ORIGINAL ARTICLE

Pharmacokinetics of intravenous busulfan as condition for hematopoietic stem cell transplantation: comparison between combinations with cyclophosphamide and fludarabine

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Abstract

Busulfan (Bu) has been used in combination with fludarabine (Flu; BuFlu) or cyclophosphamide (Cy; BuCy) as conditioning for allogeneic hematopoietic stem cell transplantation (HSCT). This multi-institutional prospective study compared pharmacokinetic (PK) parameters of Bu between BuFlu and BuCy. Plasma Bu concentrations were measured by high-performance liquid chromatography at the first dose of the first and fourth days of intravenous Bu administrations (total of 16 doses of 0.8 mg/kg). Thirty-seven patients were evaluable (BuFlu, $N = 18$; BuCy, $N = 19$). The median age was significantly higher in BuFlu. In BuFlu, the median area under the blood concentration–time curve of Bu on the fourth day was 1183 μmol min/L (range 808–1509), which was significantly higher than that on the first day [1095 μmol min/L (range 822–1453), $P < 0.01$]. In contrast, such differences were not observed in BuCy. Consistently, there was a significant decrease in the clearance of Bu on the fourth day as compared with the first day in BuFlu. These results suggest that the PK of Bu was altered during the co-administration of Flu, which was not the case with Cy. A large-scale study is required to evaluate the significance of the differences in the PK of Bu between the conditionings on HSCT outcomes.

Keywords Busulfan · Pharmacokinetics · Allogeneic stem cell transplantation

Introduction

Busulfan (Bu), an alkylating agent, has been widely used in the conditioning for autologous and allogeneic hematopoietic stem cell transplantation (HSCT), initially in combination with cyclophosphamide (Cy, BuCy) [1, 2]. BuCy has become one of the most commonly used myeloablative conditioning regimens. When oral formulations of Bu were used, blood levels of Bu varied greatly among individuals, mainly because of differences in intestinal absorption and first-pass effects [3]. As a result, graft failure and disease recurrence were associated with suboptimal blood

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concentrations of Bu. Conversely, adverse events such as sinusoidal obstruction syndrome (SOS) were reported to be increased in association with high blood concentrations of Bu [4, 5]. The intravenous formulation of Bu has contributed to the stable intraindividual and inter-individual pharmacokinetics (PK) of Bu leading to reduced toxicity and transplant-related mortality and improved survival [6, 7].

Fludarabine (Flu) is a purine analogue that suppresses lymphocyte proliferation and induces apoptosis. In the setting of allogeneic HSCT, Flu has been used as a key agent of reduced-intensity conditioning. Bu, in combination with Flu (BuFlu), has become one of the common conditionings in this setting based on its more favorable toxicity profile as compared with Cy [8, 9]. BuFlu not containing Cy can also be used in the setting of post-transplant Cy for haploidentical transplantation and is expected to be increasingly used [10].

A variety of agents can affect Bu metabolism. Among them, previous studies demonstrated a potential of Flu to interact with Bu, resulting in a significant decrease in the clearance of Bu and an increase in the area under the blood concentration–time curve (AUC) [11–13]. This was not the case in the setting of BuCy [11–15]. However, the route and the doses of Bu administration, as well as the timing of PK analysis, varied among those studies. In this prospective study, we performed a PK analysis of Bu in patients receiving 4-day fixed-dose intravenous Bu as part of BuFlu or BuCy conditioning. We compared the PK parameters between the two regimens to clarify the effect of concomitant Flu on the PK of Bu.

Patients and methods

Study design

This multi-institutional study was performed by the Kanto Study Group for Cell Therapy (KSGCT) and evaluated the influences of Flu and Cy on the PK of Bu administered intravenously in recipients of allogeneic HSCT.

This study was approved by the institutional review board of each participating center and was registered in the UMIN Clinical Trial Registry (ID UMIN000018693). All patients provided written informed consent using forms approved by each institution. The primary endpoint of this study is to evaluate the difference in the AUC between the first and fourth days of 4-day Bu administration in BuFlu. Secondary endpoints include the analysis of other PK

parameters and a comparison between BuFlu and BuCy. Based on our preliminary evaluation, a 15.2% increase in the AUC of Bu was predicted after 4-day Bu administration in BuFlu (data not shown). In our study, the optimal AUC for Bu was 950–1250 μmol min/L with a target AUC of 1250 μmol min/L [16]. Based on these data, the increase in the AUC of Bu after 4-day administration with Flu was predicted to be $1250 \times 0.152 = 190$ μmol min/L, and thus 200 μmol min/L was adopted in our study. Standard deviation was set at 270 according to a previous study [17]. With α set to 0.05 (two sided) and power $1 - \beta$ set to 0.8, the number of samples required was 17. We estimated a sampling error of 20%, and the number of samples needed was determined to be 20.

Patients

Study patients were enrolled from July 2014 to July 2018 at the participating institutions. Primary inclusion criteria were age 16 years or older; a diagnosis of acute myelogenous leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome; and administration of 4-day intravenous Bu in 16 divided doses as part of the conditioning for allogeneic HSCT in combination with Flu or Cy. Exclusion criteria were hepatic dysfunction defined by aspartate aminotransferase or alanine aminotransferase exceeding twice the normal upper limit, the use of itraconazole or metronidazole, the use of anticonvulsants other than valproic acid during the conditioning, and the use of other antineoplastic agents and/or total body irradiation as part of conditioning.

Conditioning regimen

In BuCy, patients received Bu from Day 7 to 4 and Cy on Day 3 and 2. In the BuFlu group, patients received Bu from Day 7 to 4 and Flu from Day 8 to 3. Bu was administered at 0.8 mg/kg body weight intravenously over 2 h every 6 h for 4 days (16 doses in total). All patients received valproic acid for the prophylaxis of Bu-associated seizure. Flu was administered intravenously at 25–30 mg/kg body weight for 1 h once daily. Cy was administered intravenously at 60 mg/kg body weight for 3 h once daily. The dose of Bu was calculated using actual body weight if it was less than ideal body weight (IBW) or adjusted ideal body weight (AIBW) if actual body weight was greater than ideal body weight. AIBW and IBW were calculated according to these equations:

$$\text{AIBW (kg)} = \text{IBW (kg)} + (\text{Actual body weight (kg)} - \text{IBW (kg)}) \times 1/3, \quad \text{IBW} = 22(\text{height (meter)})^2.$$

Sample collection and pharmacokinetic analysis

Blood samples (5 mL) for the PK analysis of Bu were obtained at the timing of the 1st and 13th Bu infusions. These samples were timed for the first dose before the start of Bu administration on the first day and the first dose on the last (fourth) day of Bu administration. For the two timings of analysis, blood samples were obtained just before starting an infusion of Bu, and 0, 120, and 240 min after the end of the infusion. Separated plasma samples were immediately cryopreserved until the Bu concentration measurements. Plasma Bu concentrations were measured by high-performance liquid chromatography basically as previously reported [18, 19]. The PK parameters of AUC and clearance were calculated from the blood concentration data using the PK analysis software Phoenix® WinNonlin® 7.0 (Certara LP, Princeton, NJ, USA).

Statistical analysis

Comparisons of the patient characteristics and PK parameters between the BuFlu and BuCy groups were performed using Fisher's exact test or the Mann–Whitney U test as appropriate. The PK parameters of Bu were compared between the 1st and 13th doses in the BuFlu and BuCy groups using the Wilcoxon signed-rank test. P values less than 0.05% were determined to be statistically significant. Statistical analysis was performed using JMP version 14 (SAS Institute Inc., Cary, NC, USA), and EZR [20].

Results

Patient characteristics

In total, 40 patients were enrolled into this study (BuFlu, $N=20$; BuCy, $N=20$). One patient in the BuCy group was excluded from the study because of the patient's poor general condition before initiating conditioning. Two cases in the BuFlu group were excluded from the analysis because of blood collection error ($N=1$) and outliner of AUC of Bu ($N=1$). The characteristics of the 37 evaluated patients are shown in Table 1. There were differences between BuFlu and BuCy in age, prothrombin time, estimated glomerular filtration rate (eGFR), and underlying diseases. Body weight and liver enzymes did not differ significantly between the two groups. The BuFlu group had more patients with myelodysplastic syndrome and no patients with chronic myeloid leukemia. No patients received deferasirox at the start of conditioning.

Pharmacokinetics of busulfan

The PK parameters of Bu are shown in Table 2. In the BuFlu group, the median AUC of Bu was 1183 µmol min/L (range 808–1509) on the fourth day, which was significantly higher than that on the first day [1095 µmol min/L (range 822–1453), $P<0.01$]. In contrast, although a similar trend was observed in the BuCy group, it was not statistically different [1188 µmol min/L (range 728–1654) vs. 1154 µmol min/L (range 604–1634), $P=0.08$]. In the BuFlu group, the clearance of Bu of the 4th day was significantly lower on the fourth day than on the first ($P=0.02$), whereas this was not the case in the BuCy group. In both groups, the

Table 1 Patient characteristics ($N=37$)

	BuFlu ($N=18$)	BuCy ($N=19$)	P value
Male/female	14/4	13/6	0.71
Age, years, median (range)	60 (50–66)	47 (22–54)	<0.001
Body weight, median (range) (kg)	63.8 (36.9–85)	56.5 (47.4–85.6)	0.81
Adjusted body weight, median (range) (kg)	61.7 (36.9–74.8)	56.5 (47.4–76.0)	0.93
eGFR, median (range) (mL/min)	86.5 (52.6–135.3)	119.4 (77.5–237.7)	<0.001
AST, median (range) (IU/L)	17 (8–30)	21 (12–37)	0.09
ALT, median (range) (IU/L)	14 (5–39)	22 (8–71)	0.15
PT, median (range) (%)	97 (72–106)	108 (73–121)	<0.05
Total bilirubin, median (range) (mg/dL)	0.6 (0.4–1.4)	0.5 (0.2–1.1)	0.10
Underlying disease			
Acute myeloid leukemia	12	14	<0.05
Myelodysplastic syndrome	6	2	
Chronic myeloid leukemia	0	3	

Bu busulfan, *Cy* cyclophosphamide, *Flu* fludarabine, *eGFR* estimated glomerular filtration rate, *PT* prothrombin time, *AST* aspartic aminotransferase, *ALT* alanine aminotransferase

Table 2 Pharmacokinetc parameters

	BuFlu (N=18)			BuCy (N=19)		
	1st day	4th day	P	1st day	4th day	P
AUC, median (range) (μmol min/L)	1095 (822–1453)	1183 (808–1509)	<0.05	1154 (604–1634)	1188 (728–1654)	0.08
Volume of distribution, median (range) (L/kg)	0.70 (0.59–1.07)	0.52 (0.39–0.66)	<0.01	0.67 (0.07–0.97)	0.47 (0.06–0.90)	<0.01
C_{max}, median (range) (ng/mL)	866 (630–1014)	1112 (844–1369)	<0.01	867 (644–1289)	1134 (705–1605)	<0.01
Half-life, median (range) (h)	2.88 (2.24–3.79)	3.66 (2.65–4.60)	<0.01	2.79 (1.84–4.74)	3.28 (2.16–7.57)	<0.05
Clearance, median (range) (L/h)	10.88 (7.15–16.4)	10.33 (6.61–14.0)	<0.05	10.27 (7.26–16.1)	9.76 (6.93–16.42)	0.23

Bu busulfan, *Cy* cyclophosphamide, *Flu* fludarabine, *AUC* area under the blood concentration–time curve, C_{max} maximum calculated plasma concentration

volume of distribution, maximum calculated plasma concentration (C_{max}), and half-time differed significantly between the first and fourth days. There were no statistically significant differences in the AUC or clearance of Bu between BuFlu and BuCy at any evaluation timing.

Graft failure, sinusoidal obstructive syndrome, and disease relapse

One patient experienced primary graft failure in the BuFlu group, but none did so in the BuCy group. One patient developed SOS in the BuFlu group, but none did so in the BuCy group. One patient in each group underwent allogeneic HSCT with active disease and did not achieve remission after transplantation. In addition, there were six cases of relapse in the BuCy group and two in the Bu/Flu group. The cumulative incidences of disease relapse/progression at 1 year were 11.1% (95% CI 1.7–30.4%) in the BuFlu group and 21.1% (95% CI 6.3–41.6%) in the BuCy group; the difference was not significant (P=0.13).

Discussion

In this prospective study evaluating the PK of Bu used as a part of myeloablative conditioning for allogeneic HSCT, the AUC and clearance of Bu on the fourth day of Bu administration was significantly altered as compared with those on the first day in patients receiving BuFlu. In contrast, no such alterations of PK parameters were observed in patients receiving BuCy. Our findings suggest that co-administration of Flu has the potential to affect the PK of Bu in the later phase of conditioning, increasing the AUC of Bu.

Several reports have evaluated the effect of Flu on the PK of Bu, but the results were contradictory [11, 13, 21]. In one study, the clearance of Bu was lower at the last dose of Bu than at the first dose in the 4-day Bu-containing regimen [13]. Another study demonstrated that a significant decrease in the clearance of Bu was more frequent in patients receiving Bu concomitantly or consecutively with Flu in those

receiving BuCy [11]. On the other hand, another study showed that Flu did not affect the PK of Bu [21]. Therefore, it is not conclusive that Flu has a significant effect on the PK of Bu. However, the mechanism by which Flu reduces the clearance of Bu and/or increases the AUC of Bu is unknown. Since both Bu and Flu are known to be metabolized by distinct pathways—rapid dephosphorylation in the plasma and phosphorylation by deoxycytidine kinase for Flu and glutathione S-transferases (GST) in the liver for Bu—it is impossible to speculate on the mechanisms underlying the interaction of Flu with Bu [15, 22].

There were limitations to our study. One was the small number of subjects evaluated. Another was the lack of data on glutathione S-transferase (GST) polymorphism in each patient, which was known to be associated with interindividual variation in the PK parameters of Bu [23, 24]. There are subtypes of GST as well as genetic polymorphisms in GSTA1, GSTM1, GSTP1, and GSTT1. Kusama et al. showed that 9 of 12 (75%) Japanese patients had GSTA1*A/*A, which was associated with a significantly higher clearance of Bu and a lower Bu concentration compared to GSTA1*A/*B [23]. Terakura et al. reported similar findings in Japanese patients [25].

Another limitation was the difference in age between the groups. The BuFlu group was significantly older than the BuCy group. Aging is associated with several physiological changes that can affect drug PK and pharmacodynamics [26, 27]. Age-dependent differences in the PK of Bu have been reported in children having a higher distribution volume and clearance than adults after oral Bu administration, resulting in reduced toxicity and efficacy [28]. In contrast, other investigators have shown increased Bu clearance in patients older than 60 years compared with younger patients [29]. Therefore, the effect of age on Bu metabolism has not been defined. An age-adjusted comparative study is required to determine whether aging affects the PK of Bu.

In conclusion, our results suggest that the PK of Bu was affected in combination with Flu by decreasing the clearance, and this, in turn, increased the AUC, whereas no such

effect was observed in combination with Cy. A larger scale study is required to evaluate further the effect of Flu on the PK of Bu as well as the impact of that effect on transplant outcomes.

Compliance with ethical standards

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