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RITUXIMAB DOES NOT ADVERSELY AFFECT THE STEM CELL MOBILIZATION AND ENGRAFTMENT AFTER HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN FIRST COMPLETE OR PARTIAL REMISSION

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Aims and Methods: The goal was to investigate the effect of prior combined rituximab (R) and intensive chemotherapy on peripheral blood stem cell mobilization and their engraftment after stem cell transplantation (ASCT) in 69 patients with poor-risk, diffuse large B-cell lymphoma (DLBCL).

Results: A statistically comparable median number of CD34+ stem cells was collected in both groups ($13.80x10^6$/kg in the non-R group and $7.81x10^6$/kg in the R group; p = 0.110). A trend toward greater number of CFU-GM was found in the non-R group ($98.1x10^4$/kg) compared to the R group ($76.6x10^4$/kg; p = 0.068). The non-R patients had a much higher median number of BFU-E ($90.9x10^4$/kg) than the R patients ($31.3x10^4$/kg; p = 0.001).

Conclusions: Hematopoietic engraftment was rapid for both groups and no different between them. The 3-year event-free survival was 90.4 % in the R group and 67.2 % in the non-R group (p = 0.04), but there was no significant difference in the 3-year overall survival (94,7 % vs 83,5 %; p = 0.179).

INTRODUCTION

The addition of rituximab (humanized chimeric anti-CD20 monoclonal antibody, R) to chemotherapy (CT) leads to higher response rates and improved survival for patients with diffuse large B-cell lymphoma (DLBCL)[1,2]. It has also been reported that R is effective in removing circulating B cells from peripheral blood and this might be a useful in vivo purging agent before stem cell harvesting[3]. Several studies have indicated that the combination of R with chemotherapy does not compromise the mobilization and engraftment of autologous peripheral blood stem cells (PBSC) in lymphoma patients and some have also pointed to improved patient survival[4-6]. However, the overall feasibility and effectiveness of adding R to intensified first-line CT followed by autologous stem-cell transplantation (ASCT) in poor-risk patients remains to be established[7]. In this retrospective study, the effect of prior combined R and CT administration on peripheral blood stem cell mobilization and post-transplant engraftment was analyzed and compared with the results of patients previously treated without R.

PATIENTS AND METHODS

Patient population

From May 1999 to August 2007, sixty-nine ASCTs for DLBCL were performed in poor-risk patients younger than 65 years who had achieved complete remission (CR) or partial remission (PR) after induction CT (n = 31) or R with CT (n = 38). Poor risk was defined as International Prognostic Index (IPI) 3–5 or age-adjusted IPI (aaIPI) 2–3 (for patients younger than 60 years), IPI 1–2 or aaIPI 1 with one or more additional adverse prognostic parameters (bulky disease more than 10 cm in largest diameter, beta-2-microglobulin more than 3.0 mg/l, bcl-2 protein expression in immunohistochemical staining), and failure to achieve CR irrespective of the patient's initial IPI/aaIPI. Three patients were primarily treated with 6 courses of the ProMACE-CytaBOM regimen, and 3 patients with 6 courses of the PACEBO regimen. All other patients received intensive sequential CT consisting of 3 courses of PACEBO, 1 course of IVAM (with ifosfamide 1500 mg/m^2 and methotrexate 3 g/m^2), and 1 course of HAM (cytosine arabinoside 2 g/m^2 twice daily on days 1 to 2, mitoxantrone 10 mg/m^2 on days 2 to 3). R 375 mg/m^2 was administered on day 1 of the CT regimens in 38 (55 %) patients. The treatment protocols were reviewed and approved by our institutional review board, the Independent Ethics Committee of the University Hospital Olomouc, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Mobilization and harvesting of CD34+ cells

PBSC were mobilized after CE (cyclophosphamide 4 g/m^2 on day 1, etoposide 200 mg/m^2 on days 1 to 3) in 6 cases or after HAM in 60 cases followed by 5 μg/kg

Table 1. Results of peripheral blood stem cells collection.

Variable	Chemotherapy (n=31)	R-chemotherapy (n=38)	p-value
Median number of mononuclear cells ($x10^8$/kg)	3.77	5.24	0.800
Median number of CD34+ cells ($x10^6$/kg)	13.80	7.81	0.110
Number of patients with $\geq 5.0x10^6$ CD34+ cells/kg	26 (84 %)	35 (92 %)	0.480
Median number of CFU-GM ($x10^4$/kg)	98.1	76.6	0.068
Median number of BFU-E ($x10^4$/kg)	90.9	31.3	0.001

body weight of filgrastim from day 8. The PBSC were collected by leukapheresis (COBE Spectra cell separator, Lakewood, CO, USA) with a targeted minimum cell count > $2x10^6$/kg body weight of CD 34+ cells and then cryopreserved without purging or CD34+ selection. The BEAM conditioning regimen (BCNU 300 mg/m^2, etoposide 800 mg/m^2, cytosine arabinoside 1600 mg/m^2, and melphalan 140 mg/m^2) was given to all 69 patients. The cryopreserved cells were reinfused on day 0.

Analysis of harvested stem cells

The sample of leukapheresis product (50 µl) was stained with phycoerythrin-conjugated (5 µl) anti-CD34 (Clone 581, Immunotech, Marseille, France). Cells were subsequently lysed for 15 minutes, centrifuged, washed twice, and resuspended in PBS and analyzed with the FACSCalibur flow cytometer (BectonDickinson, San Jose, CA, USA). All samples were gated on a leukocyte window and 60,000 events were collected in this region. A double-layer methylcellulose progenitor cell assay system with GM–CSF and EPO was used to quantify granulocyte-macrophage colony-forming units (CFU-GM) and burst-forming units-erythroid (BFU-E), as previously described[8-10]. MNCs were plated at 20.000 cells per 35-mm culture dish (1 ml). Cultures were incubated at 37 °C for 14 days in a 100 % humidified incubator with 5 % CO_2 and 10 % O_2 in air. Day-14 GM-CFC-derived colonies were defined as any colony containing more than 40 translucent cells; day-14 BFU-E-derived colonies were defined as any colony. CFU-GM and BFU-E were counted using an inverted microscope.

Engraftment kinetics, response and statistical methods

Neutrophil recovery was defined as the first of 3 consecutive days with a neutrophil count greater than $0.5x10^9$/l; platelet recovery was defined as the first of 3 consecutive days with an unsupported platelet count greater than $20x10^9$/l. The times to recover $1.0x10^9$/l granulocytes, $50x10^9$/l and $100x10^9$/l platelets after ASCT were also recorded. The numbers of granulocytes and platelets and Hb values were analyzed on day +100 after ASCT. Response to therapy was assessed using the International Working Group criteria published in 1999[10]. Survival curves were constructed based on the Kaplan-Meier methodology. The statistical analyses were performed using Statistica for Windows 7.1 (StatSoft Inc., 2005) and SPSS 12.0.1 (SPSS Inc., 2003).

RESULTS

Patient prognostic characteristics and response to induction chemotherapy were well-balanced between the CT and R-CT groups. Statistically comparable cumulative numbers of mononuclear cells (MNCs) were collected in both groups, with medians of $3.77x10^8$ MNCs/kg (range, 1.52 to $11.81x10^8$/kg) in the non-R group, and $5.24x10^8$ MNCs/kg (range, 2.17 to $17.89x10^8$/kg), in the R group (p=0.800). The same was true for CD34+ stem cells – $13.80x10^6$ CD34+cells/kg (range, 2.76 to $39.50x10^6$/kg) were collected in the non-R group and $7.81x10^6$ CD34+ cells/kg (range, 3.15 to $37.30x10^6$/kg) in the R group (p=0.110). There was no significant difference in the numbers of those with more than $5.0x10^6$/kg CD34+ cells/kg between non-R patients (84 %) and R patients (92 %; p=0.480). A trend toward a greater number of CFU-GM was observed in the non-R group (median, $98.1x10^4$/kg; range, 24.2 – $269.1x10^4$/kg) in comparison with the R-group (median, $76.6x10^4$/kg; range 11.5 – $216.9x10^4$/kg) (p=0.068). A statistically significant difference between the non-R and R groups was found in the number of BFU-E. The non-R patients had a much higher median number of BFU-E ($90.9x10^4$/kg; range, 8.8 – $337.0x10^4$/kg) than the R patients ($31.3x10^4$/kg; range, 1.4 – $191.0x10^4$/kg) (p=0.001) (Table 1). Hematopoietic engraftment was rapid, with median neutrophil recovery of 11 days in both groups (p=0.420) and median platelet recovery of 10 days in the non-R and R groups (p=0.530). The times to recover $1.0x10^9$/l granulocytes, $50x10^9$/l and $100x10^9$/l platelets after transplantation were nearly similar in the two groups and were statistically non-significant as well as the numbers of granulocytes and platelets and hemoglobin values on day +100 after ASCT. No differences in toxicity or serious complications in the non-R and R groups after ASCT were found. No toxic or transplant-related death was reported. The median follow-up

was 59.9 and 29.2 months for the non-R and R groups, respectively. The number of relapses was higher in the non-R patients (9/31; 29 %) compared with the R patients (2/38; 5 %) (p=0.009). The administration of R was associated with lower number of deaths due to lymphoma progression (3 % vs. 19 %, p=0.04). The probability of 3-year event-free survival (EFS) was 77.2 % (95 % CI, 66 to 89 %) in all 69 patients. The 3-year EFS was significantly higher in the R group (90.4 %) than in the non-R group (67.2 %) (p = 0.04) (Fig. 1a). The probability of 3-year overall survival (OS) was 88.4 % (95 % CI, 80 to 97 %) but no significant difference between the R (94.7 %) and non-R groups (83.5 %) was noted (p=0.179) (Fig. 1b). No patient developed a second malignancy or myelodysplastic syndrome to the date of analysis.

DISCUSSION

R combined with CT is a safe and effective first-line treatment for patients with CD20+ DLBCL. Previous studies have reported improved remission and survival rates in low-risk young patients and patients older than 60 years[1,2]. Little data are available regarding the effectiveness and safety of R administered in combination with intensified CT eventually followed by PBSC mobilization, harvesting and ASCT after myeloablative CT[4,7,12,13]. This study represents a retrospective analysis of patients with poor-risk DLBCL treated with myeloablative CT with ASCT in first CR or PR in our center. The aim was to investigate whether repeated use of R with CT pre-PBSC mobilization results in impaired stem cells yield, prolonged engraftment, increased complications or worse survival of these patients.

The results show that prior R-CT had no significant effects on mobilization of MNCs or CD34+ cells. R-CT even resulted in slightly higher but non-significant optimal mobilization rate (≥5.0x10^6/kg CD34+ cells/kg in 92 % vs. 84 % of patients). These findings are consistent with earlier studies which indicate that R has no negative affects on PBSC mobilization kinetics[6,14,15]. In this study, the in vitro growth of harvested progenitor cells was investigated and the median number of CFU-GM was shown to be higher in patients previously treated only with CT (98.1x10^4/kg) in comparison with those treated and mobilized after R-CT (76.6x10^4/kg), although the statistical significance was only borderline (p=0.068). Significant difference between the two groups was found for BFU-E

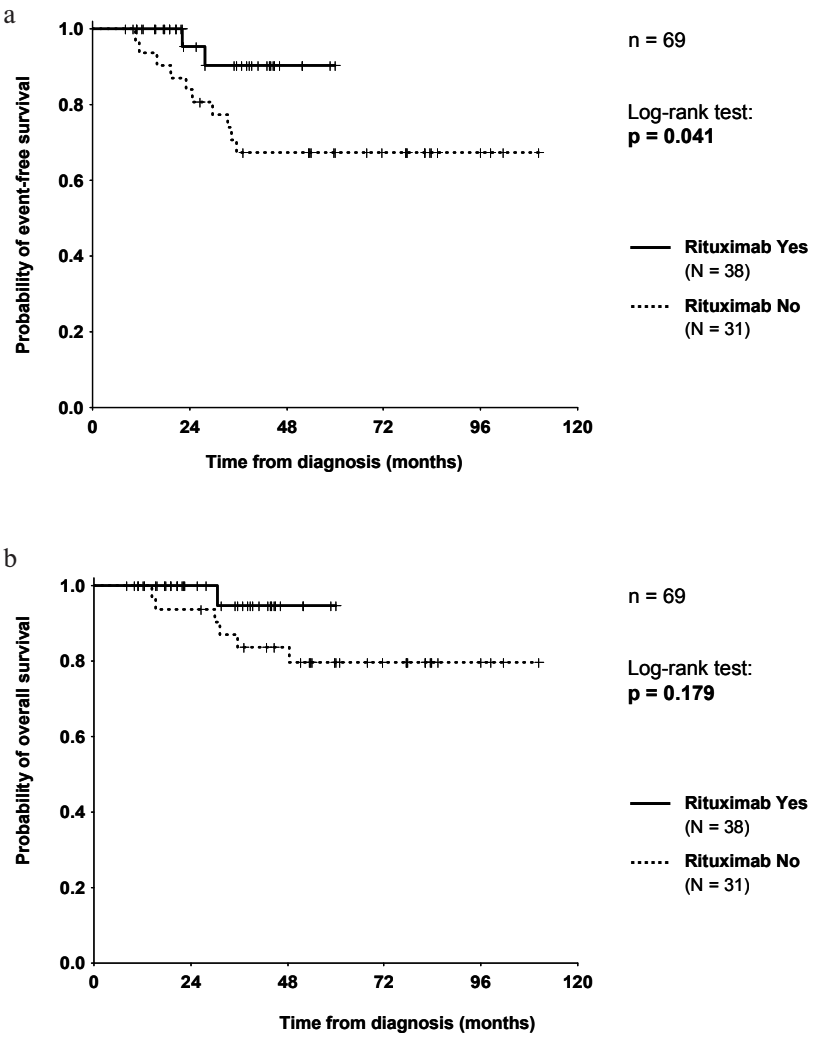

Fig. 1. EFS (a) and OS (b) for DLBCL patients initially treated with rituximab plus chemotherapy or with chemotherapy alone.

progenitor cells. The non-R patients had a BFU-E median number of $90.9x10^4$/kg whereas the R-CT patients had the BFU-E median of $31.3x10^4$/kg ($p = 0.001$). The etiology and importance of this phenomenon is unclear. Activation of the complement and some inflammatory cytokines (i.e. tumor necrosis factor-alpha) by rituximab may lead to non-specific bystander lysis or inhibition of some stem cells compartments[16,17]. On the other hand, a majority of studies confirm that only CD34+ cell subsets are significantly associated with short-term and long-term hematopoietic reconstitution[18]. Our data support previous publications reporting that R-CT has no impact on the engraftment and function of PBSC after ASCT and that this approach may improve survival of DLBCL patients[14,15,19]. In this study, the times to recover granulocytes and platelets after transplantation were similar in the non-R and R groups as well as the numbers of granulocytes and platelets and hemoglobin values on day +100 after transplantation. Also, no difference was found in toxicity and serious complications after ASCT, no toxic or transplant-related death, second malignancy or myelodysplastic syndrome were reported. Although the follow-up was significantly different in the non-R and R groups, the number of relapses and deaths due to lymphoma progression was significantly higher in the non-R patients. The 3-year EFS was significantly higher in the R group (90.4 %) than in the non-R group (67.2 %) ($p = 0.04$), but the OS mainly due to lower number of patients and salvage therapy with rituximab or allogeneic transplantation in relapsed non-R patients was not different. The conclusion is that R with intensive CT has no negative affect on the mobilization and engraftment of PBSC and reduces relapse rate in poor-risk DLBCL patients.

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