An Evaluation of Busulfan Pharmacokinetics in Patients Undergoing Hematopoietic Stem Cell Transplantation

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Background: Busulfan (BU) pharmacokinetics (PK) are shown to be highly variable and thus their evaluation is critical for the success of hematopoietic stem cell transplantation (HST) in Caucasians. However, there are no data available for Japanese patients.

Methods: BU PK were evaluated in seven Japanese adult patients who underwent allogeneic HST. Four patients received 16 doses of 1 mg/kg of oral BU every 6 h for over 4 days followed by 120 mg/kg of intravenous cyclophosphamide, while three patients were given eight doses of 1 mg/kg of oral BU over 2 days in addition to 180 mg/kg of intravenous fludarabine with or without 2 Gy of total body irradiation. Blood samples were collected for PK analysis after the sixth dose of BU was administered.

Results: The average plasma BU concentrations at steady state (Css) ranged from 745 to 2422 ng/ml. Four of seven patients had BU Css >1000 ng/ml, the previously defined concentration associated with an increased risk of regimen-related toxicity (RRT). Indeed, one of them developed hepatic veno-occlusive disease. On the other hand, no severe toxicity greater than grade II except stomatitis was observed in the remaining patients whose Css were <1000 ng/ml.

Conclusion: A possible increased risk of RRT associated with high plasma BU concentrations should be kept in mind after oral administration of BU. A prospective trial of adjusting BU doses depending on the BU PK is warranted for Japanese patients.

Key words: busulfan – pharmacokinetics – regimen-related toxicity – veno-occlusive disease

INTRODUCTION

Total body irradiation (TBI) in combination with cyclophosphamide (CY) is the standard myeloablative conditioning regimen for allogeneic hematopoietic stem cell transplantation (HST). Busulfan (BU) was introduced as an alternative to TBI, and cumulative data indicate that the therapeutic efficacy of a preparative regimen with BU and CY is equivalent to TBI and CY in patients with chronic myeloid leukemia (CML) (1). However, veno-occlusive disease (VOD) of the liver seems to be experienced more in BU-treated patients (2).

The standard dose of BU is 1 mg/kg body weight administered orally every 6 h. As for alkylating agents in general, the therapeutic effect of BU is closely correlated with the area under the plasma concentration–time curve (AUC) or the average plasma concentrations at steady state (Css) (3). It is demonstrated that AUC or Css varies widely from one patient to another after oral BU, and excessively high BU concentrations are associated with an increase in hepatic VOD (4,5), while lower levels are related to a high relapse rate in patients with CML (6) and graft rejection in children (7,8). Monitoring of BU concentrations and the dose adjustment according to the BU levels is shown to reduce the risk of severe toxicity in patients with myelodysplastic syndrome (MDS) and CML (9,10).

BU, which causes DNA damage and induces cells to undergo cell cycle arrest, is metabolized mainly in the liver through conjugation with glutathione by glutathione S-transferase (GST). High hepatic GST activity correlates with high BU clearance and low plasma BU concentrations (11), indicating that conjugation with glutathione is an effective mechanism to detoxify BU metabolically. A recent study has shown that the polymorphisms of GST genes are associated with the risk of developing hepatic VOD in patients undergoing HST (12). Ethnic variation is also demonstrated in relation to a gene deletion polymorphism of GST (13), suggesting that BU metabolism is influenced by race. The pharmacokinetics

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(PK) of BU have been extensively investigated in Caucasians and few studies have focused on Asian people (14).

We have recently developed a high-performance liquid chromatographic method to determine BU concentrations (15). By using this method, we undertook the BU PK study in seven Japanese adult patients who received oral BU for allogeneic HST.

**PATIENTS AND METHODS**

**PATIENTS AND TREATMENT**

We prospectively evaluated BU PK in seven Japanese adult patients who underwent allogeneic HST for hematological malignancies. Four patients received 16 doses of BU at 1 mg/kg every 6 h over 4 days followed by 120 mg/kg of CY, while three patients received BU as a part of a reduced-intensity preparative regimen which was given in eight doses over 2 days in addition to 180 mg/kg of fludarabine (FLU) with or without 2 Gy of TBI. Prophylaxis against graft-versus-host disease (GVHD) consisted of a combination of cyclosporin and methotrexate in five patients who were transplanted with either bone marrow (BM) cells or peripheral blood stem cells (PBSCs) from their human leukocyte antigen (HLA)-matched siblings, while cyclosporin and mycophenolate mofetil were given in two patients undergoing unrelated cord blood (CB) cell transplantation. Sodium valproate was given to all patients as prophylaxis against BU-related seizures, and fluconazole, ciprofloxacin and aciclovir were administered as prophylaxis against fungal, bacterial and herpes infection, respectively. Patients’ characteristics are shown in Table 1. All patients provided an informed consent to participate in this study.

**PK STUDIES**

Blood samples were collected for analysis in heparinized tubes at 30, 60, 120, 240 and 360 min after the sixth dose of BU was taken orally. They were stored on ice until the plasma was separated and the separated plasma was frozen at −20°C until analysis. BU concentrations were measured by a high-performance liquid chromatographic method as previously described.

### Table 1. Patient characteristics and busulfan pharmacokinetic parameters

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age</th>
<th>Sex</th>
<th>Disease</th>
<th>Donor</th>
<th>Graft</th>
<th>HLA compatibility</th>
<th>Conditioning regimen</th>
<th>CL/F(l/h/kg)</th>
<th>Vd/F(l/kg)</th>
<th>t1/2(h)</th>
<th>kα(h⁻¹)</th>
<th>Css(ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>F</td>
<td>MDS</td>
<td>Sibling</td>
<td>BM</td>
<td>6/6</td>
<td>BU 16 mg/kg + CY 120 mg/kg</td>
<td>0.16</td>
<td>0.81</td>
<td>2.6</td>
<td>1.64</td>
<td>745</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>ML</td>
<td>Sibling</td>
<td>PBSC</td>
<td>6/6</td>
<td>BU 16 mg/kg + CY 120 mg/kg</td>
<td>0.21</td>
<td>0.64</td>
<td>2.1</td>
<td>2.60</td>
<td>827</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>F</td>
<td>AML</td>
<td>Sibling</td>
<td>PBSC</td>
<td>6/6</td>
<td>BU 16 mg/kg + CY 120 mg/kg</td>
<td>0.10</td>
<td>0.48</td>
<td>3.4</td>
<td>3.39</td>
<td>1676</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>M</td>
<td>AML</td>
<td>Sibling</td>
<td>PBSC</td>
<td>6/6</td>
<td>BU 16 mg/kg + CY 120 mg/kg</td>
<td>0.19</td>
<td>0.71</td>
<td>2.6</td>
<td>2.56</td>
<td>861</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>F</td>
<td>ML</td>
<td>Sibling</td>
<td>PBSC</td>
<td>6/6</td>
<td>BU 8 mg/kg + FLU 180 mg/m²</td>
<td>0.11</td>
<td>0.51</td>
<td>3.2</td>
<td>3.18</td>
<td>1747</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>F</td>
<td>AML</td>
<td>Unrelated</td>
<td>CB</td>
<td>5/6</td>
<td>BU 8 mg/kg + FLU 180 mg/m² + TBI 2 Gy</td>
<td>0.07</td>
<td>0.60</td>
<td>6.0</td>
<td>0.54</td>
<td>2422</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>M</td>
<td>ML</td>
<td>Unrelated</td>
<td>CB</td>
<td>5/6</td>
<td>BU 8 mg/kg + FLU 180 mg/m² + TBI 2 Gy</td>
<td>0.15</td>
<td>0.68</td>
<td>3.3</td>
<td>0.75</td>
<td>1146</td>
</tr>
</tbody>
</table>

MDS, myelodysplastic syndrome; ML, malignant lymphoma; AML, acute myeloid leukemia; BM, bone marrow cells; PBSC, peripheral blood stem cells; CB, cord blood cells; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; TBI, total body irradiation; CL/F, clearance; Vd/F, volume of distribution; t1/2, elimination half-life; kα, absorption rate constant; Css, average plasma concentration at steady state.

### Table 2. Clinical outcome

<table>
<thead>
<tr>
<th>UPN</th>
<th>Heart</th>
<th>Bladder</th>
<th>Kidney</th>
<th>Lung</th>
<th>Liver</th>
<th>CNS</th>
<th>Stomatitis</th>
<th>GI</th>
<th>Hepatic VOD</th>
<th>Acute GVHD</th>
<th>Event-free survival</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17 months</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>2 months</td>
<td>Relapse</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>I</td>
<td>I</td>
<td>21 months</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>I</td>
<td>19 months</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>III</td>
<td>27 months</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>I</td>
<td>I</td>
<td>4 months</td>
<td>Died of Aspergilosis</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td>13 months</td>
<td>–</td>
</tr>
</tbody>
</table>

CNS, central nervous system; GI, gastrointestinal organ system; VOD, veno-occlusive disease; GVHD, graft-versus-host disease.
to All seven patients achieved engraftment. Neutrophil recovery
between doses.

REGIMEN-RELATED TOXICITY (RRT)

RRT was scored using the criteria described by Bearman et al.
(17). The overall grade was calculated from the maximum toxic-
ity grade for the bladder, renal, pulmonary, hepatic, central
nervous system, mucosal and gastrointestinal organ systems
which the patients experienced during the first 28 post-
transplant days. The diagnosis of VOD was made clinically
based on the standard criteria of a bilirubin >2 mg/dl and at
least two of the following signs or symptoms: hepatomegaly
with right upper quadrant pain, ascites or weight gain >5% from
the baseline (18).

RESULTS

BUSULFAN PK

Table 1 presents the PK data of BU. The median clearance
(CL/F) was 0.15 l/h/kg (0.07–0.21 l/h/kg), the median volume
of distribution (Vd/F) 0.64 l/kg (range 0.48–0.81 l/kg), the
median elimination half-life (t1/2) 3.2 h (range 2.1–6.0 h)
and the median absorption rate constant (ka) 2.56/h (range
0.54–3.39/h). The average plasma concentrations of BU Css
ranged from 745 to 2422 ng/ml (median 1146 ng/ml).

ENGRAFTMENT

All seven patients achieved engraftment. Neutrophil recovery
to >0.5 x 10^9/l and platelet recovery to 50 x 10^9/l occurred on
days 11–28 (median 13 days) and on days 13–75 (median 14
days), respectively.

RRT

Previous studies indicate that BU Css of >1000 ng/ml is asso-
ciated with an increased risk of developing hepatic VOD (4,5).Four of seven patients were treated with 16 mg/kg of BU and
120 mg/kg of CY. One of them developed hepatic VOD. This
was a 21-year-old female patient (UPN 3) with acute myeloid
leukemia (AML) in second remission. She underwent allogene-
ic HST from her HLA-matched brother. Hematological reco-
very was quite rapid; neutrophil counts reached >0.5 x 10^9/l on
day 11 and platelet counts >50 x 10^9/l on day 13. However,
serum levels of total bilirubin rose to 9.6 mg/dl on day 20 with
no significant increase in liver transaminase and alkaline phos-
phatase levels. Hepatomegaly and ascites became apparent in a
few days. She fulfilled the clinical criteria of hepatic VOD. She
became anuric, with serum creatinine levels up to 4.6 mg/dl,
requiring hemodialysis on day 23. Her BU Css came back
to 1676 ng/ml. Fortunately, her liver and renal function
returned to normal and the ascites disappeared within 3
weeks. She has been alive in remission for 21 months.

There were three remaining patients who also received BU
and CY. No grade II–III toxicity except stomatitis was experi-
enced in these patients whose BU Css were 745, 827 and
861 ng/ml, respectively.

Three patients received a reduced-intensity conditioning
regimen consisting of a total of 8 mg/kg of BU. They had
BU Css of 1745, 2422 and 1146 ng/ml, respectively. They
were all higher than 1000 ng/ml, but no grade II–III RRT
was observed.

CLINICAL OUTCOME OF THE HST

There were two patients who ended up with recurrence of the
underlying disease or fatal complication. A 60-year-old female
(UPN 2) with malignant lymphoma in third remission received
allogeneic HST following a BU and CY conditioning regimen,
but had recurrence of the disease after 2 months. Another
patient was a 65-year-old female (UPN 6) with AML in second
relapse, who underwent unrelated umbilical cord transplan-
tation following a reduced-intensity conditioning, and died of
Aspergillus pneumonia at 4 months after HST. The remaining
five patients have been alive in remission for 13–27 months
(median 19 months) after HST.

DISCUSSION

This study represents the first PK data of oral BU for Japanese
adult patients. A BU Css level below 600 ng/ml is shown to
 correlate with the increased risk of graft rejection (7). It
was fortunate that all patients achieved BU Css of higher
than 600 ng/ml, and therefore, the influence of low BU con-
centrations on the risk of poor engraftment was not evaluable
from our small study.

More than half (four out of seven) of the patients had BU Css
higher than 1000 ng/ml and, one of them developed hepatic
VOD. On the other hand, no severe toxicity greater than
grade II except stomatitis was observed in the other three
patients whose Css were below 1000 ng/ml. The number of
patients evaluated in the present study was too small to draw
a definitive conclusion. However, an increased risk of RRT
associated with high plasma BU concentrations was suggested
in Japanese adults after the oral administration of BU in
combination with CY.

In contrast, three patients receiving BU as a reduced-
intensity conditioning had high BU Css, but no severe RRT
was observed. In these cases, the total dose of BU was half of
that given in the BU–CY regimen. This may suggest that
not only BU Css but also the total dose is important to predict
adverse events of BU. A prospective trial monitoring BU con-
centrations and adjusting BU doses depending on the PK is
warranted for Japanese patients who are planning to undergo
HST especially when a high and full dose BU-containing con-
ditioning regimen is used.
Acknowledgments

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