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CASE REPORT / PRACA KAZUISTYCZNA

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THE USE OF MESENCHYMAL STEM CELLS IN THERAPY OF STEROID-RESISTANCE CHRONIC REFRACTORY GRAFT-VERSUS-HOST DISEASE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

ZASTOSOWANIE MEZENCHYMALNYCH KOMÓREK MACIERZYSTYCH W LECZENIU PRZEWLEKŁEJ OPORNEJ CHOROBY PRZESZCZEP PRZECIWKO GOSPODARZOWI PO PRZESZCZEPIENIU KOMÓREK KRWIOTWÓRCZYCH

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Summary

Introduction. Chronic graft-versus-host disease (GVHD) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation. Mesenchymal stem cells (MSC) are involved in tissue repair and modulating immune responses. We present report of two cases of use of third-party donor mesenchymal stem cells in the therapy of steroid-resistant chronic GVHD.

Case reports. The doses of MSC in both patients were 1.08 and 1.4 x 10^6 cells per kg body weight respectively. Time from diagnosis of GVHD to infusion of MSC was 420 and 330 days, respectively. Both patients had single infusion of MSC. We did not observe any adverse effects after both infusions of MSC. We obtained initially a partial response and then complete response in the first patient and a partial response in the second patient. In the first patient we achieved improvement of skin involvement resulting in reduction of immunosuppression. In the second patient with scleroderma we achieved only a minor improvement.

Conclusion. Administration of MSC is a safe method and might by an effective therapy for patients with chronic refractory GVHD.

Streszczenie

Wstęp. Przewlekła postać choroby przeszczep-przeciwko-gospodarzowi (chronic graft-versus-host disease, GVHD) jest zagrażającym życiu powikłaniem po przeszczepieniu allogenicznych komórek krwiotwórczych. Mezenchymalne komórki macierzyste (MSC) mają znaczenie w procesach reparacji tkankowej oraz modulowania odpowiedzi immunologicznej. W pracy przedstawiamy opis dwóch przypadków zastosowania MSC od dawcy niezwiązanego z pacjentem, tzw. third-party donor, w terapii steroido-opornej przewlekłej GVHD.

Opis przypadku. Zastosowane dawki MSC u pacjentów wynosiły odpowiednio 1,08 oraz 1,4 x 106 komórek na kg cc. Czas od rozpoznania GVHD do infuzji MSC wynosił odpowiednio 420 i 330 dni. U obydwu pacjentów dokonano pojedynczych infuzji MSC. U żadnego z nich nie obserwowano niepożądanych reakcji po zastoso-

waniu MSC. U pierwszego pacjenta uzyskano początkowo częściową poprawę, a po kilku miesiącach remisję całkowitą wszystkich zmian związanych z GVHD. Dzięki temu możliwe było znaczne zredukowanie dawek leków immunosupresyjnych. U drugiego pacjenta uzyskano tylko przejściową nieznaczną poprawę stanu twardzinowej skóry.

Wniosek: Zastosowanie MSC jest metodą bezpieczną i może być efektywną terapią u pacjentów z przewlekłą postacią choroby GVHD.

Key words: chronic graft-versus-host disease, mesenchymal stem cells, children
Słowa kluczowe: przewlekła choroba przeszczep przeciwko gospodarzowi, komórki mezenchymalne, dzieci

INTRODUCTION

Chronic graft-versus-host disease (cGVHD) is an important complication after hematopoietic stem cell transplantation (HSCT), which might be associated with high transplant rate mortality (TRM) and reduction in quality of life of the otherwise cancer-free patients [1-3]. The main issue are patients who did not respond to first- and second-line therapy with combinations of corticosteroids and calcineurin inhibitors [4].

Nowadays, no standard therapy of chronic refractory GVHD is available. In order to improve the prognosis of these patients new therapeutic approaches are being sought. One of them might be the use of mesenchymal stem cells (MSC).

MSC are multipotent non-hematopoietic stem cells that can differentiate into fibroblasts, osteoblasts, chondrocytes and adipocytes [5-9]. They have been used to repair injured tissues. It is also believed that they have immunomodulatory properties, including inhibition of T cell proliferation, inhibition of the activity of cytotoxic T cells, inhibition of differentiation and maturation of dendritic cells, B cells and NK cells [10, 11]. MSC have been used for prophylaxis and treatment of acute GVHD, as well as for the treatment of refractory chronic GVHD [12-15]. In all these studies it was emphasized that the treatment of MSC is safe for the patient [15-17].

For the evaluation of the response to the treatment of GVHD and organ scoring the National Institutes of Health (NIH) consensus criteria are being used worldwide [18]. These criteria distinguish severe, moderate and mild grades of cGVHD. As a definition of complete remission (CR), the resolution of all clinical manifestations of the involved organs is required. Good partial response (PR) is defined as reduction of more than 75% pathologic changes, PR is defined as reduction of more than 50% but less than 75%, and minor PR as reduction of more than 25% but less than 50%. No response (NO) is defined as no improvement of all affected organs is found [15].

In this paper we report two cases of use of third-party donor mesenchymal stem cells in therapy of steroid-resistant chronic GVHD.

Patient 1

17-year old boy, diagnosed with severe aplastic anemia, after failure of immunoablative therapy, underwent bone marrow transplantation from HLA-matched brother. Conditioning regimen consisted of cyclophosphamide 50 mg/kg/daily, days from -6 to -3 and antithymocyte globulin (Thymoglobulin, Genzyme) 3,75 mg/kg total (divided into days -3 and -2). Due to primary graft failure, he had another transplantation of peripheral blood hematopoietic stem cells from the same donor at day +35. For GVHD prophylaxis the patients received cyclosporine 3 mg/kg/day and for prophylaxis of infectious complications ciprofloxacin, acyclovir and trimetoprim-sulfametoxazol. After successful hematological engraftment, he developed acute graft-versus-host disease II grade, with progression to chronic GVHD, limited to the skin. He was treated with prednisolone and cyclosporine. However, periodically exacerbation of the GVHD was observed, and 18 months after transplantation he developed steroid-resistant skin and ocular GVHD, with involvement of 80% of skin surface and dry-eye syndrome. He experienced infectious complications and an increase of creatinine concentration. At this time, the decision of therapy with MSC was undertaken.

After agreement of Local Bioethical Committee, third-party donor MSC (obtained from Polish Stem Cells Bank) were administered at the dose of 1.08×10^6 cells/kg body weight. No adverse effect were observed during and after the MSC transfusion. The patient continued therapy with steroids and cyclosporine. 3 month later he presented slight improvement in skin symptoms and resolution of ocular symptoms (minor PR). At 6 months after MSC therapy, further improvement of skin changes was observed that

enabled to reduce immunosuppression (PR). At 9 months after MSC therapy, all skin changes have resolved (CR).

Patient 2

16 year old boy, with relapsed acute lymphoblastic leukemia, underwent peripheral blood hematopoietic stem cell transplantation from HLA-matched sister. Conditioning regimen consisted of Total Body Irradiation (TBI), 12 Gy divided into 6 doses delivered over 3 days (days -6,-5,-4), and etoposide 60 mg/kg at day -3. For GVHD prophylaxis he received cyclosporine 3 mg/kg/day and for prophylaxis of infectious complications ciprofloxacin, acyclovir and trimetoprim-sulfametoxazol.

Five months after transplantation, he developed chronic generalized sclerodermatous GVHD involving 80% of skin, without preceding acute GVHD. He was initially treated with prednisolone and cyclosporine. Due to prednisolone-resistance, he was referred for phototherapy. No improvement was observed and patient's mobility deteriorated. At 15^{th} month after transplantation, the decision of therapy with mesenchymal stem cells was undertaken.

After agreement of Local Bioethical Committee, third-party donor MSC were administered at the dose of $1.4x10^6$ cells/kg body weight. No adverse effect were observed during and after the MSC transfusion. The patient continued therapy with steroids and cyclosporine, as well as physiotherapy. At 3 and 6 months afterward, he presented a slight improvement (minor PR). Then he was switched to sirolimus therapy with more pronounced improvement (PR).

MSC infusion

MSC were obtained from the Polish Stem Cell Bank (Polski Bank Komórek Macierzystych, Warszawa). The MSC were derived from Wharton's jelly (WJ) of umbilical cord from third party unrelated donors. Cells were frozen and transported in liquid nitrogen. Before MSC infusion, patients were pre-medicated with hydrocortisone and clemastine. MSC were thawed in water bath and administered during 10 minutes infusion.

DISCUSSION

Chronic GVHD significantly increases the morbidity and mortality after allogeneic hematopoietic stem cell transplantation [1, 2, 3, 19]. The treatment of chronic GVHD is still unsatisfactory, because only about 50% of patients respond to front-line therapy with steroids and calcineurin inhibitors [4, 20]. MSC, due to their unique immunomodulatory properties, could provide an alternative therapeutic method [10, 11]. Initially, promising results of the treatment of acute GVHD were obtained [13, 14, 17]. Afterwards, Weng et al. reported outcomes of 19 patients treated with MSC for refractory chronic GVHD with a median dose 0.6×10^6/kg body weight. They obtained a partial or complete response in 73.7% and reduction or discontinuation of immunosuppression in 71.4% of patients with oral mucosa, GI tract, liver and skin involvement [15].

So far only 7 patients with acute GVHD were treated with MSC in Poland. The median dose was 1.5×10^6 cells per kg body weight and the median time from diagnosis of GVHD was 28 days. The number of infusion was from 1 to 4. Recently, another patient with chronic GVHD was treated with MSC. The authors achieved a partial or complete remission in four patients [21].

Our report relates to the use of MSC in therapy of chronic steroid-resistant GVHD. The doses were comparable to those given by other authors and were respectively 1.08 and 1.4×10^6 cells per kg body weight in the first and second patient and the time from diagnosis of GVHD to infusion of MSC was respectively 420 and 330 days in the first and second patient. The patients had a single infusion of MSC. We obtained initially PR and then CR in the first patient and PR in the second patient. In the first patient we have achieved improved skin and reduced immunosuppression. In the patient with scleroderma we have achieved only a minor improvement. Our patients received a single dose of MSC. It is suggested that clinical benefit might not depend on the number of MSC but it may be a result of a production of growth factors or temporary immunosuppression [15]. In other studies almost half of the patients had received a single dose of MSC, while the remaining patients have received 2-4 infusions [15, 21].

MSC are used in regenerative medicine because they can differentiate to several mesenchymal tissues. The use of MSC was described in hemorrhagic cystitis, thrombotic microangiopathy symptoms, and refractory cytopenias after HSCT [5-9]. The MSC have also immunomodulatory effect and low immunogenicity, thus MSC are useful in transplant setting. MSC can inhibit T-cell proliferation after stimulation by allo-

antigens and mitogens and prevent cytotoxic T-cells activity [10, 11]. Weng et al. detected that analysis of lymphocyte subsets can help to assess whether MSC might participate in the amelioration of GVHD. They observed that the CD8+CD28+ cells decreased when the GVHD improved. In contrast, the CD8+CD28- cells and CD5+CD19+ cells increased after MSC infusions. Probably, CD8+CD28- cells served as regulatory cells to induce immune tolerance and CD5+CD19+ cells protected from autoimmunity by producing cytokines such as IL-10 [15, 22-24].

In conclusion, our experience is that the infusion of third party donor WJ-derived MSC is a safe procedure with clinical effect that can ameliorate symptoms and allow a reduction of immunosuppressive treatment in patients, even though MSC were applied in very advanced phase of disease as an ultimate salvage therapy. In comparison to the published data, possibly earlier application can lead to better clinical response. Further studies concerning pathophysiology, optimal doses, number of infusions and time intervals as well as the timing of MSC infusion are warranted.

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