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Autologous mesenchymal stromal cells of hemoblastosis patients efficiently support hematopoietic recovery after stem cell transplantation

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Summary

Mesenchymal stromal cells (MSCs) derived from bone marrow possess immunoregulatory activity and are able to support hematopoiesis. Unfortunately, data concerning the biological properties of MSCs in various pathologies is poor and often discrepant. In this study, we demonstrated that MSCs derived from bone marrow of patients with hemoblastoses have fibroblast-like morphology and a typical phenotype. Moreover, the patients' MSCs possess well-defined hematopoietic-supporting activity coupled with decreased immunosuppressive potential. These properties prove the clinical application of co-transplantation of autologous hematopoietic stem cells and MSCs in oncohematology to achieve a rapid hematopoietic recovery. Therefore we investigated the safety and hematopoietic effects of MSCs in patients with hematological malignancies receiving peripheral blood hematopoietic stem cell (PBSC) transplantation. We revealed the decreasing of the period of neutropenia and thrombocytopenia in the patients with hematological tumors after high-dose chemotherapy, when autologous PBSC were co-transplanted with ex vivo expanded autologous MSCs. Our results show that co-transplantation of autologous MSCs with PBSC is feasible and safe. The shortening of hematopoietic recovery time suggests that MSC may have a positive impact on hematopoiesis.
Keywords: hemoblastosis, bone marrow, mesenchymal stromal cells, stem cell transplantation

Introduction

Multiple studies have been performed investigating the kinetics, safety and efficiency of mesenchymal cell isolation, characterization, culture-expanding, and clinical approaches [26, 10, 1, 31, 14, 18, 21, 27, 28]. In recent years the biological activity of MSC has been very actively discussed for their potential use for suppression of autoimmune and transplant-related reactions, but also with the aim of speeding up of hematopoiesis recovery after bone marrow transplantation [15, 16, 29, 24]. Many existing protocols suppose the use of autologous MSCs [23, 9]. However, the use of autologous MSCs may be limited in a clinical setting, due to imperfect and often controversial knowledge of the functional competence and integrity of immunosuppressive and hematopoietic-supporting potentials of these cells [19, 5, 8, 4]. As shown earlier, bone marrow-derived mesenchymal stromal stem cells provide support for hematopoiesis in vitro and in experimental animal models [3, 15, 16, 24]. In this study we compared the morphology, immunophenotype, and immunoregulatory activities of MSCs derived from the bone marrow of healthy donors and patients with hematological malignancies. Our results demonstrated that MSCs derived from bone marrow suffering from hemoblastoses can be successfully expanded in culture and infused along with PBSCs after high-dose chemotherapy without any toxicity. Moreover,

one of the promising results is the decrease in the period of critical neutropenia and thrombocytopenia in the patient after high-doses chemotherapy, when PBSCs and MSCs are co-transplanted. Our results show that co-transplantation of MSCs is associated with rapid hematopoietic recovery when PBSC counts are suboptimal.

Patients and methods

From April 2005 through June 2007, after obtaining written informed consent, 39 patients who were eligible for high-dose chemotherapy and PBSC transplantation were enrolled into single center investigation of the feasibility, safety, and hematopoietic effects of autologous culture-expanded MSCs (Table 1). The clinical trial protocol and the consent form were approved by the Local Ethics committee of the Institute of Clinical Immunology SB RAMS.

Patients with lymphomas and leukemia were required to have an Eastern Cooperative Oncology Group performance status of 0 or 1 and were required to have adequate visceral organ function, including a left ventricular ejection fraction of at least 50%, forced expiratory volume in 1 second, serum direct bilirubin less than 2.0 mg/dl, and an actual or calculated creatinine clearance greater than 60 ml/min. Also all patients with hematological diseases underwent restaging evaluation with blood count, biochemical activity, trepanobiopsy, and computed tomography.

At the start of therapy, a neutrophil count greater than 1.2x106/ml and a platelet count greater than $100x10^6$/ml were required.
Patients were excluded if they had bone marrow involvement or active infection. Patients were excluded for evidence of tumor on routine histological staining of bilateral paraffin-embedded posterior iliac crest bone marrow biopsy specimens. In the "refractory group", the patient was required to have no effects after standard chemotherapy.

SLE patient eligibility depended on a refractory to standard immunosuppression therapies and either organ- or life-threatening visceral involvement. The inclusion criteria were: not controlled with pulse therapy Cy glomerulonephritis (World Health Organization (WHO) class III–IV), central nervous system (CNS) lupus, vasculitis involving the lung or heart, or transfusion-dependent autoimmune cytopenias. Evaluation of eligible patients by two independent rheumatologists and transplant physicians, informed consent, and approval by the ethical committee were part of the protocol of Tyndall et al. [29]

High-Dose Chemotherapy and PBSC Infusion

The PBSC mobilization regimen consisted of Cyclophosphamide 4.0 g/m^2 IV infusion over 6 hours on day 1, along with Mesna (first 3.0 g/m^2 IV, then 500 mg every 3 hours orally/IV for eight doses). On day 7 after completion of the Cyclophosphamide, patients began subcutaneous injections of recombinant human G-CSF (Neupogen, Granocyte, Leukostim) 10 mg/kg/d. On recovery of neutrophils to a level greater than $1.2x10^9$/l (usually 12 to 15 days after Cyclophosphamide treatment), patients underwent a leukapheresis procedure using AS TEC 204 (Fresenius, Germany) or Spectra LRS 07 (COBE, Lakewood, CO) apheresis equipment. Cells were cryopreserved using a controlled-rate liquid nitrogen freezer using previously published methods [10]. After PBSC procurement, high-dose chemotherapy with BEAM (BCNU 300 mg/m2 on day -7, Etoposid 800 mg/m2 and Ara-C 800 mg/m2 on days -6, -5, -4, -3, Melphalan 120 mg/m2 on day -2) for NHL and HD patients, Alceran (200 mg/m^2 on day -2) for patients with AL, Cy (200-140 mg/kg in divided doses for 4 days -6, -5, -4, -3 with/or not ATG (total doses 60 mg/kg) for patients who suffered from SLE were administered. PBSCs were thawed and infused 24 hours after the completion of high-dose chemotherapy.

Phenotypic analysis of mobilized cells (expression of CD 34 and CD45) was performed using multicolor flow cytometry (FACSCalibur, Becton Dickinson). Moreover, cancer cell contamination of every PBSC specimen was excluded by the use of cytological analysis.

	Control	MSC supported
Median age, years (range)	32 (17–57)	31 (7–55)
Gender (male/female)	16/23	22/21
Diagnoses		
HD	CR 4 PR 8 Refractory 3	CR 0 PR 5 Refractory 3
NHL	CR 4 PR 2 Refractory 2	CR 4 PR 6 Refractory 4
MM	CR 2 PR 2 Refractory 3	CR 0 PR 2 Refractory 2
AML (1st remission)	2	11
ALL (2nd remission)	4	4
SLE (SLEDAI >16)	3	2
Conditioning regimens	BEAM for HD and NHL; mono-Melphalan 140–200/m2 for AML, ALL and MM; Cyclophosphamide 120 mg/kg for SLE	
Mean dose of CD34+/CD45+ cells	5.57/kgx106(1.81–9.5x106/kg)	5.64/kgx106(2.5–8.7x106/kg)
Mean dose of MSCs	--	6.64x106 (0.8–23.0x106)

Table 1. Characteristics of patients who were treated with (n=39) and without (n=42) mesenchymal stem cells

Supportive Care

Before, during, and for 24 hours after treatment with high dose Cyclophosphamide, patients received hyperhydration with forced diuresis and Urometexan (Mesna) infusion for the prevention of hemorrhagic cystitis. Patients were treated in a single room without air filtration. All patients followed a standardized supportive care protocol including antiemetic therapy, analgesia for mucositis, transfusion support, and venoocclusive disease prophylaxis. A low microbial diet, oral daily Fluoroquinolone (1 g/d) changed to intravenous Cefepime on neutropenic fever, Fluconazole (400 mg/d) and Acyclovir (10 mg/kg/d) and aerosolized Amphotericin B (10 mg twice daily) were started upon admission and discontinued when the ANC rebounded to $0.5x10^9$/l. Platelets irradiated with 25 Gy and red blood cells were given to maintain a platelet count greater than 20 000/µl and a hemoglobin level greater than 8.0 g/dl. For the first 6 months after transplantation, patients were treated orally with daily oral Fluconazole and Trimethoprim/Sulfamethoxazole three times a week.

Harvesting and Ex Vivo MSC Culture

A median of 50 ml of bone marrow aspirate was obtained under sterile conditions by puncture of posterior iliac crests of patients or donors (n=9) under local anesthesia during standard pre-transplant staging approximately 2 days before high-dose Cyclophosphamide mobilization and 30 days before scheduled PBSC infusion. Washed heparinized bone marrow cells were re-suspended in phosphate-buffered solution (PBS, Sigma-Aldrich, Germany) and overlaid on Ficoll density gradient (1.078 g/l), then centrifuged for 20 min at 1000 g. A median of 320x106 (range 250–600 x106) mononuclear cells (MNC) were collected from the interface, washed three times in PBS, and re-suspended in α-modified minimum essential medium (αMEM, Sigma-Aldrich, Germany) containing 100 µg/ml gentamycin and 5% human platelet lysate (PL). After the cell number was counted, 30 ml of cell suspension was plated in a 175 cm2 plastic culture flask (Nunc, Denmark).

It is important to note that for clinical use with a therapeutic intent we used 5% platelet lysate to replace fetal calf serum (FCS) in the MSC culture. Platelet lysate was obtained from several allogeneic platelet units prepared by machine trombocytaphereis using AS TEC 204 (Fresenius, Germany). The platelet units were frozen at -40 C, thawed, mixed, aliquoted and lysate frozen at -20 C until use.

MSCs were cultured in humidified incubators with 5% CO2 and initially allowed to adhere for 72 hours, followed by a media change every 3 days. When cultures reached more than 90% confluence, adherent cells were detached with 0.05% trypsin-EDTA (Sigma-Aldrich, Germany) and replated at a density of 0.8x106 per 175 cm^2 flask until processing for cryopreservation. Harvested MSCs from 1-2 passages were cryopreserved with a rate-controlled freezer (Planer Kryo 560-16) in 10% human Albumin solution (Microgen, Russia) and in a final concentration of 10% Dimethylsulfoxide (Sigma-Aldrich, Germany) and 10% Hydroxyethylstarch in freezing bags.

Cell cultures were tested for sterility weekly and before cryopreservation (Municipal Hospitals N1, Microbiology Laboratory, Novosibirsk) for the presence of bacterial/fungal contamination via microbiological cultural tests. All cell manipulations were performed in a sterile class II biological safety cabinet.

The number of MSCs was estimated by the quantity of colony forming unit-fibroblasts (CFU-F). For that, 106 of bone marrow MNCs were cultivated in Petri dishes for 14 days and stained using Giemsa protocol, then the number of spindle-shaped cell colonies consisting of more than 50 cells were counted.

Flow Cytometry

Phenotypic analysis of MSCs (expression of CD3, CD14, CD16, CD20, CD34, CD73, CD90, CD105 and HLA-DR molecules) was performed using multicolor flow cytometry (FACSCalibur, Becton Dickinson). Cells were detached with 0.05% trypsin-EDTA (Sigma, USA), washed with PBS plus 2% bovine albumin, fixed in 1% paraformaldehyde, blocked with 10% normal goat serum, and incubated separately with appropriate primary antibodies (BD Bioscience, USA). Non-treated MSCs were used as negative control.

The immunosuppressive properties of MSCs

To study MSC effects on immune cell function, MSCs were first cultivated for 24 h in flat-bottomed 96-well or 24-well plates in α-MEM/20% fetal calf serum (FCS, Gibco, USA). After this, peripheral blood MNCs (PBMNCs) from donors were added to MSC monolayer and stimulated with mitogens or alloantigens (mixed lymphocyte culture, MLC). For MLC, 0.1x106 PBMNCs from two donors were cultivated for 5 days in RPMI-1640 medium supplemented with 0.3 mg/ml L-glutamine, 100 µg/ml Gentamycine and 10% of heat-inactivated donor AB (IV) serum. Mitogen-induced proliferative response was studied in 3-day cultures of donor PBMNCs, which were activated by Concanavalin A (ConA, 15 µg/ml, Sigma-Aldrich, Germany) or 1 µg/ml monoclonal antibodies against CD3 (anti-CD3, Becton Dickinson, USA). Cell proliferation was measured by incorporation of 3H-thymidine that was added at 1 µCi/well during the last 18 h of cultivation. Proliferative response of MNCs cultivated without MSCs was used as control.

Support for hematopoiesis by MSC (CFU Assay)

To estimate MSCs‘ effect on colony-forming activity of hematopoietic progenitor cells, bone marrow MNC were grown in “complete” methylcellulose medium (Methocult H4344, Stem Cell Technologies, Vancouver, BC), containing hSCF, hGM-CSF, hIL-3, and hEPO at a density of $25x10^5$/ml. Autologous MSC were added to MNCs in MNC: MSC ratios of 1:1 and 10:1 in triplicates. Cultures were grown at 37°C, 5% CO2 for 14 days, then the numbers of erythroid- and granulocyte-macrophage colony-forming units (CFU-E and CFU-GM, respectively) were calculated. Colonies were considered if consisted of at least 50 cells.
MSC Infusion

On the day of infusion, cryopreserved units were thawed ex tempore in a 37°C water bath, washed twice with PBS, transferred

into 60-ml syringes with 10% human Albumin and infused into patients one hour after the PBSC infusion through a side port of a running 0.9% saline IV infusion into a central catheter over 10 to 20 minutes. Patients were premedicated with Acetaminophen (650 mg) and Diphenhydramine. Vital and clinical signs and symptoms were monitored at the time of infusion and every 15 minutes thereafter for 3 hours, followed by every 2 hours for 6 hours and every 8 hours for 3 days. There was no immediate or delayed toxicity related to MSC infusion. None of the patients experienced allergic reactions or respiratory symptoms.

Statistical analysis

Statistica 6.0 software for Windows, StatSoft Inc. USA was used for basic descriptive, and correlation analysis of data. The statistical significance was assessed with the Mann-Whitney U-test. The T-test was used to analyze the difference in means when comparing two groups. In assessing correlation, we used 2D scatterplots estimated correlation coefficient r and P-value for Rank Order Correlation Spearmen test. A p-value of less than 0.05 was considered statistically significant.

Results

MSC characteristics

At the first stage we compared the quantity of MSCs in bone marrow of patients with hemoblastosis and healthy donors. Since MSCs were first described as fibroblast-like cells capable of forming colonies [13], the initial content of MSC precursors among bone marrow MNCs was measured as a colony-forming unit fibroblast (CFU-F) number. The mean number of CFU-F in the control group was 36±3 colonies per 106 bone marrow-derived MNCs (min-max range 16–70). The CFU-F number in patients with hemoblastosis was 21±3 ($p<0.05$). The range of values in the patient group widely varied (from 1 to 67 CFU-F), and 70% of samples showed CFU-F numbers that were below the lower quartile value of control group (<25 CFU-F). The most significant decrease of CFU-F number was revealed in patients with NHL (16±5, $p<0.01$). In patients with MM and HL number of MSC precursors was also decreased (19±10 and 26±7, respectively; $p>0.05$). Decreasing CFU-F numbers mean that MSC progenitors in bone marrow of investigated patients are present at a lower amount.

Taking into consideration the MSC deficiency in patients with hemoblastosis, it seemed important to evaluate their ability for expansion in vitro. With that aim we analyzed the growth rate of MSC by measuring cultivation time until confluence. In healthy volunteers, confluent growth in MSC cultures was reached by day 15±0.5 (range 11–22 days). Meanwhile, in the patient group 80–90% confluence took on average 26±2 days (range 10–50); 23±3 days in patients with HL ($p<0.01$); 29±3.5 days in patients with NHL ($p< 0.01$), and 30±4 days in patients with MM ($p<0.01$). It seems to be important that despite a low CFU-F number in patient cultures it was possible to isolate and in vitro expand MSCs from patients with hematological malignancies.

MSC immunophenotype

Further, we characterized the immunophenotype of MSCs. Classically, MSCs are defined as cells expressing STRO-1, CD105 (SH2), CD71, CD73 (SH3, SH4), CD90 (Thy1) surface markers and not expressing lineage (CD3, CD14, CD20, CD16) and hematopoietic (CD34, CD45) markers along with HLA-DR antigen [11, 30]. We observed that on the first passage the MSCs of healthy volunteers expressed CD73 (88±3.7%), CD90 (83±2.6%) and CD105 (90±6.3%), while only some few MSCs expressed CD34 (0.7±0.3%), CD3 (3.9±1.5%), CD20 (5.3±2.3%), CD16 (5.6±1.7%), CD14 (5.6±2.3%), and HLA-DR (11.1±1.6%).

The MSCs of patients with ALL, HL, NHL and MM were found to express a similar immunophenotype. However, several differences were revealed as well. Firstly, CD73, CD90 and CD105-positive cells were lowered (78±6.8, 71±8.7 and 82±10% correspondingly; $p>0.05$). Secondly, in contrast to healthy volunteers, MSCs of patients with hemoblastosis showed a two-fold increased number of HLA-DR-positive cells (23±4.8 vs 11.1±1.6%, $p<0.05$), which is in agreement with published data [6].

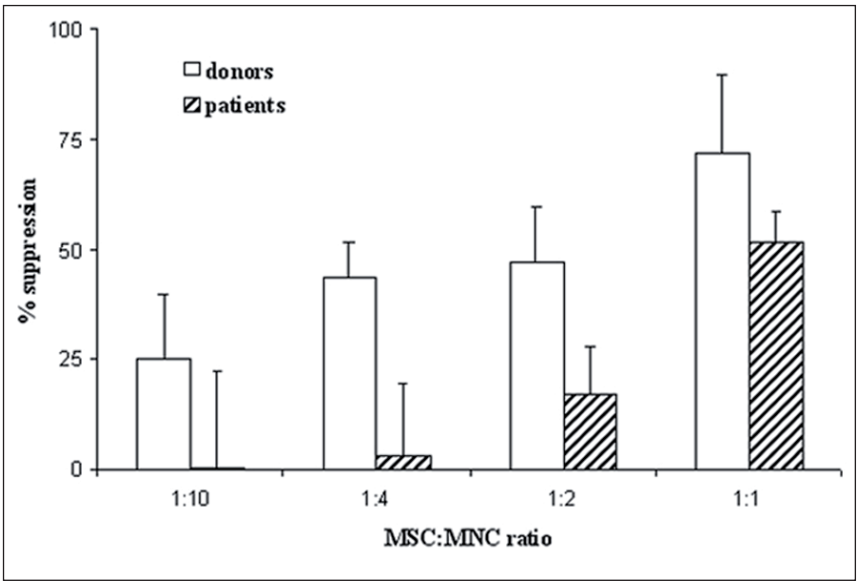
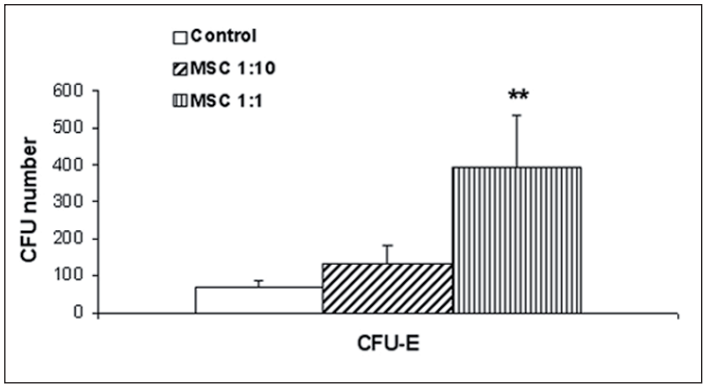


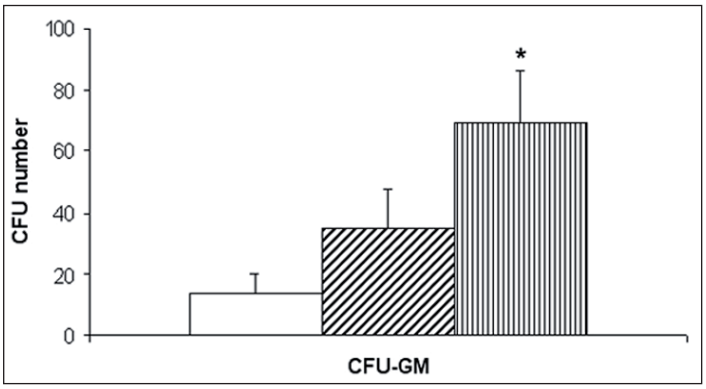
Figure 1. Effects of donor and patient MSCs on proliferative response of T-cells in MLC.
MNCs from two donors (both 0.1×10^6 per well) have been cultivated for 5 days in the absence (control) or presence of MSCs of healthy donors (n=7) or hematologic patients (n=10). Cellular uptake of ^{3}H-thymidine added at 18 hours before the end of cultivation was used to measure cell proliferation rate. Results are expressed as a suppression rate in percents calculated using the following formula: $(1 - \frac{cpm_{MNC+MSC}}{cpm_{MNC}}) \times 100\%$, where $cpm_{MNC+MSC}$ is proliferation rate of MNCs in presence of MSCs, and cpm_{MNC} is proliferation rate of MNCs in absence of MSCs (control).

Immunosuppressive properties of MSCs

Evaluation of the immunosuppressive activity of MSCs showed that donor MSCs rendered a dose-dependent suppressive effect on T-cell proliferation in mixed lymphocyte cultures (Fig. 1) as well as in cultures stimulated with ConA or monoclonal anti-CD3 antibodies (data not shown). The most pronounced effect was ob-

served at the MSC:MNC ratio of 1:1. However, even at lower concentrations of MSCs (MSC:MNC ratio of 1:2 and 1:4) their





Figures 2a/ 2b. Effects of MSCs on colony-forming activity of bone marrow MNCs.
Bone marrow MNCs of hematologic patients (n=9) were cultivated for 14 days in absence (control) or presence of autologous MSC at various concentrations (1:1 and 1:10) in semi-liquid methylcellulose medium. At the end of cultivation the amounts of erythroid (A) and granulocyte-macrophage (B) colonies per 10^5 MNCs were calculated.
* - pU < 0,05 and ** - pU < 0,01 – versus control; Mann-Whitney U-test.

suppressive activity remained high, and averaged 44–47%. In patients with hemoblastosis a significant suppressive effect of MSCs was registered only at the highest concentration of MSCs in culture (MSC:MNC ratio of 1:1). Nevertheless, even in this case the level of immunosuppressive activity of patient MSCs was lower compared to the level in control group (52±7 and 72±18%, correspondingly; p>0.05), varying from 18 to 64%. Reduction of MSC dose in mixed lymphocyte cultures strongly decreased ability of MSCs to block T-cell proliferative response.

Support of hematopoiesis by MSC

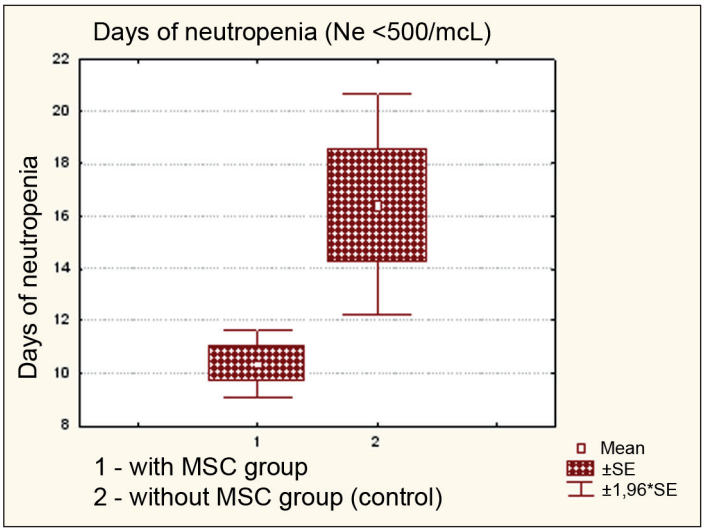
One of the promising fields of therapeutic application of MSCs is their use for the rapid recovery of hematopoiesis after bone marrow transplantations. This statement is based on the ability of mesenchymal cells to support the hematopoietic progenitors [9, 15]. However, the question about the integrity of hematopoiesis-supporting potential of MSCs in case of hemoblastosis still remains open. Assessment of the colony-forming potential of patient bone marrow MNCs under co-incubation with autologous MSCs showed that the presence of MSCs (in ratio of 1:1) increased the number of CFU-E and CFU-GM approximately five times (Fig. 2). It should be noted that even in the presence of lower MSC concentration (in ratio of 1:10) a two-fold increase of hematopoietic colonies was also registered. The ability of MSCs to support hematopoietic progenitors in vitro was observed in all investigated cases (ALL, n=3; HL, n=3; NHL, n=3).

Hematopoietic engraftment and clinical outcome

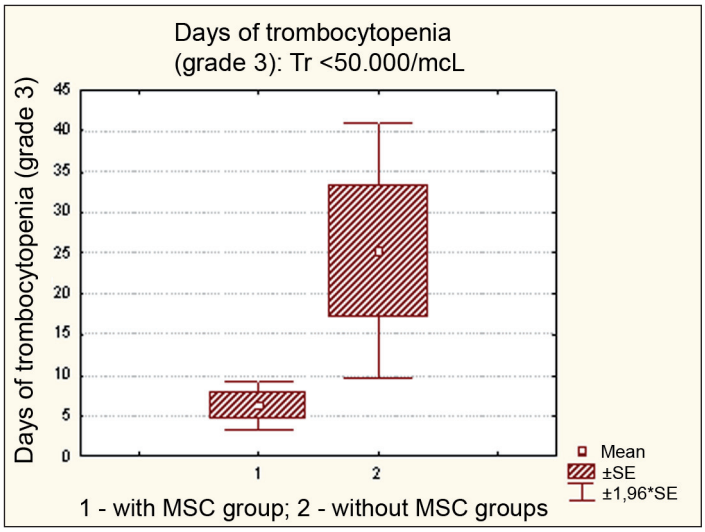
Patients of both groups received a PBSC infusion containing equal doses of CD34+cells: the mean number of CD34+ mononuclear cells was 5.57x106/kg in the control group and 5.64x106/kg in the group with MSC co-transplantation. The dose of mesenchymal cells infused varied from 0.8x106 to 23x106 (mean 6.64x106).

We revealed significant prompt hematopoietic engraftment in the MSC supported group. Patients in this group recovered hematopoiesis with mean neutrophil recovery (500/ml) in 10.3 days (range 3 to 17 days), whereas a mean neutrophil recovery of 16.3 days (range 12 to 21 days) was seen in the MSC unsupported group (p=0.02) (Fig. 3). Additionally, the platelet count recovery of greater than 50,000/ml without transfusion support, occurred in 6.4 days (range 4 to 10 days), when MSCs were co-transplanted and 24.5 days (range 10 to 39 days), respectively (p=0.05) (Fig. 4).

There was no significant correlation between the number of MSCs and days of neutropenia or thrombocytopenia. However,



Figures 3. Effects of MSCs infusions on neutrophil recovery
The t-test was used to evaluate a difference in period of neutrophil recovery



Figures 4. Effects of MSCs infusions on neutrophil recovery
The t-test was used to evaluate a difference in period of platelets recovery

we found a significant invert correlation between the number of MSCs and days of neutropenia in selected group, when infused PBSC were $<3.0x10^6$/kg.

No differences in analysis of severity and rate of complication, transfusion dependence, total or disease-free survival between the groups compared were revealed. Patients underwent standard restaging evaluation with computed tomography 42 days after transplantation and every 3 months thereafter. All patients evaluated on Day +60 were free of symptoms and clinical findings. Median follow-up of the patients is 9 months (range 4 to 22 months). Four patients died as a result of early disease progression (NHL, n= 2; HD, n=1; MM, n=1). All of them had refractory disease before PBSC transplantation. Of the 34 remaining patients, 33 are without evidence of disease; one has a stable disease (plato-phase in MM).

Transplant-related mortality / Toxicity

Two patients died on Day +7 and +21 due to transplant-related complications. There was one patient who died before engraftment. The cause of death in patient 1 on Day +7 was pneumonia and Candida sepsis as background. Patient 2 recovered hematopoiesis on Day +11, but died from pneumonia on Day +21. Progression of HD: lymphoadenopathy in the mediastinum was also revealed on autopsy of patient 2. Similar toxicity and causes of death were found in the control group: 3 fatal outcomes due to infection among 42 transplanted patients.

Discussion

The work presented here originated from the question of whether MSCs isolated from bone marrow of patients with hematologic malignancies possess intact functional properties including immunosuppressive activity and hematopoiesis-supporting ability. The transplantation of autologous bone marrow is widely used in clinical practice, but very little data exists on the characteristics of MSCs from the bone marrow of these patients, and the reported results often differ from one other.

Some authors have shown a statistically significant decrease of CFU-F numbers in cases of acute myeloid leukemia either in primary patients and patients after PCT courses [5, 6, 8] or for patients suffering from lymphomas, acute lymphoid leukemia and multiple myeloma [6, 23]. Notable is that the CFU-F number in patients varies from total absence to donor values. Moreover, neither sex nor age or involvement of bone marrow or time of the last PCT course affects the number of CFU-F [23]. In this study we also found a decrease in the CFU-F numbers for bone marrow samples obtained from patients with hemoblastosis. However, despite a low CFU-F number in patients our data display the principal possibility of isolation and in vitro expansion of MSC from patients with hematological malignancies.

Moreover, our results demonstrated a decrease in the proliferative capacity of in vitro expanded patient MSCs. For achieving confluence growth of patients' MSCs, cultivation time needed to be twice as long. On the one hand, this may be caused by inhibition of proliferative potential in the patients' MSCs, and on the other hand it may be caused by initially lowered content of MSC in bone marrow MNCs under hematological malignancies.

Our data shows that the MSCs of patients with ALL, HL, NHL, and MM do not express linear and hematopoietic antigens but are positive for some "stromal" markers. Along with that we managed to elicit some other special features. Namely, in the MSC population there was a lower number of CD73, CD90, and CD105-positive cells. Moreover, patient MSCs exhibited a two-fold increased number of HLA-DR-positive cells. Our data corresponds with Campioni D. et al [6], who used 4-color flow cytometry to show significant decreases in CD73, CD90, and CD105 expression on MSCs of 43 hematological patients. We have found this fact just as a tendency but this probably was caused by small array of patients (n=16) or by using 1-2-color flow cytometry.

We further found that in contrast to donor cells, patient MSCs in some cases did not suppress but even increased a proliferative response in MLC (data not shown), which corresponds with published data [17]. The low number of such observations did not allow us to connect this feature either with a distinct nosologic form of hemoblastosis, nor with particular phenotype or morphology of MSCs. One of the possible reasons for this phenomenon could be the ability of MSCs to secrete IL-7, which induces and supports lymphocyte proliferation [9].

Finally, in all investigated cultures (ALL, HL, NHL) we observed the ability of MSCs to support growth of hematopoietic progenitors in vitro. Published data concerning this issue can not be called univocal. For example, it was demonstated that the stromal cells of patients with acute myeloid leukemia possessed a low potential to stimulate growth of allogeneic hematopoietic precursors [8]. Meanwhile Mayani et al. [19] have shown that stromal cells of patients with AML display intact hematopoiesis-supporting activity, which strongly depends on the presence of CFU-F.

Summarizing the given experimental data we can conclude that MSCs of patients with hemoblastosis used in our study correspond to the Minimal criteria for defining multipotent mesenchymal stromal cells settled in the International Society for Cellular Therapy position statement [11]. These cells are characterized by adhesiveness, show fibroblast-like morphology, and a specific phenotype. Moreover, patients' MSCs display immunosuppressive and hematopoiesis-supporting activity. However, they have a number of distinct features. For instance, patient stromal cells in comparison with healthy donor cells contain lower numbers of CD73, CD90, and CD105-positive cells and a two-fold increased number of HLA-DR-positive cells. In addition, MSCs in hemoblastosis display lower levels of suppressive activity that is only become apparent at high concentrations of MSCs. It is important to note that described features of MSCs in hemoblastosis are not crucial for their hematopoiesis-supporting activity. This fact in combination with the ability of MSCs to expand ex vivo represents the basis for the clinical application of hematopoietic stem cell co-transplantation with mesenchymal stromal cells in onco-hematology for the acceleration of hematopoiesis recovery.

Based on our clinical findings, we propose that culture-expanded autologous MSCs can be used to improve the rate and quality of

hematopoietic engraftment, particularly in patients who previously received stroma-damaging therapy. Indeed, our report demonstrates that autologous MSCs can be successfully culture-expanded in FCS-free conditioning media, and infused IV for therapeutic intent with efficacy and without toxicity into different nosologic groups of patients at the time of PBSC transplantation. We have optimized MSC culture expansion methods to generate optimal numbers of autologous MSCs in a relatively short period of time for clinical use with a therapeutic intent without xenogenic components (e.g., FCS) [7].

Patients treated with high-dose chemotherapy generally experience complete and rapid neutrophil and platelet engraftment when supported with mobilized PBSCs containing >2–3 106 CD34+ cells. On the other hand, patients receiving lower doses of CD34+ cells are at increased risk for delayed platelet engraftment [3, 2].
Unsuccessful mobilization of CD34+ cells is commonly associated with extensive previous therapy, which is also associated with microenvironment damage. Coupled with low doses of CD34+ cells, an abnormal marrow microenvironment increases the risk of delayed engraftment. Sequential use of high-dose chemotherapy is also toxic to the marrow microenvironment, as evidenced by the delayed hematopoietic recovery after transplantation despite infusion of equal numbers of stem cells [12, 20, 22, 25]. Therefore, attempts to protect and improve the bone marrow microenvironment ex vivo are likely to better hematopoiesis engraftment after suboptimal CD34+ transplantation.

In conclusion our results indicate that the proposed cellular therapy protocol is feasible and may have a number of beneficial clinical effects in the setting of hematopoietic stem-cell transplantation. We therefore suppose studying it in more depths in randomized trials.

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Клеточная терапия и трансплантация (КТТ), том 1, номер 4

Аутологичные мезенхимальные стромальные клетки больных гемобластозами эффективно поддерживают восстановление кроветворения после трансплантации стволовых кроветворных клеток

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Резюме

Мезенхимальные стромальные клетки (МСК), выделенные из костного мозга, обладают иммунорегуляторной активностью и способны поддерживать гемопоэз. К сожалению, данные, характеризующие биологические свойства МСК при различных патологических состояниях, очень немногочисленны и зачастую противоречивы. В настоящей работе мы показали, что МСК, полученные из костного мозга больных гемобластозами, имеют морфологию фибробластоподобных клеток и характерный фенотип. Более того, МСК больных обладают хорошо выраженной способностью к стимуляции гемопоэза, в сочетании со сниженным иммуносупрессорным потенциалом. Эти свойства МСК послужили основанием для клинического применения котрансплантации аутологичных гемопоэтических стволовых клеток и МСК в онкогематологии с целью ускорения восстановления гемопоэза. Нами были исследованы безопасность и гемопоэтические эффекты мезенхимальных стромальных клеток у пациентов с гемобластозами, которым выполнялась трансплантация периферических стволовых кроветворных клеток (ПСКК). Использование ex vivo культивированных МСК, котрансплантированных с аутологичными ПСКК, позволило нам выявить снижение периодов критической нейтропении и тромбоцитопении у больных гемобластозами после высокодозной химиотерапии. Полученные результаты демонстрируют возможность и безопасность совместных трансплантаций МСК и ПСКК. Сокращение периода восстановления кроветворения свидетельствует о позитивном влиянии МСК на гемопоэз.

Ключевые слова: гемобластозы, костный мозг, мезенхимальные стромальные клетки, трансплантация стволовых клеток