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Case Report

Effect of autologous stem cell transplantation combined with DC-CIK cell immunotherapy on relapsed and refractory lymphoma

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Abstract: Purpose: To investigate the clinical efficacy and treatment-related side effects of $CD34^+$ autologous stem cell transplantation combined with DC-CIK immunotherapy in patients with relapsed and refractory lymphoma. Methods: The study included 24 patients with relapsed and refractory lymphoma for $CD34^+$ autologous stem cell transplantation combined with DC-CIK immunotherapy. Survival analysis was done using the Kaplan-Meier survival curve analysis. Results: All the 24 patients were successfully transplanted, with a success rate of 100%. 10 cases achieved complete remission (41.7%), 4 achieved partial remission (16.7%), 7 were in stable status (29.2%), 3 achieved progressive disease (12.5%). Among 24 patients, 2 patients died of serious infections (8.3%) and 22 patients had a mean survival time of 26 months with an overall survival rate of 70.8%. 11 cases achieved long-term disease-free survival (45.8%). 3 patients had mild allergies and transient fever during CIK cell reinfusion. Conclusion: Autologous stem cell transplantation combined with DC-CIK immunotherapy on relapsed and refractory lymphoma patients achieved good clinical efficacy and safety with fewer side effects.

Keywords: Relapsed and refractory lymphoma, autologous transplantation, DC-CIK, immunotherapy

Introduction

Lymphoma is a malignant tumor originating in the hematopoietic and lymphoid tissues. Along with social and economic development in recent years, the incidence of malignant lymphoma in our country rapidly increases. It is one of the major malignant tumors causing serious health concerns in patients. About 20-30% of malignant lymphomas develop into refractory and relapsed lymphomas in the early stage and during the course of treatment [1].

Hematopoietic stem cell transplantation is one of the important treatment options for patients in later stage of lymphoma. However, allogeneic hematopoietic stem cell transplantation has many limitations for its application, such as difficulty in finding a donor, high mortality rate, expensive cost, various other complications and relatively low quality of life. The use of autologous stem cell transplantation can effectively avoid the above drawbacks and has good clinical efficacy. In recent years, it has attracted much attention in clinical medicine [2, 3].

Tumor biological immunotherapy is a matter of great concern in recent years. Tumor immunotherapy was one of the 10 major scientific and technological breakthroughs in 2014 [4]. Immunotherapy uses human body's own immune cells and processes them before transfusing back into the body of patients. In this way, the specific or non-specific immune killing effect of these cells shows no effect on the normal cells and tissues of the body. Dendritic cells (DCs) are the most powerful antigen-presenting cells and cytokine-induced killer (CIK) cells are the immune effect or cells with killing activity. DCs when contacted by antigens (Ags) of autologous lymphoma get activated and these provide strong activation signals for CIK, inducing

Table 1. Clinical features of 24 cases of patients with relapsed and refractory lymphoma

Clinical features	Value (%)
Age	
≤60 years old	24 (100)
Gender	
Male	14 (58.3%)
Female	10 (41.7%)
Diagnosis	
HL	6 (25%)
NHL	18 (75%)
B Symptoms	
Positive	9 (37.5%)
Negative	15 (62.5%)
Ann Arbor staging	
I-II	6 (25%)
III-IV	18 (75%)
IPI scoring	
0-1	7 (29.2%)
2-3	16 (66.7%)
4-5	1 (4.1%)
Pretransplantationstatus	
CR+PR	8 (33.3%)
SD+PD	16 (66.7%)
Pretreatment regimen	
BEAM	14 (58.4%)
R+BEAM	5 (20.8%)
Others	5 (20.8%)

pan-specific killing that targets different epitopes of lymphomas [5]. CIK cells containing Ag information actively identifies tumor cells and has higher killing activity than the pure CIK cells, leading to significant decrease in tumor reduction rate. DC-CIK cells carrying autologous lymphoma antigen produces active immunity with passive immunity and maximizes antitumor effects [6, 7]. We selected 24 cases of patients with relapsed and refractory lymphoma, that were admitted to our hospital between 2011 and 2015 and were treated with $CD34^+$ autologous stem cell transplantation combined with DC-CIK immunotherapy.

Patients and methods

The study included 24 patients with relapsed and refractory lymphoma that were admitted to our hospital from 2011 to January 2015. Lymphoma diagnosis was in line with WHO standards. No minimum inclusion criteria were set. Patients had an average age of 36.17 years (15-58 years). Follow-up time ranged from 3-42 months, with a median follow-up time of 13 months. Before transplantation, all 24 patients had relapse or progression after receiving many times of first-line chemotherapy. The clinical features of 24 patients are shown in **Table 1**.

Preparation of DC-CIK carrying autologous lymphoma antigen

Peripheral blood of 40 ml was withdrawn from patients. Platelets and red blood cells were removed by gradient centrifugation. Medium, serum and DMSO were added in proportion for liquid nitrogen cryopreservation. This was used for loading autologous lymphoma antigen. Another part of peripheral blood mononuclear cells (PBMCs) of 1.0×10^{10}-5.0×10^{10} were collected. After adherence for 2 hours, cell suspension was extracted for CIK culture. GM-CSF and IL-4 (both had final concentration of 125 ng/ml) were added to the remaining adherent cells. Fresh factors were supplemented every 3 days to the culture. On day 6, autologous Ag (ie Ag-DC) was added with a final concentration of 5 ug/ml to the culture. PBMC and Ag-DC cultured for 8 days were selected and added 10 μl of FITC-labeled CD83 monoclonal antibody (mAb), CD86 mAb and PE-labeled HLAII mAb respectively, followed by flow cytometry measurement. Patient PBMC cell suspension was added with RPMI 1640 solution (CIK culture medium) that contained IFN-γ (1000 U/ml), CD3 mAb (100 ng/ml), IL-2 (500 U/ml) and IL-1 (100 U/ml). On day 7, CIK was collected and divided into 2 groups: one group was with CIK cells and another group consisted of CIK cells co-cultured with Ag-DC in 1:10 (Ag-DC-CIK). After grouping, the two groups of cells were cultured for another 24 h. PBMC and CIK, Ag-DC-CIK cells cultured for 8 days were chosen for detection. FITC-labeled mAb was added for detection of CD3, CD8, CD56 expression. Cells were harvested and frozen in liquid nitrogen to prepare for reinfusion.

Mobilization and collection of peripheral blood stem cells

G-CSF of 5-10 μg/Kg was used for mobilization of peripheral hematopoietic stem cells. 5-6 days after mobilization, blood cell separator (Fresenius SE & Co. KGaA) was used to collect

peripheral blood stem cell. $CD34^+$ cells were counted and diluted with PBS/EDTA buffer containing human serum albumin. MNC number in cell suspension was adjusted to 4×10^{10}, with about 4×10^8 of $CD34^+$ cells. CD34 monoclonal antibody was added and incubated for a total of 30 min at room temperature with 2 times of washing. CliniMACS cell enrichment device was used for cell sorting (select cell isolation procedures, load samples on column and start automatic sorting procedures). After completion of sorting, $CD34^+$ cells enriched bags were removed and examined for the percentage, absolute number and cell viability of each cell subset in $CD34^+$ group, $CD34^-$ group and eluent, respectively. The total number of MNCs and $CD34^+$ cells, recovery rate, purity and other indicators were calculated.

Treatment process

① Depending on the type of lymphoma, BEAM or R + BEAM therapeutic regimen were adopted for pretreatment, respectively. ② After pretreatment, reinfusion of cryopreserved $CD34^+$ hematopoietic stem cells began on day 0 of peripheral blood stem cell transplantation. ③ Immunotherapy began from 8 to 13 days after transplantation (4 or 6 according to the number of cells), DC-CIK transfusion was conducted in patients, respectively. Before reinfusion, contaminated cells were excluded. Cells were then centrifuged and re-suspended using 100 ml physiological saline which contained 5 g of human serum albumin solution and 200,000 U of IL-2. Intravenous infusion was carried out within 2 hours after intramuscular injection of 25 mg Phenergan. ④ Hematopoietic recovery and reconstruction was done after transplantation. G-CSF, IL-11 and other hematopoietic growth factors were given to promote hematopoietic reconstitution.

Clinical efficacy evaluation

① Hematopoietic reconstitution time; ② Short-term efficacy and relapse: According to the IHP2007 revised edition of lymphoma efficacy evaluation criteria [8], evaluation included complete remission (CR), partial response (PR), stable disease (SD) and progressive disease (PD); ③ Long-term effects after transplantation was also studied. Follow-up was conducted to record patients' overall survival time and disease-free survival time; ④ Adverse reactions of DC-CIK cell therapy was also recorded.

Statistical analysis

SPSS 16.0 was used for statistical analysis. Survival analysis was analyzed using the Kaplan-Meier survival curve analysis. $P<0.05$ was considered to be statistically significant.

Results

All patients underwent the treatment and most patients received BEAM regimen for pretreatment. 5 patients were given R + BEAM regimen. One patient was given reduced BEAM regimen due to poor physical condition. 5 patients were given non-BEAM regimen. One patient was treated with local radiotherapy before pretreatment to reduce tumor burden. One patient had a short-term relapse after transplantation and was given the second autologous transplantation. The number of autologous stem cells in reinfusion and the number of DC-CIK cells varied, with $CD34^+$ cell reinfusion average number of 14.43×10^6/kg ($0.87\text{-}290\times10^6$/kg) and DC-CIK cell reinfusion average number of 6.28×10^8 ($4.5\text{-}8.7\times10^8$) in 24 cases of patients. At the end of treatment, all patients were subjected to regular follow-up in our hospital wards.

Short-term efficacies of all patients were successfully transplanted, with a success rate of 100%. The average time for neutrophil recovery (Neu≥0.5×10^9/L) was 8 days, with a median recovery time of 11 days. The average time for platelet recovery (20×10^9/L) was 13 days, with a median recovery time of 16 days. Among the 24 patients, 10 cases achieved complete remission (CR rate = 41.7%), 4 cases achieved partial remission (PR rate = 16.7%), 7 cases achieved stable disease (SD rate = 29.2%), 3 cases achieved progressive disease (PD rate = 12.5%).

The follow-up deadline was May, 2015 Out of 24 patients, two patients lost during follow-up. Among the 22 evaluable cases, 2 patients died within two months of transplantation due to severe infection (8.3%). The average survival time of 22 cases was 26 months, with the median survival time of 22 months (2-42 months). By the end of follow-up, 17 patients survived, with an overall survival rate of 70.8% (OS =

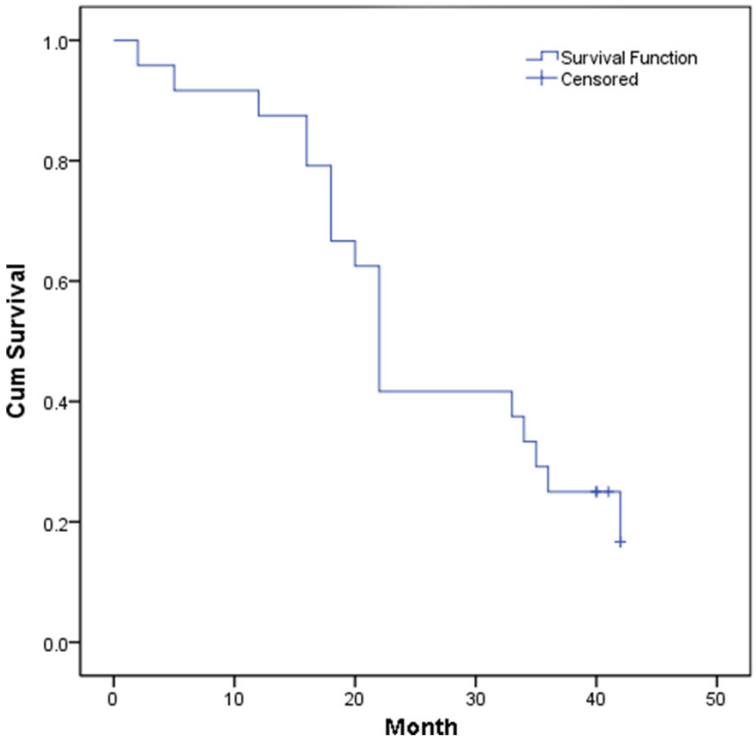

Figure 1. Survival function of 24 cases relapsed and refractory lymphoma patients who received autologous stem cell transplantation combined with DC-CIK cell immunotherapy (n = 24).

70.8%). Among the surviving patients, 11 cases had long-term disease-free survival (DFS = 45.8%); 5 cases had tumor recurrence after 2 years of transplantation, for whom PR was achieved after first-line chemotherapy treatment; 1 case had short-term relapse and achieved PR after second-time transplantation and immunotherapy. Among the 5 patients who died, two cases died within 2 months after transplantation due to severe infection; 2 cases had short-term relapse and refused second-time transplantation, then died with other diseases during the treatment of second and third-line induced chemotherapy; 1 case died with second-time tumor (**Figure 1**).

Adverse events were observed among the 24 patients who had sequential DC-CIK cell therapy after transplantation, 3 patients had transient fever during CIK cell infusion, with body temperature between 38.3-39.1°C. Body temperature returned to normal after physical cooling or administration of antipyretic. One patient had skin urticaria accompanied with itching during the infusion. After anti-allergy treatment was given, the infusion was continued without any discomfort. At the end of follow-up period, no patients showed liver and kidney dysfunction, change in hemogram or other symptoms.

Discussion

Currently there is still a lack of the standard treatment for relapsed and refractory lymphoma. The main treatment is combined chemotherapy, hematopoietic stem cell transplantation and clinical trials of new drugs. Combined chemotherapy is relatively poor in efficacy, high in recurrence rate and low in long-term survival rate. While the wide application of allogeneic hematopoietic stem cell transplantation is limited because of its high transplant-related mortality and donor sources. Autologous hematopoietic stem cell transplantation can effectively avoid transplant-related adverse reactions and has advantages like good patient tolerance, unlimited sources, rapid hematopoietic implantation, mature technique, operation easiness and high remission induction rate. But there are still defects like relapse. According to the 9th version of NCCN guidelines, patients with failure of second and third line chemotherapy or relapsed lymphoma should be treated with autologous hematopoietic stem cell transplantation as soon as possible. Multiple combination chemotherapy which aimed in reducing the relapsed lymphoma after autologous bone marrow transplantation effectively increased the disease relapse, thus reducing the relapse rate and prolonging the survival time of the patients [3]. Furthermore, use of conventional high-dose therapy with autologous peripheral blood stem cell transplantation in the treatment of relapsed and refractory lymphoma of patients who are aged and having impaired hematopoiesis, had a better hematopoietic recovery [9]. In our study, all patients were treated with autologous peripheral blood stem cell transplantation. No transplant-related deaths occurred; implantation time was short with rapid hematopoietic recovery, low rate of seri-

ous infections, no bleeding, no organ dysfunction or other complications. The safety was relatively high.

The characteristics of DC-CIK anti-tumor cell therapy included: ① CIK anti-tumor activity significantly increased after being stimulated by DC. ② When DC is co-cultured with CIK cells, the expression of DC maturation surface markers CD86, CD80, CD40 and human leucocyte antigen increased, and IL-12, IFN-γ secretion in cell supernatant increased significantly, increasing the antigen-presenting specificity of DC and co-stimulatory factors. ③ DC cells activated CIK cell proliferation, resulting in a large number of efficient immune cells. ④ Co-culture of DC and CIK cells can reduce the number of immunosuppressive T cells (Treg, namely $CD4^+$ $CD25^+$ cells) and its secretion of IL-10 within CIK cell population, weakened the inhibitory effect of Treg on anti-tumor immune cells [10]. We performed selected $CD34^+$ hematopoietic stem cell transplantation combined with DC-CIK treatment on 24 patients with relapsed and refractory lymphoma. The anti-tumor immune ability of patients after transplantation was effectively improved. The purposes of removing residual tumor cells in the body, increasing remission rate and prolonging survival had been achieved. As a new adoptive immunotherapy, the combination of DC-CIK with conventional tumor treatments like surgery, chemotherapy and radiotherapy, realizes the multidisciplinary cancer treatment, achieves good efficacy and is worthy of clinical application.

Our study results showed, ① Relapsed and refractory patients were mainly with NHL. First-line chemotherapy was effective at early stage and its efficacy gradually diminished along with the prolonged treatment time. Second and third-line chemotherapy was partly effective but was unable to maintain disease remission. Autologous stem cell transplantation was the ultimate choice of means in these patients. ② Stem cell transplantation combined with DC-CIK immunotherapy significantly improved treatment response rate of patient and reduced the side effects caused by chemotherapy. ③ Autologous hematopoietic stem cell transplantation reconstructed the immune and hematopoietic systems in patients. DC-CIK reinfusion killed tumor cells and the potential tumor cells mixed within the grafts through a variety of ways, meanwhile did not harm normal tissue cells, eliminating the recurrence risk and improving overall survival rate and quality of life of patients.

In summary, our analysis suggested that the treatment of selected $CD34^+$ autologous stem cell transplantation combined with DC-CIK immunotherapy on relapsed and refractory lymphoma patients achieved good clinical efficacy and safety and with fewer side effects which is worthy of clinical promotion.

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Disclosure of conflict of interest

None.

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