Possibilities for the Use of Autologous Mesenchymal Stem Cells in the Therapy of Radiation-Induced Lung Injuries

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Possible therapeutic effect of systemic (intravenous) transplantation of autologous mesenchymal stem cells was studied in experiments (C57Bl/6 mice) and pilot clinical trial. Clinical trial was performed on 11 patients with radiation-induced lung injuries developed after combined chemotherapy and radiation therapy for lymphogranulomatosis or breast cancer. The patients were subjected to single transplantation of mesenchymal stem cells and course of standard pharmacotherapy. The method for isolation of autologous mesenchymal stem cells was licensed. The transplantation of mesenchymal stem cells was followed by a decrease in the mortality rate of mice with radiation-induced lung injury. Clinical trial showed that cell therapy with autologous mesenchymal stem cells does not induce progression of the underlying oncological disease. Parameters of spirography, immune status, lung scintigraphy, and markers for inflammation and tissue hypoxia in the patients remained practically unchanged 1 year after the treatment. These clinical signs reflect stabilization of the radiation process.

Key Words: *radiation-induced lung injuries; autologous mesenchymal stem cells*

Radiation-induced lung injury is one of the most severe complications of treatment for some oncological diseases. Radiation pneumonitis and pulmonary fibrosis are observed in 8.9-32 and 12-95% patients, respectively [1,2]. Pulmonary fibrosis leads to lung deficiency, which has an adverse effect on the quality of life in patients. Hence, the development of efficient therapeutic methods for radiation injuries (*e.g.*, radiation-induced lung injury) is an urgent problem.

Microcirculatory disturbances with impairment of tissue reparation and subsequent depletion of the vascular bed in the zone of irradiation play a major role in the pathogenesis of radiation injuries [3,5]. It was hypothesized that the vascular endothelium originates from hemopoietic islets of the extraembryonic and embryonic mesenchyme [21]. Embryological studies showed that mesenchymal cells with hemangioblastic potencies are formed due to splanchnotome migration from the mesoderm [22]. Peripheral and central hemopoietic germ cells develop into the endothelial and hemopoietic differone, respectively. Therefore, bone marrow (BM) hemangioblasts circulating in the peripheral blood serve as precursors for blood cells and vascular cells [8,13,19].

Transplantation of mesenchymal stem cells (MSC) and fixation of these cells in the pulmonary vascular bed of patients with pulmonary fibrosis will be probably followed by an increase in capillary blood flow in the zone of injury and induction of

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reparative processes. This hypothesis is derived from published data on high plasticity of MSC and their ability to produce cells of the vascular endothelium [15] and lung parenchyma [23]. However, there is no general agreement on this problem [11, 16]. Further studies are required to evaluate the role of MSC as endothelial precursor cells for lung vessels and vascular parenchyma after radiation exposure.

Stem cell transplantation is an example of introduction of the results of basic studies in the field of molecular and cellular biology into medical practice. This method holds much promise for substitution of abnormal and aged cells and tissues. Studies at the Medical Radiological Research Center (Russian Academy of Medical Sciences) were designed to obtain transplantable cell cultures from BM MSC, to develop new experimental models for studying the effect of transplanted cultures from MSC, and to provide the scheme of limited clinical trials with transplantation of cell cultures.

Here we studied the effect of MSC transplantation in mice with radiation-induced lung injury. A pilot clinical trial was performed to evaluate the effect of autologous MSC transplantation on the development of radiation injuries in irradiated lung tissue of patients with oncological diseases and radiation disorders.

MATERIALS AND METHODS

Experimental part of the study. Possible therapeutic effect of intravenous (systemic) transplantation of MSC was studied in male C57Bl/6 mice aging 3 months, weighing 19-21 g, and subjected to local irradiation of the thorax. This mouse strain is characterized by high radiosensitivity (test for radiation-induced lung injury). Previous studies showed that 40-60% animals die from pneumonitis 6 months after thoracic irradiation in a dose of 12 Gy [9, 17]. The mice were anesthetized with Nembutal (60 mg/kg intravenously) and exposed to local γ-irradiation of the thorax using a Luch device (12 Gy, dose rate 45.4 cGy/min). The suspension of mouse MSC (0.2 ml) was administered into the caudal vein 1 day after irradiation (treatment group). MSC were genetically labeled with green fluorescent protein (GFP). The dose of MSC was 10^6 cells per mouse. Irradiated animals of the control group received physiological saline (0.2 ml intravenously) instead of GFP-MSC suspension. Each group consisted of 20 specimens. The mortality rate of mice from radiation pneumonitis was recorded over 6 months.

In series II we studied the possible mechanisms for the therapeutic effect of systemic transplantation of MSC in irradiated animals. The accumulation of GFP-MSC (2×10^6 cells intravenously) in the lung tissue was evaluated in non-irradiated C57Bl/6 mice (control group) and animals with local irradiation of the thorax (1 day before cell transplantation, treatment group). The mice were subjected to pharmaceutical euthanasia (70 mg/kg nembutal) 24 h after stem cell transplantation. The suspension of lung cells in physiological saline was obtained by mechanical disintegration of the right lung. The cell suspension was passed through needles of decreasing diameter. The ratio of GFP-labeled cells was estimated by means of flow cytofluorometry [10].

The results of these experiments allowed us to obtain the license for the production and clinical use of MSC (Federal Service on Surveillance in Healthcare, Russian Ministry of Healthcare and Social Development; No. FS-2006/206 of August 11, 2006). The protocol was discussed by the Scientific Council of the Center and approved by the Ethics Committee.

Clinical part of the study. Eleven patients with radiation-induced lung injury (9 patients with radiation-induced lung fibrosis and 2 patients with late radiation pneumonitis) were subjected to MSC autotransplantation in combination with standard therapy [2]. The mean age of patients was 32 years. Radiation therapy was prescribed for breast cancer (BC, 4 patients) or lymphogranulomatosis (LGM, 7 patients). The mean total irradiation dose at the area of lung projection was 43.6 Gy. The mean period of MSC treatment after beam therapy was 2.3 years.

The cells were obtained by bone marrow puncture under sterile conditions (0.5-1.0 ml) and placed in tubes with heparin (100 U/ml puncture sample). Erythrocytes were precipitated at room temperature for 1-2 h. The supernatant was taken by a Pasteur pipette. The cells were washed with medium 199 (Biolot medium, reagents, and plates) and centrifuged at 1000 rpm for 10 min. The pellet was resuspended in a growth medium: RPMI-1640 medium with 100 U/ml penicillin, 100 ng/ml amphotericin, 2 mM L-glutamine, and 20% fetal bovine serum. Culturing was performed in Carrel flasks (bottom area 25 cm^2) with 5×10^6-10^7 BM cells in 8 ml growth medium in a thermostat at 37°C. Gaseous mixture containing 5% CO_2 was passed through flasks during medium replacement or reinoculation of cells into new culture flasks. After attaining confluence, the cells were reinoculated using 0.25% trypsin into 25-cm^2 culture flasks and then into 175-cm^2 flasks. The yield of human MSC was $(1\text{-}2)\times10^8$ cells by the 5th-6th week. This amount of cells was required for BM transplantation to the donor organism [7].

TABLE 1. Radiation-Induced Mortality and Mean Lifespan of C57Bl/6 Mice (*M*±*m*)

Group	Radiation-induced mortality, %	Lifespan, days
Control	55±11	147±16
Treatment	10±7*	156±21

Note. Here and in Table 2: *$p<0.05$ compared to the control group.

Standard therapy for late pneumonitis and lung fibrosis included treatment with broncholytic and mucolytic agents, antihistamine drugs, vitamins, nonsteroid antiinflammatory drugs, and antibiotics (when indicated). The patients were hospitalized before and 1 year after transplantation of autologous MSC (at 3-month intervals). All patients were subjected to a detailed clinical examination and laboratory assays (biochemical blood test, spirography, study of immune parameters, roentgenography of the lungs in 2 projections, and radioisotope scintigraphy of the lungs with 99 mTc-labeled microspheres of human serum albumin). The quality of life of patients was estimated using a QOli-NS questionnaire. The patient was asked to evaluate physical activity, psychological status, relationships with other people, and state of health (in points) [18]. Patient's general state was scored by a physician using the Karnovsky index.

RESULTS

The effect of systemic transplantation of MSC on radiation-induced mortality of C57Bl/6 mice was studied over 6 months after local irradiation of the thorax. Systemic transplantation significantly decreased the mortality rate of mice over 6 months after irradiation (χ^2=8.6, Table 1). The time of death did not differ in mice of both groups. These data suggest that death of animals is realized via the same mechanism.

Comparative study was performed to evaluate the accumulation of GFP-MSC in the lungs of irradiated and non-irradiated C57Bl/6 mice. Thoracic irradiation with 12 Gy was followed by increased accumulation of transplanted stem cells in the lung

TABLE 2. Number of GFP-Labeled Cells in Suspension of Lung Cell from C57Bl/6 Mice (*M*±*m*)

Group	Number of mice	Ratio of GFP-labeled cells in suspension of lung cell, %
Control	5	2.9±0.3
Treatment	5	5.3±0.8*

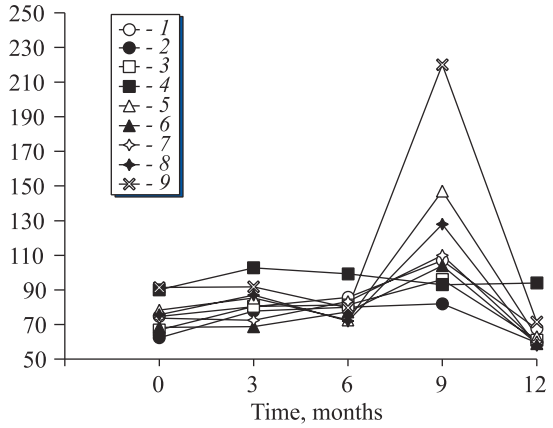

Fig. 1. Results of spirography after MSC autotransplantation. Lung vital capacity (LVC, liters, *1*); forced LVC (liters, *2*); forced expiratory volume over the 1st second (liters, *3*); Tiffeneau index (%, *4*); peak expiratory flow rate (EFR, liters/sec, *5*); peak forced EFR (liters/sec, *6*); maximum EFR at 75% forced LVC (liters/sec, *7*); EFR 50% (liters/sec, *8*); EFR 25% (liters/sec, *9*).

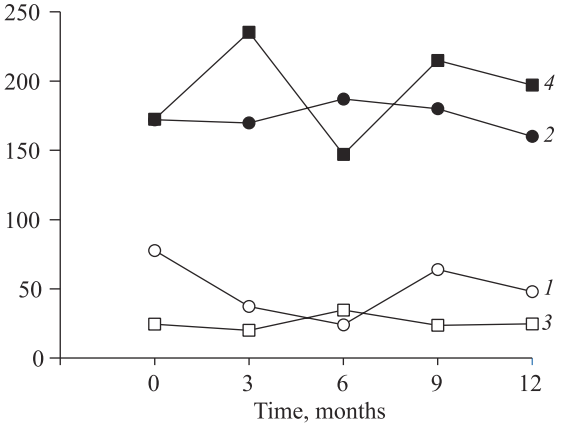

Fig. 2. Results of biochemical blood tests in patients after MSC autotransplantation. Myoglobin (ng/ml, *1*); seromucoids (mg/liter, *2*); bicarbonates (mol/liter, *3*); α_1-antitrypsin (mg/dl, *4*).

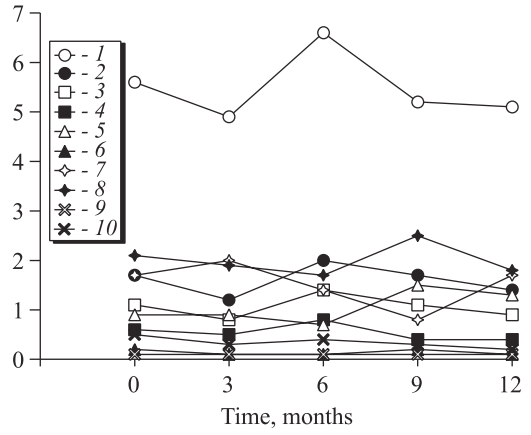

Fig. 3. Average immune parameters after MSC autotransplantation. Leukocytes (10^9 cells/liter, *1*); lymphocytes (10^9 cells/liter, *2*); $CD3^+$ (10^9 cells/liter, *3*); $CD8^+$ (10^9 cells/liter, *4*); immunoregulatory index (*5*); $CD19^+$ (10^9 cells/liter, *6*); IgM (g/liter, *7*); IgA (g/liter, *8*); circulating immune complexes (optical density units, *9*); $CD16^+$ (10^9 cells/liter, *10*).

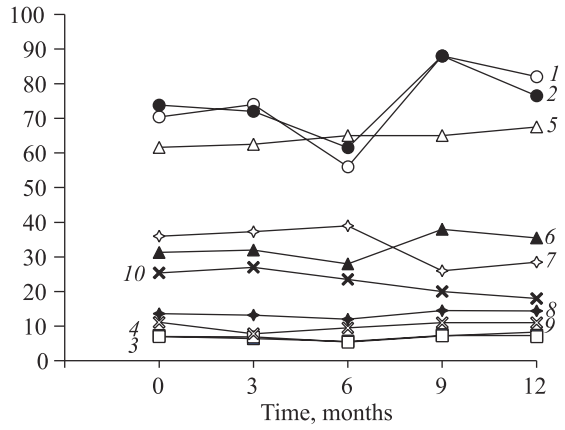

Fig. 4. Immune parameters after MSC autotransplantation. Phagocytic index, 30 min (%, *1*); phagocytic index, 120 min (%, *2*); phagocytic number, 30 min (number of engulfed microbes, *3*); phagocytic number, 120 min (*4*); $CD3^+$ (%, *5*); $CD4^+$ (%, *6*); $CD8^+$ (%, *7*); IgG (g/liter, *8*); $CD19^+$ (%, *9*); $CD16^+$ (%, *10*).

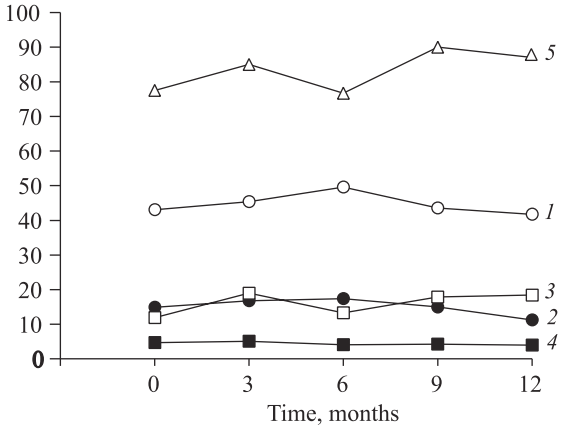

Fig. 5. Self-assessment of health (QOLi-NS questionnaire) and Karnovsky index. Physical activity (points, *1*); self-assessment of health (points, *2*); psychological status (points, *3*); relationships with other people (points, *4*); Karnovsky index (%, *5*).

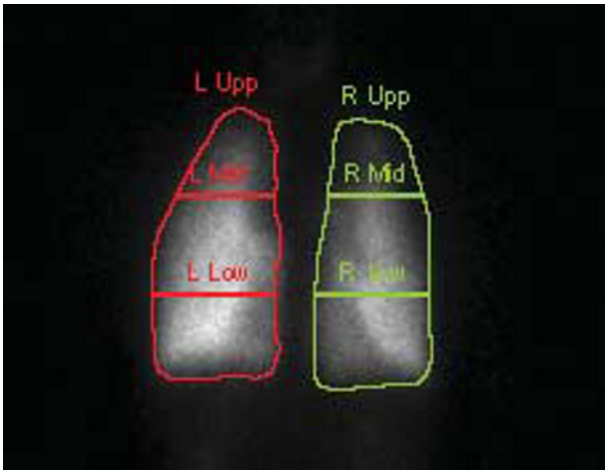

Fig. 6. Scintigraphy of the left and right lobe of the lungs in a patient with late radiation-induced lung fibrosis: irregular reduction of perfusion in the upper part of the lungs.

tissue (by 2 times, Table 2). Similar results were obtained for other organs and tissues of animals under various conditions of MSC labeling [6,12]. MSC enter the abnormal tissue and initiate regenerative processes. They reduce the severity of early or late radiation injury and damage due to inflammatory or autoimmune processes [14,20].

Descriptive analysis of the pilot clinical trial is shown in Figs. 1-5.

In none of the patients progression of the underlying disease was observed over 1 year after transplantation of autologous MSC. No adverse changes were found in inflammatory tests, indexes of tissue hypoxia, and parameters of external respiration and immune state. Self-assessment of health status and Karnovsky index remained practically unchanged 1 year after autotransplantation. X-ray examination revealed a decrease in the degree of infiltration and stabilization of lung fibrosis.

According to the results of radioisotope scanning, vascular perfusion in the pulmonary circulation before MSC transplantation and 3 months or 1 year after this treatment was 95, 100, and 97.6%, respectively (Fig. 6).

Radiation-induced tissue injury is related to microcirculatory deficiency, endothelial damage, obliteration of small vessels, and loss of parenchymal cells [4]. Progression of radiation injury is associated with arteriocapillary fibrosis, which contributes to the loss of parenchymal cells. Clinically, these changes are manifested in the persistent inflammatory process. It results in death of highly-specialized tissues, changes in the architectonics, and increase in the severity of functional insufficiency. In this trial, the patients with radiation-induced lung injury were treated with standard drugs and autologous MSC. It was a safe approach to the treatment of patients receiving radiation therapy for LGM and BC. Radiation-induced changes in the lungs were stabilized 1 year after the start of treatment. The first results of our study should be analyzed in further investigations. This approach holds much promise for the therapy of patients with radiation injuries to vital organs.

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