ORIGINAL RESEARCH

Xiaoning Wang, MD
Ying Zhang, MM
Ting Fan, MB
Haibo Liu, MM
Mengchang Wang, MD
Huasheng Liu, MD
Mei Zhang, MD
Pengcheng He, MD

Department of Hematology, The First
Affiliated Hospital of Xi'an Jiaotong
University, Xi'an, Shaanxi, China

Effective mobilization with etoposide and cyclophosphamide for collection of peripheral blood stem cell in patients with multiple myeloma

Abstract

Purpose: To evaluate the efficacy and toxicity of etoposide and cyclophosphamide for mobilization peripheral stem cells in multiple myeloma patients.

Methods: We retrospectively analyzed 46 patients with multiple myeloma who underwent peripheral blood stem cell collection for upfront autologous hematopoietic stem cell transplantation in the First Affiliated Hospital of Xi'an Jiaotong University between January 2010 and July 2019. The mobilization protocols included cyclophosphamide 2.0 g/m² with G-CSF (CTX group) before January 2015, and two-days of 5 mg/kg.d etoposide and 1.0 g/m².d cyclophosphamide with G-CSF (EC group) after January 2015.

Results: The success rate of harvest ($\geq 2 \times 10^6$ cells/kg) during the first mobilization attempt was 82.1% in the EC group and 50.0% in the CTX group, and the rate of adequate harvest ($\geq 4 \times 10^6$ cells/kg) was 57.1% in the EC group and 15.8% in the CTX group. After the second mobilization, a sufficient number of CD34⁺/kg cells for an auto-HSCT was obtained for all patients in the EC group and the majority (68.4%) of patients in CTX group. There was no significant difference of non-hematological adverse events between two groups. The mean neutrophil engraftment time was 11.22 ± 1.56 days and 9.89 ± 2.81 days for the CTX and EC groups, respectively ($P > 0.05$). Platelet engraftments were significantly faster in the EC group than the CTX group ($P < 0.05$). There was no significant difference of one-, three- and five-years overall and progress free survival between the two groups ($P > 0.05$).

Conclusion: The etoposide and cyclophosphamide regimen could be an effective and safe method for mobilization in patients with multiple myeloma.

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Correspondence to:
Pengcheng He
Email: xykcls@163.com

Introduction

High-dose melphalan followed by autologous hematopoietic stem cell transplantation (AH SCT) is currently considered as the standard treatment for eligible multiple myeloma (MM) patients. Tandem AH SCT may provide additional survival advantage in selected patients, especially those not achieving very good partial response after the first AH SCT. Salvage AH SCT for relapses after the first AH SCT may also yield a survival advantage. Enough autologous stem cells mobilization and collection was the key factor for successful AH SCT. The mobilization procedure usually aims to enable the collection of adequate PBSCs for at least two transplants.

Cyclophosphamide ($2-4 \text{ g/m}^2$) followed by granulocyte-colony stimulating factor (G-CSF) was the most commonly used mobilization chemotherapy [1]. There are many side effects of high or intermediate cyclophosphamide mobilization, including hemorrhagic cystitis, renal insufficiency and febrile neutropenia. Etoposide is another drug that is used for mobilization. The use of a combination of etoposide and cyclophosphamide for autologous hematopoietic stem cell mobilization remains controversial [2-3].

At our institute, we used a modified protocol of an etoposide and cyclophosphamide (EC regimen) for mobilization in MM patients eligible for ASCT, and we found that this protocol might be as effective as the combination of CTX and G-CSF for stem cell mobilization. Thus, we retrospectively analyzed the efficacy and safety of EC regimen in comparison with that of previous methods including CTX and G-CSF for mobilization.

Methods

Patients

Data of 46 MM patients who underwent peripheral blood stem cell collection (PBSCC) for upfront ASCT in the First Affiliated Hospital of Xi'an Jiaotong University between January 2010 and July 2019 were retrospectively analyzed. The study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All patients gave informed consent. Induction treatment before AH SCT included VAD (vincristine, doxorubicin and dexamethasone), TD (thalidomide and dexamethasone), bortezomib-containing regimens and other chemotherapies.

Mobilization chemotherapy

The mobilization protocols of autologous peripheral blood stem cells (PBSC) included the following: 1)

cyclophosphamide 2.0 g/m^2 intravenous infusion on day 1 and day 2, together with mesna 2.4 g/m^2 intravenous infusion followed by 10 ug/kg/day G-CSF in two divided doses started when patients had neutropenia (CTX group); and 2) etoposide 5 mg/kg.d day 1 and day 2 combined with cyclophosphamide 1.0 g/m^2 intravenous infusion on day 1 and day 2 (EC group).

Hydration was used to prevent cyclophosphamide toxicity. The inclusion of etoposide was not due to patient randomization. Instead, after January 2015, the mobilization protocols for MM patients was etoposide and cyclophosphamide.

Collection of PBSC

Peripheral blood stem cell collection was initiated by the Cobe Spectra I and II when the total white blood cell count was higher than $4 \times 10^9/\text{L}$. The total blood volume of one apheresis was 1.2 L. Yields of $\text{CD}34^+$ cells per kilogram of patient body weight $<2.0 \times 10^6/\text{kg}$, $>2.0 \times 10^6/\text{kg}$ or $>4.0 \times 10^6/\text{kg}$ were defined as failure, success or adequate mobilization, respectively.

AH SCT conditioning regimen

The conditioning regimen for AH SCT was melphalan 200 mg/m^2 intravenous infusion over 2 h on day -2 or 140 mg/m^2 if serum creatinine $>2.0 \text{ mg/dL}$ intravenous infusion on day -2 and PBSC was infused on day 0. The G-CSF was given with a dose of 10 ug/kg/day started on day +4 and continued until neutrophil engraftment.

Hematopoietic engraftment

Neutrophil engraftment was defined as an absolute neutrophil count $0.5 \times 10^9/\text{L}$ for three consecutive days and platelet engraftment as platelet count $20 \times 10^9/\text{L}$ for seven consecutive days without platelet transfusion.

Statistical analysis

Fisher's exact test or χ^2 analysis was used to make comparisons between categorical data. Continuous data were compared with t test. Statistical analyses were carried out using the software SPSS 18.0 for Windows. A p value of less than 0.05 was considered significant.

Results

Patient characteristics

A total of 46 patients were involved, 28 of whom received the EC regimen mobilization while 18 patients received cyclophosphamide alone. Patients baseline characteristics are described in Table 1. Although induction treatment prior to ASCT was heterogeneous due to the nature of

retrospective study, most patients were treated with bortezomib-based regimens (n=16), lenalidomide-based

regimens (n=10), thalidomide-based regimens (n=8) and VAD regimen (n=8). The other baseline characteristics were comparable between two groups ($P>0.05$).

TABLE 1. Characteristics of patients with different mobilization regimen

Patient characteristics	CTX group (n=18)	EC group (n=28)	P
Median age in years (range)	45 (22-56)	54 (35-65)	0.064
Male: female ratio	14:4	19:9	0.466
Type of multiple myeloma: # patients			0.146
IgG	4	12	-
IgA	1	4	-
Other	13	12	-
Stage: # patients			0.392
I	1	5	-
II	3	6	-
III	14	17	-
A	16	25	0.964
B	2	3	-
Median no. of prior chemotherapy cycles (range)	4 (3-11)	4 (2-11)	0.542
Median no. of prior chemotherapy regimens (range)	2 (1-4)	2 (1-4)	0.613
Prior chemotherapy regimen: # patients			
VAD	6	2	0.022
PAD/BCD/BTD	4	12	0.037
TD/TAD/TCD	4	4	0.488
RD/RVD	2	8	0.221
others	2	2	0.551
Prior radiation therapy: # patients (%)	2 (11.11)	0 (0.00)	0.071
Prior stem cell toxic chemotherapy (melphalan): # patients (%)	2 (11.11)	2 (11.11)	0.641
Median interval from diagnosis to mobilization in months (range)	5 (3-16)	5 (3-13)	0.783
Cytogenetic abnormalities: # patients (%)	6 (33.33)	9 (32.14)	0.933
Response after induction therapy: # patients (%)			
CR	10 (55.56)	19 (67.85)	0.398
VGPR	6 (33.33)	6 (21.43)	0.540
PR	2 (11.11)	3 (10.71)	0.641

Abbreviations: BCD, bortezomib, cyclophosphamide and dexamethasone; BTD, bortezomib, thalidomide and dexamethasone; CR, complete remission; CTX, cyclophosphamide; EC, etoposide and cyclophosphamide; PAD, bortezomib, adriamycin and dexamethasone; PR, partial remission; RD, lenalidomide and dexamethasone; RVD, lenalidomide, bortezomib and dexamethasone; TAD, thalidomide, adriamycin and dexamethasone; TCD, thalidomide, cyclophosphamide and dexamethasone; TD, thalidomide and dexamethasone; VAD, vincristine, adriamycin and dexamethasone; VGPR, very good partial remission.

Mobilization efficacy

The success rate of collection for an auto-HSCT ($\geq 2 \times 10^6$ cells/kg) during the first mobilization attempt was 82.1% ($n=23/28$) in the EC group and 50.0% ($n=9/18$) in the CTX group, and for two auto-HSCTs ($\geq 4 \times 10^6$ cells/kg) was 57.1% ($n=16/28$) in the EC group and 15.8% ($n=3/19$) in the CTX group, there was a significant difference between the two groups ($P=0.021$, $P=0.005$) (Figure 1). After the second mobilization attempt, a sufficient number of $CD34^+$ /kg cells for an AHSCT was obtained for all patients in the EC group and 68.4% ($n=13/19$) patients in the CTX group. The median number of $CD34^+$ cells collected in the first day of apheresis was 4.3×10^6 /kg and 2×10^6 /kg in the EC and CTX groups, respectively. The median number of $CD34^+$ cells collected in the second day of apheresis was 3.14×10^6 /kg and 1×10^6 /kg in the EC and CTX group respectively. The failure rates of mobilization were 22.2% ($n=4/18$) and 0 ($n=0/28$), respectively.

FIGURE 1. Number of $CD34^+$ cells collected in the two groups * $P<0.05$, ** $P<0.01$

Adverse events

The main adverse event was agranulocytosis, which was observed in 45 patients (97.8%) (17 cases in the CTX group and 28 cases in the EC group). As a result, four cases in the CTX group and four cases in the EC group (17.3%) developed grade 3 febrile neutropenia. Grade 4 thrombocytopenia was observed in 39 patients (84.7%) (12 cases in the CTX group and 27 cases in the EC group), and 43 patients (93.5%) received platelet transfusions. Red

blood cell transfusions were also required in 22 patients (47.8%). The non-hematological adverse effects during mobilization were compared between two groups with no significant difference (Figure 2).

FIGURE 2. Non-hematological adverse effects of the two groups

Engraftment

The mean days until neutrophil engraftment post-ASCT was 11.22 ± 1.56 days for CTX group and 9.89 ± 2.81 days for the EC group, there was no significant difference ($P=0.089$). The mean platelet engraftment time was 10.67 ± 2.97 days in the EC group. One patient in the CTX group had platelet engraftment failure, and the platelet engraftment time for seven patients in the CTX group was more than 14 days. Platelet engraftments were significantly faster in the EC group than the CTX group ($P<0.05$; $P=0.031$, respectively).

Survival

The one-, three- and five-years overall survival (OS) of patients in CTX group were 94.4%, 76.7% and 70.8%. And the one-, three- and five-years OS of patients in the EC group were 95.0%, 70.4% and 70.4%, respectively (Figure 3). There was no significance difference in OS between two groups ($P=0.774$). The one-, three- and five-years progress free survivals (PFS) of patients in the CTX group were 88.9%, 71.1% and 65.2%, respectively. And the one-, three- and five-years PFS of patients in the EC group were 90.0%, 74.3% and 74.3%, respectively. There was no significance difference in PFS between the CTX and EC groups ($P=0.771$).

Discussion

Multiple myeloma is the leading indication of AHSCT. Collection of sufficient amounts of hematopoietic stem cells was the essential factor for successful AHSCT. For

MM patients, the most commonly used approach to stem cell mobilization was CTX plus G-CSF. Despite the success of CTX+G-CSF for PBSC mobilization in MM, there was still almost 5-10% patients with mobilization failure, depending on patient age, duration of disease and the type of induction chemotherapy [4-6]. The standard dosage of CTX for mobilization was 3-4 g/m² for mobilization. It was reported that high dose cyclophosphamide (3-4 g/m²) with growth factor mobilization was associated with a higher incidence of post-transplant non-staphylococcal bacteremia and a prolonged engraftment if progenitor cell reinfusion occurred before 30 days after first apheresis, suggesting a potential bone marrow microenvironment damage with high-dose CTX [7-8]. Thus, the development of a safer mobilization method could improve HSC mobilization in patients with MM.

Park et al. [9] reported that single-dose etoposide (375 mg/m²) could be an effective and safe method for mobilization. Neutrophil and platelet engraftment were significantly faster in single-dose etoposide than high-dose (3.5 g/m²) cyclophosphamide ($P < 0.001$), but the adverse events were severe than with CTX. Plerixafor is a novel mobilization agent for patients, but is expensive and so was mainly used for patients with mobilization failure [10-12].

It has been reported that etoposide combined with CTX, when used for mobilization, was better than CTX alone. Demirer et al. [13] reported that 68% patients achieved the target level of 5×10^6 CD34⁺ cells/kg in a median of three (range 1-8) collections, 77.19% patients achieved the target level of 2.5×10^6 CD34⁺ cells/kg after mobilization with CTX (4 g/m²) or etoposide (600 mg/m²) combined with CTX (4 g/m²). The majority of patients in the study of Gojo et al. [14] obtained more than 5×10^6 CD34⁺/kg after mobilization with CTX (4.5 g/m²) or etoposide (2 g/m²) combined with CTX (4.5 g/m²), but 75.5% of patients who received etoposide combined with CTX required hospitalization mainly for treatment of neutropenic fever. In our study, auto-HSCT ($\geq 2 \times 10^6$ cells/kg) during the first mobilization attempt was 82.1%

FIGURE 3. Overall survival and progress free survival of patients in two groups A) OS; B) PFS.

($n=23/28$ patients) in the EC group. The target rate of mobilization with the EC regimen is the same, and the high-quality mobilization rate is lower than literature reports. But the adverse event was tolerable, because we decrease the dosage of VP-16 (10 mg/kg) and CTX (2 g/m²). Also, it showed that EC regimen mobilization promoted platelet engraftment but did not affect OS and progress-free

survival, compared with the CTX mobilization. This suggests that the EC regimen was an effective and safe mobilization regimen.

This study also has two important limitations. It was a retrospective research and the number of patients was small. Thus, it needed to be further investigated by random prospective clinical trial.

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