at a median of thirteen days (range 11-24 days) in the BuIV group and twelve days (range 11-16 days) in the BuPO group and did not differ between the two groups.

Table 2. Patients characteristics.

	Busulfan IV (n=15)	Busulfan PO (n=12)	P
Median age at transplantation (years)	4	4,5	NS
Diagnosis			
Neuroblastoma	11	9	NS
Ewing Sarcoma	3	3	
Burkitt Lymphoma	1	0	
Conditioning regimen			
Bu-Mel	14	11	NS
Other	1 (Bu-Mel-Arac)	1 (Bu)	
Sources of stem cells			
PBSC	15	11	NS
BM	0	1	
VOD			
No	15	8	0,028
Yes : mild	0	1	
moderate	0	2	
severe	0	1	
Platelets > 20x109/l			
No	0	0	NS
Yes	15	12	
Mean duration in days (range)	34 (10-258)	33 (11-88)	NS
PMN >0,5 x 109/l			
No	0	0	NS
Yes	15	12	
Mean duration in days (range)	13 (11- 24)	12 (11-16)	NS
Best status after transplantation			
CR	13	7	NS
PR	0	1	
VGPR	0	1	
Not evaluated	0	1	
Progression	2	2	

PBSC: peripheral blood stem cells, BM: bone marrow, CR: complete remission, PR: partial remission, VGPR: very good partial remission, NS: not significant

Median time to platelet reconstitution (platelets > $20x10^9$/l) was similar in both groups as shown in *table 2*.

Using Mann Whitney test, the incidence of VOD was statistically higher (P=0,028) in the BuPO group (n=4) than in the BuIV group (n=0). Of the four patients with VOD, one patient had a severe VOD, two patients a moderate VOD and one a mild VOD. (*Table 1*).

Survival and Event Free Survival

The median follow-up post SCT as of February 2011

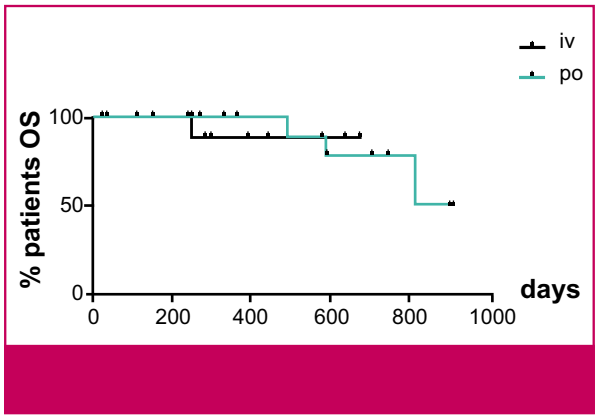

Figure 1. Overall survival of the BuIV group versus BuPO group.

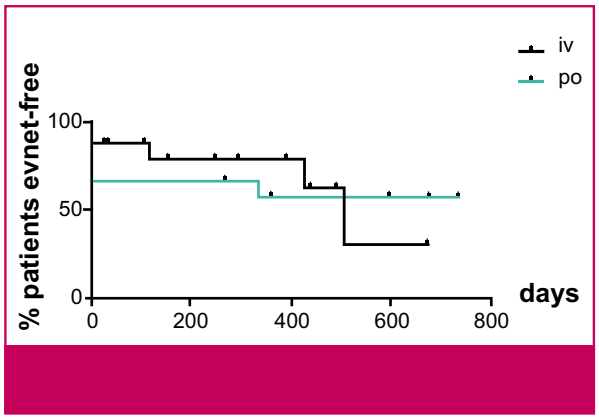

Figure 2. Event-free survival of the BuIV group versus BuPO group.

was 280 days in the BuIV group and 645 days in the BuPO group. In total, 22 patients (81,5%) were alive of whom 18 in continuous complete remission (CR).

Within BuIV group, two patients have never been in CR after SCT. Relapse (n=3) or progression (n=2) occurred in five patients. Median duration between SCT and first relapse or progression was 228 days (range 115 - 504 days). One of the five patients died from disease progression and another patient is still receiving palliative care.

In the BuPO group. Complete remission was achieved in seven patients. Among the seven patients in CR, one subsequently relapsed. Progression was noted in four other patients, of whom one had previously achieved partial remission and one Very Good Partial Response (VGPR). Relapse or progression occurred within a median time of 336 days after SCT. Three out of five patients died of progressive disease.

No significance differences between the two groups have been found in terms of relapse, disease-free survival and overall survival after SCT. (*Fig 1 and 2*)

Discussion

Busulfan and melphalan are chemotherapy agents commonly used in conditioning regimes for autologous transplantation in children and adolescents. The European Bone Marrow Transplantation (EBMT) Group has reported its experience of HSCT in the paediatric age, showing that the Bu-Mel conditioning was the most successful combination in the EBMT solid tumour registry data resulting in significantly better survival rates in neuroblastoma and Ewing's tumours.[1-4]

Over the past twenty years several studies have reported wide individual variability in Bu disposition after oral administration, being two to three-folds higher in younger children than in adults and older children. The erratic intestinal absorption of oral Bu as a result of vomiting and gastro-intestinal permeability as well as the variable clearance in paediatric patients contributes to the inter-individual variability.[5,8-10] With the development of an intravenous formulation of Bu, a more accurate control of both the inter- and intra-patient variability in adults has been provided and further improved the Bu-based conditioning therapy. Despite these formulations, considerable inter-individual variability has persisted in children and has been correlated with graft rejection and disease relapse, as well as with the severity of conditioning regimen toxicities, especially VOD.[11-17]

In a study of Vassal et al, assessing the pharmacokinetics of BuIV in children, the age-related variation of BuIV clearance could be explained by a log-linear relationship between absolute clearance and absolute body weight. Therefore, Bu was administered at five-fixed dose levels defined by body weight as part of Bu-Mel regimen (1mg/kg for < 9kg; 1,2 mg/kg for 9 to > 16kg; 1,1 mg/kg for 16-23kg; 0,95 mg/kg for > 23 kg-34 kg; 0,8 mg/kg for > 34 kg). This results in reliable engraftment with mild to moderate toxicity compared with the oral Bu preparation and is similar to our findings.[13,15]

All our patients showed profound pancytopenia after transplantation. Successful engraftment as measured by ANC > $0,5x10^9$/l occurred in all 27

Key messages for clinical practice

1. More predicable pharmacokinetics after intravenous busulfan than with the oral formulation of busulfan.
2. Busulfan and melphalan are most commonly used as myeloablative chemotherapeutics in children and young adolescents undergoing autologous for Ewing Sarcoma's and neuroblastoma.
3. Busulfan has been widely used as a chemotherapeutic agent in high-dose preparative regimens in children undergoing both allogeneic or autologous hematopoietic stem cell transplantation for malignant and non-malignant disorders.
4. Intravenous administration of busulfan leads to mild to moderate toxicity with a lower incidence of hepatic veno-occlusive disease.

children. Time and rate to engraftment was similar in both groups.

HVOD is one of the most common and severe side effects of HSCT. High plasma levels of the cytoreductive agents commonly used in the conditioning regimen of autologous HSCT, such as Bu and Mel, have been associated with increased risk of HVOD. This has been attributed to Bu-mediated depletion of hepatic gluthatione, which in turn predisposes hepatocytes to injury from ensuing melphalan. The enzyme responsible for the busulfan metabolic clearance is the gluthatione-s-transferase (GST). Four main subfamilies of GST (A1, M1, T1 and P1) have been described. The persistence of inter-individual variations after intravenous administration of Bu, may be in part explained by GST polymorphisms such as homozygous deletions of GST-M1gene.[2,16-19] Arsan et al observed an increase in the incidence of HVOD in the GSTM1-null patients, due to altered metabolism of these drugs, generating toxic metabolites and thus resulting in increased risk of HVOD.[17] However, data regarding the incidence of HVOD after BuIV seems controversial. Some authors have shown comparable incidence to that observed with oral Bu while in other studies the prevalence was lower after BuIV compared to BuPO. Children in our study who developed HVOD all received BuPO followed by Mel. No HVOD has been documented in our patients after BuIV. These results were clearly not due to shorter follow-up times in the BuIV group since VOD most often occurs within the first thirty days of haematopoietic stem cell transplantation.

Despite the fact that not all children were in CR at the time of transplant and the shorter follow-up in the BuIV group, the rate of disease recurrence was similar in both groups and EFS and OS were at least comparable to what is expected with oral Bu-based conditionings in this setting (fig 1 and 2). The majority of the children were treated for a neuroblastoma. In previous reports, high-dose therapy followed by HSCT plays an important role in the control of this disease with an estimated OS ranging from 37% to 64% and EFS around 34%.[3,4,21,22] Ladenstein et al emphasized the superiority of the use of Bu-Mel over other regimens in high-risk Neuroblastoma and Ewing's sarcoma.[16,22,23]

Conclusion

Despite the small number of patients, this non-randomised controlled design study confirmed the apparent benefit of intravenous busulfan in children undergoing an autologous HSCT in terms of incidence of VOD. The use of a five-level dose schedule defined by body weight resulted in an efficient engraftment with no marked increase in non-haematological toxicity compared with oral Bu.

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