·CLINICAL RESEARCH·

Efficacy and Toxicity of Intravenous Busulfan-based Conditioning before Allogeneic Peripheral Blood Stem Cell Transplantation for Leukemia

TONG Xiu-Zhen, XU Duo-Rong, ZOU Wai-Yi, LI Juan, LUO Shao-Kai, PENG Ai-Hua, ZHANG Guo Cai, ZHENG Dong

Department of Hematology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, 510080, P. R. China

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Correspondence to: LI Juan
Tel: 86-20-87755766-8831
Fax: 86-20-87332150
E-mail: tongxz05@163.com

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[ABSTRACT] **BACKGROUND & OBJECTIVE**: Busulfan (Bu) is commonly used as a component of conditioning regimens for hematopoietic stem cell transplantation. Erratic gastrointestinal absorption as a result of oral administration of Bu not only affects the efficacy, but also increases the risk of toxicity. This study was to analyze the efficacy and toxicity of intravenous Bu and cyclophosphamide (Cy) conditioning before allogeneic peripheral blood stem cell transplantation (allo-PBSCT) for leukemia. **METHODS**: Fifteen leukemia patients received intravenous Bu/Cy conditioning before allo-PBSCT, while 20 patients received oral Bu/Cy conditioning. The responses and adverse events of the 2 groups were assessed. **RESULTS**: All 15 patients in intravenous Bu/Cy group had hematopoietic engraftment. The median time of engraftment was 12 (9–15) days for neutrophils and 15 (11–24) days for platelets. Of the 15 patients, 6 (40.0%) developed acute graft-versus-host disease (aGVHD), including 4 cases of grade Ⅰ–Ⅱ aGVHD and 2 cases of grade Ⅲ–Ⅳ aGVHD; during conditioning, 7 (46.6%) had vomiting, 1 (6.7%) had oral mucositis, 1 (6.7%) had hemorrhagic cystitis, 2 (13.3%) had hepatic damage, none developed seizure. With a median follow-up of 180 days (range, 35–420 days), 14 (93.3%) patients were alive, 1 died of severe aGVHD accompanied fungal infection of the lung and central nerve system. The occurrence rates of hepatic damage and oral mucositis were significantly lower in intravenous Bu/Cy group than in oral Bu/Cy group (13.3% vs. 60.0%, 6.7% vs. 80.0%, $P<0.01$). There were no significant differences in hematopoietic reconstruction, aGVHD, stomatitis, gastrointestinal reaction, and hemorrhagic cystitis between the 2 groups ($P>0.05$). **CONCLUSION**: The intravenous Bu/Cy conditioning before allo-PBSCT for leukemia has clear efficacy with low extramedullary toxicity.

KEYWORDS: Leukemia; Stem cell transplantation, allogeneic; Busulfan; Cyclophosphamide; Pretreatment; Efficacy; Toxicity

1. Introduction

Oral administration of busulfan (Bu) at a large dose combined with cyclophosphamide (Cy) has been extensively applied in hematopoietic stem cell transplantation (HSCT) as the pretreatment regimen. One major disadvantage of oral administration is a high incidence of complications, such as hepatic veno-occlusive disease[1]. An ideal conditioning regimen should decrease the toxicity of allogeneic peripheral blood stem cell transplantation (allo-PBSCT), while has no influence on its efficacy. In 2000, Andersson *et al.* [2] introduced intravenous Bu into the HSCT conditioning regimen, and achieved good effects. However, this regimen is rarely adopted and reported in China.

This study was to analyze the efficacy and toxicity of intravenous Bu in combination with Cy (Bu/Cy) as the conditioning regimen before allo-PBSCT for leukemia.

2. Materials and Methods

2.1 Clinical data

Fifteen leukemia patients received Bu/Cy pretreatment were admitted for transplantation between Nov. 2005 and Jan. 2006, with a median age of 30 (15-48) years. Eight cases were 15-29 years, four cases were 30-39 years, and three cases were 40-48 years. There were nine males and six females. Eight cases had acute myeloid leukemia, six cases had acute lymphoblastic leukemia, and one case had chronic myeloid leukemia in the chronic phase. All diagnoses were confirmed by blood morphological examination and cytogenetic examination. Among these patients, 13 achieved complete remission after the first therapy, one case achieved complete remission after the second therapy, and 2-3 treatment courses were performed before transplantation for 13 cases. Among the donors in intravenous Bu/Cy group, there were 11 males and four females, with a median age of 29 (12-51) years. The genders of eight donors were not consistent with the hosts. Thirteen cases had siblings with completely matched human leukocyte antigen (HLA), one case received half matched transplantation from his mother, and one case received donation from an unrelated donor.

Oral Bu/Cy group had 20 cases, all of which were leukemia patients admitted for transplantation from Jan. 2002 to Oct. 2005, with a median age of 19 (15-44) years. There were 12 males and eight females; there were 12 cases of acute myeloid leukemia, seven cases of acute lymphoblastic leukemia, and one case of chronic myeloid leukemia in the chronic phase. There was no significant difference in gender, age, gender combination between the donors and the hosts, transplantation style, and the interval from diagnosis to transplantation, and so on between the two groups (P>0.05).

2.2 Methods

2.2.1 Mobilization and collection of peripheral blood stem cells (PBSCs)

Granulocyte-macrophage colony stimulating factor (GM-CSF) at a dose of 5μg·(kg·d)$^{-1}$ was administered four days before transplantation for continuously 5-6 days. CS3000 plus blood cell separator was applied to perform PBSC separation on the fifth day. The amount of circulating blood was 10-12L, and the median amount of collected mononuclear cells was 6.67 × 10^8/kg [(4.3-12.1) × 10^8/kg]

2.2.2 The pretreatment regimen

Fifteen patients started to receive Bu at a dose of 0.8mg·(kg·d)$^{-1}$ intravenously within 2h seven days before transplantation, once every six hours for four days in total. Then Cy at a dose of 60mg·(kg·d)$^{-1}$ was intravenously dripped for two days. The patients rested for one day before re-infusion of stem cells.

In the oral Bu group, 20 cases received oral Bu at a dose of 1mg·(kg·d)$^{-1}$, once every six hours for four days in total. If the patient vomited, additional Bu was administered according to the vomit time and volume. The rest procedures were the same as described for intravenous Bu/Cy group.

2.2.3 Graft versus host disease (GVHD)

The Cyclosporine A plus short-term methotrexate regimen was used: cyclosporine A at a dose of 2 mg·(kg·d)$^{-1}$ was dripped intravenously one day before transplantation. Oral cyclosporine A at a dose of (5-8) mg·(kg·d)$^{-1}$ was applied when the patient could eat. Sixty days after transplantation, the dose was decreased by 5% every two weeks, and the drug was withdrawn half a year later. Methotrexate was administered at a dose of 15 mg/m^2 on the first day after transplantation, and at a dose of 10 mg/m^2 on the third, sixth and eleventh day after transplantation, together with calcium folinate. When acute GVHD occurred, methylprednisolone or other immunosuppressive agents was used.

2.2.4 Hepatic veno-occlusive disease

Prostaglandin E1 at a dose of 5μg·(kg·d)$^{-1}$ was administered for 30 days in total.

2.2.5 Prevention of hemorrhagic cystitis (HC)

Hyperhydration and alkalinizing was performed for diuresis 6 h before the pretreatment. The urine volume was maintained above 2500ml/m^2 daily; and the pH value of urine was maintained between 6.8-7.2 till 2 days after transplantation. Mesna was applied on the day when Cy was infused, at a dose of 120% Cy.

2.2.6 Prevention of cytomegalovirus (CMV) infection

Ganciclovir at a dose of 500mg·d^{-1} was used before transplantation for continuously 7 days to prevent CMV infection. Re-use of ganciclovir was determined by the results of

CMV detection after transplantation.

2.2.7 Detection of graft survival

Peripheral hemogram, ABO blood group, sex chromosome and polymerase chain reaction on short tandem repeat loci were evaluated respectively to judge the engraftment.

2.2.8 Standards of complication diagnosis and toxicity grading[3]

(1) Hemorrhagic cystitis was graded as grade 0, no cystitis; grade Ⅰ, microscopic hematuria; grade Ⅱ, gross hematuria; grade Ⅲ, blood clot was observed in urine; grade Ⅳ, urethral obstruction was induced by blood clot. (2) Oral mucositis was graded as grade 0, no mucositis; grade Ⅰ, red mucosal spot with pain; grade Ⅱ, red mucosal spot, ulcer, the patient could still take common food; grade Ⅲ, ulcer, the patient could take fluid food; grade Ⅳ, the patient could not take food. (3) Gastrointestinal reactions were graded as grade 0, no gastrointestinal reaction; grade Ⅰ, nausea; grade Ⅱ, light vomiting; grade Ⅲ, vomiting, and therapy was needed; grade Ⅳ, vomiting could not be controlled. (4) Diagnosis criteria of HOVD were classified as hyperbilirubinemia (34.2μ mol/L), with at least two of the following symptoms: liver enlargement (painful), ascites, increased body weight by 5.0% above the base line.

2.2.9 Follow- up

The patients were followed up at clinic or by random visits, from Jan. 2002 till Jan. 2007.

2.3 Statistical methods

SPSS10.0 software was applied to perform data analysis. Ratios were tested using chi-square test. Means were compared using *t* test. $P<0.05$ was set as the significant level.

3. Results

3.1 Hematopoietic engraftment and graft survival in intravenous Bu/Cy group

All 15 patients in intravenous Bu/Cy group had hematopoietic engraftment. The median time of neutrophils reaching 0.5×10^9/L and platelets reaching 20×10^9/L after engraftment were 12 (9-15) days and 15 (11-24) days, respectively. Fifteen patients, who were complete chimeras, were confirmed donor transplantation. Their bone marrows were found complete remission 30 days after transplantation.

Nine cases (60.0%) were transfused with erythrocytes, twice in average (1-7 times). Fifteen cases received platelet transfusion, four times (1-6 times) in average.

3.2 aGVHD in intravenous Bu group

Six cases had aGVHD (40.0%), including four cases of grade Ⅰ-Ⅱ aGVHD and two cases of grade Ⅲ-Ⅳ aGVHD. The four cases of grade Ⅰ-Ⅱ aGVHD were patients with completely matched transplantation, who were responsive to methylprednisolone. Two cases of grade Ⅲ-Ⅳ intestinal aGVHD received half matched and non-sibling donor transplantation, and their conditions were improved after administration of methylprednisolone and anti-CD25 antibody. The condition of one of the two patients was not controlled. Of 10 cases who survived more than 150 days after transplantation, four cases (40.0%) developed chronic GVHD (c-GVHD), all of which were limited type.

3.3 Toxicity of intravenous Bu group

Seven cases (46.6%) had vomiting, four of which were grade Ⅰ-Ⅱ and two were grade Ⅲ. Vomiting appeared mainly on the third day (from the second to the forth day) after intravenous administration of Bu, and the median duration was 5 days (4-8 days). Six cases had diarrhea: two of them had diarrhea on the third day after Bu administration, lasting for 3-6 days; four cases had diarrhea on the 13th day after transplantation, with a duration of 4 days. The conditions were improved after the symptomatic treatment.

One case (6.7%) had oral mucositis, which appeared on the 5th day after transplantation, lasting for 4 days. One case (6.7%) had hemorrhagic cystitis, which was observed on the 18th day after transplantation (grade Ⅲ). Hemorrhagic cystitis lasted for 21 days, and the symptoms disappeared after liquid transfusion and alkalization of urine. There was no epileptoid convulsion or interstitial pneumonia.

Two cases (13.3%) had hepatic damage. One case had increased aminotransferase on the 3rd day after transplantation, who recovered after protective liver therapy. Another case had 63 nmol/L of aspartate aminotransferase and 213 nmol/L of alanine aminotransferase on the 1st day after transplantation; on the 18th day, his hepatic function was aggravated, and aspartate aminotransferase and alanine aminotransferase increased to 206 nmol/L and 632 nmol/L, respectively; his liver function was recovered after protective liver therapy and anti-GVHD therapy for 3 weeks. No case had hepatic veno-occlusive disease.

3.4 Survival of patients in intravenous Bu group

With a median follow-up period of 180 days (36-420 days), 14 (93.3%) patients were alive, and one case with graft from non-sibling died from severe aGVHD accompanied by fungal infection in lung and the central nerve system on the 97th day after transplantation.

3.5 Comparison of toxicity between intravenous and oral Bu conditioning regimens

The occurrent rates of hepatic damage and oral mucositis were significantly lower in intravenous Bu/Cy group than those in oral Bu/Cy group ($P<0.01$). Compared to oral Bu group, hepatopoietic reconstruction was a little quicker and incidences of gastrointestinal reactions, HC and aGVHD were slightly lower in intravenous Bu group; but the differences were not significant ($P>0.05$) (Table 1).

Table 1 Efficacy and toxicity of intravenous versus oral busulfan-based conditioning before allogeneic peripheral blood stem cell transplantation for leukemia

Group	Cases	Engraftment time (days)		aGVHD	Vomiting	OM	HC	Hepatic damage
		ANC>0.5×10⁹/L	PBC>20×10⁹/L	[cases (%)]	[cases (%)]	[cases (%)]	[cases (%)]	(%)
Introvenous Bu/Cy	15	12.0±3.0	16.0±5.0	6 (40.0)	7 (46.6)	1 (6.7)	1 (6.0)	2 (13.3)
Oral Bu/Cy	20	15.1±4.8	19.1±8.3	9 (45.0)	14 (70.0)	16 (80.0)	4 (20.0)	12 (60.0)
P value		>0.05	>0.05	>0.05	>0.05	<0.01	>0.05	<0.01

Bu, busulfan; Cy, cyclophosphamide; ANC, absolute neutrophil count; PBC, platelet blood cell count; aGVHD, acute graft-versus-host disease; OM, Oral mucositis; HC, hemorrhagic cystitis. The data of engraftment time are presented as mean±SD of relevant groups.

4. Discussion

Bu is commonly applied in the pretreatment of allo-PBSCT, but its oral dose can not be properly administrated in patients. Takamatsu *et al.*[4] detected stable plasma concentrations of Bu in seven patients administrated with Bu orally. Their plasma concentration of Bu ranged from 745-2 422 μg/ml, with a difference as high as three times, which could affect the toxicity and therapeutic effects of the drug. The pharmacokinetic study on oral Bu suggests that a plasma concentration of Bu lower than the optimal dose would increase the risk of recurrence and rejection, while a high plasma concentration would increase the incidences of gastrointestinal toxicity, hepatic toxicity, mucositis, aGVHD, and the death rate related to transplantation[5]. Andersson *et al.*[6] confirmed that the pharmacokinetics of intravenous administration of Bu were highly consistent in different patients and hematopoietic engraftment could be guaranteed in 86.0% patients. Compared with oral administration of Bu, the conditioning toxicity caused by intravenous Bu could be lowered, and the treatment related mortality was decreased to 9.8% within100 days.

In this essay, it was indicated that hematopoietic engraftment was 100.0% when intravenous Bu/Cy was applied as components of the conditioning regimen. The conditions of patients in our study were consistent with other literatures[6]. Compared to oral Bu group, the hematopoietic reconstruction was quicker in intravenous Bu group, suggesting that intravenous Bu/Cy could realize stable engraftment of allo-PBSCT from siblings or irrelevant donors.

In cases received the intravenous Bu/Cy conditioning regimen, the total vomiting rate was 46.6% (seven cases), which was similar to the other reports (43.0%). When patients took Bu orally, even active symptom control against vomiting could not avoid severe gastrointestinal reactions. Sometimes, the patients have to stop the treatment, so that the conditioning regimen is needed to be changed. For patients who received intravenous Bu, their pharmacokinetic variances could be avoided, thus to guarantee the stability of the drug concentration.

Oral mucositis occurred in only one patient (6.7%) administered with intravenous Bu, and the incidence was significantly lower than that in oral Bu group (80.0%), which was consistent with findings in literatures[7]. The incidence of oral mucositis in intravenous Bu group was extremely low, which could decrease the sufferings of patients and benefit transplantations. This might be due to the first pass effect of the liver, or rescue of MTX using calcium folinate.

The incidence of hepatic damage in intravenous Bu group was lower that that in oral Bu group, with no occurrence of hepatic veno-occlusive disease. It was reported that, hepatic damage would occur in 80.0% patients administered with Bu orally[8]. Kashyap *et al.*[9] also confirmed that, the incidence and

mortality rates of HVOD were obviously lower in intravenous Bu group than that in oral Bu group, which were 8.0% and 33.0%, and 3.3% and 20.0%, respectively. Intravenous Bu also significantly increased the survival rate of patients within the first 100 days after transplantation. Lee *et al.* [10] observed 241 allo-PBSCT cases, 186 of which received oral Bu/Cy, and 55 received intravenous Bu/Cy; the incidences of hepatic veno-occlusive disease were 41.7% and 18.5%, respectively. The incidence of hepatic veno-occlusive disease in intravenous Bu group was obviously lower, than that in oral Bu group. There was no difference in the incidence of aGVHD or hemorrhagic cystitis between the two groups.

The intravenous Bu conditioning regimen could improve the long-term therapeutic effect of transplantation, better than that of the oral Bu regimen. Shimoni *et al.* [11] reported that, when the intravenous Bu conditioning regimen was adopted to treat 43 cases of malignant hematologic diseases before allo-PBSCT, the patients achieved 2-year survival and disease free survival of 63.0% and 44.0%, respectively. Aggarwal *et al.* [12] reported autologous bone marrow transplantation using intravenous administration of Bu as the conditioning measure, which exerted a better therapeutic effect against severe non-Hodgkins lymphoma than using oral Bu; the 5-year survival was 58.0% in intravenous Bu group and 28.0% in oral Bu group; recurrence related mortalities were 3.0% and 28.0% in the two groups respectively. In our study, until the last visit, only one of the 15 patients died from severe aGVHD, the rest 14 cases (93.3%) were survived. The follow-up period was short in this study, so the long-term therapeutic effects should be further studied.

In summary, when intravenous Bu is adopted to replace oral Bu in the conditioning regime, patients obtain more stable hematopoietic reconstruction and engraftment; the toxic or side events are lowered, especially oral mucositis and hepatic toxicity; the therapeutic effects of transplantation are improved.

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