Long-Term Effects of Stem Cells on Total-Body Irradiated Mice

M V Vyalkina, I B Alchinova, E N Yakovenko, Yu S Medvedeva, I N Saburina and M Yu Karganov

Institute of General Pathology and Pathophysiology, Baltiyskaya str., 8, Moscow, 125315, Russian Federation

E-mail: nedzumy@bk.ru

Abstract. C57Bl/6 mice were exposed to γ-radiation in a sublethal dose of 7.5 Gy. In 3 hours injection 10⁶/mouse of bone marrow multipotent mesenchymal stromal cells stem cells intravenously to experimental group was done. Methods used: body weight measurement, open field behavior, subfraction composition of blood serum (laser correlation spectroscopy, LCS), histological examination of the spleen, liver, and pancreas, count of T and B cells, white blood formula. After 1.5 and 3 months the general trend towards intermediate position of the parameters observed in the experimental between those in intact and irradiated controls attests to partial protective/restorative effects of the injected cells.

1. Introduction
Bone marrow multipotent mesenchymal stromal cells (BM MMSC) are the second major stem cell population in bone marrow. They are used for restorative organ therapy, because they can be relatively easy isolated from a small volume of bone marrow aspirate and expanded in a necessary amount in a short period of time. In vitro BM MMSC are able to differentiate into cells of different types of connective tissues – bones, cartilage, tendons, skeletal muscles, adipose tissue, as well as supporting stroma. The accumulated preliminary data showed multiple mechanisms and pathways of BM MMSCs contribution to injury reparation. Systemic administration of BM MMSC is accompanied with integration of injected cells into parenchyma of recipient’s organs, especially in the regions of lung, liver, kidney, muscle and pancreas injury.

Current concepts on radiation-induced insults are based on the assumption that an effective treatment modality should be administered immediately, within a few hours after radiation exposure to protect and prevent the death of the critically irradiated cells. Cell therapies to reconstitute failing organs, aside from the hematopietic bone marrow (BM) stem cells transplantation, by indefinitely replacing the affected cells in most cases are not highly practical. Multipotent mesenchymal stromal cells from different sources, mainly from the BM, cord blood and adipose tissues were proposed for regenerative medicine [1].

2. Experimental Design, Instruments and Methods

2.1. BM MMSC preparation
To obtain the primary human BM MMSC culture, the mononuclear cell fraction was isolated from bone marrow of healthy donors’ iliac crest by centrifugation in Ficoll gradient. The obtained cell suspensions were centrifuged for 7 minutes at 1000 rot/min, the pellets were resuspended in growth medium (α-MEM, 17 % fetal calf serum (FCS), L-glutamine (2mM), 1% penicillin-streptomycin). Cells were seeded on Petri dishes in high density (1×10^5 cells/cm²). In 24 hours floating cells were removed with growth medium, the adhered culture was washed with Hank’s solution, then the fresh growth medium was added, and cells were further incubated for 4-8 days. When the primary culture reached 70% confluency, cells were washed with Versene solution, incubated in 0.25% trypsin solution (37°C, 3-5 min), discarded from plastic and centrifuged (7 min, 1000 rot/min). The cells in the pellet were resuspended in growth medium to inactivate trypsin, centrifuged one more time and seeded on Petri dishes at low density (100 cells/cm²) in full growth medium (DMEM/F12 (1:1), L-glutamine (2mM), 1% penicillin-streptomycin, 10 ng/ml basic fibroblast growth factor (bFGF), 1:100 insulin-transferrin-selenite, 10% FCS).

During culturing process, BM MMSC culture became less heterogenic and after the second passage was mainly (more than 90%) represented with small cells with the average diameter of 6-9 µm.

The obtained BM MMSC culture expressed such surface markers as CD73 (95%), CD90 (79%), CD105 (98%) and did not express CD14 (1,1%), CD34 (1,31%), CD 45 (0,6%), CD49b (1%). The resulting marker profile is an important criteria of purity and quality of BM MMSC culture with the prevalence of precursor cells, which possess high clonogenic, proliferative and differentiation potential. These obtained and characterized BM MMSC cultures were further used in experimental work.

2.2. Experimental groups

The study was performed on C57Bl/6 mice weighing 25-27 g. The mice were exposed to γ-radiation in a sublethal dose of 7.5 Gy at 5.4 R/min radiation intensity. Groups: intact control (n=11), irradiation (n=19), experiment, irradiation + injection 10^6/mouse of the studied stem cells intravenously (n=41), green protein, irradiation + injection of the studied cells labeled with green protein (n=3). Tests were performed 3 and 6 weeks and 3 months after irradiation/injection.

2.3. Methods

2.3.1. Body weight. High radiation doses cause bowel and liver damages during the first weeks after irradiation, which impairs digestion and leads to changes in body weight. In our previous experiments we have found interstrain differences in changes of body weight after irradiation, which may be caused by the development of metabolic syndrome [2]. The animals were weighed on a Mettler Toledo scales after 1-h food deprivation.

2.3.2. Open field behavior. The effect of the potent stress factor on the behavior of rodents was studied using the open field test. Vertical and horizontal activities were measured over 3 min. The testing was performed in a square chamber (45×45 cm) at 40 lux illumination. In this test, behavioral phenomena caused by the conflict between fear motivation and exploratory activity are studied.

2.3.3. Subfraction composition of blood serum - Laser correlation spectroscopy. The method of laser correlation spectroscopy (LCS) is based on the analysis of the spectrum of quasielastic light scatter during coherent monochromatic laser irradiation of microparticles in biological fluids [3]. The distribution histograms in the serum provide qualitative information on the mean particle sizes and their relative content. Strict correspondence of certain fragments of the spectrum to biological nature of serum components can be determined after additional studies.

Blood was taken routinely: 100 µl of whole blood is transferred to plastic tubes with 100 µl physiological saline. The samples were left at room temperature for 0.5-2 h and then centrifuged at 5000 rpm for 15 min. The supernatant was collected in plastic tubes.
2.3.4. Histological examination of the spleen, liver, and pancreas. After the end of the experiment, the animals were sacrificed by anesthetic overdose and the organs (liver and intestine) were taken for histological examination for evaluation of the damaging effects of irradiation. The organs were fixed in 4% neutral formalin. The slides were prepared routinely.

For simplification of data processing, three injury severity degrees were specified depending on the severity of morphological changes [4].

Cytometry and white blood formula were performed routinely.

3. Results and Discussion

After 3 weeks: no differences by body weight were revealed between the irradiated controls and experimental mice. By the parameters of horizontal and vertical locomotor activities and leukocyte and T lymphocyte counts, the experimental mice were between the intact control and irradiated mice. No differences by B cell count were revealed between the irradiated controls and experimental group (figure 1).

In the subfraction spectrum of blood serum, a tendency towards accumulation of small particles is noted, which is a typical effect of radiation. It can be hypothesized that the increase in the contribution of small particles into light scatter during the 3rd week is related to tissue destruction. Similar processes are observed in groups of irradiated mice, but injection of cells probably levels out the individual differences (figure 2).

Histological analysis revealed that severe abnormalities of the spleen, liver, and pancreas were considerably less incident in the experimental group in comparison with the control groups (intact and irradiated). In the blood, an increase in neutrophil count (stab and segmented) and a decrease in lymphocyte count were noted both in the experimental and irradiated control group in comparison with intact control.

After 6 weeks: the mean body weight increased in all groups; the experimental group occupies an intermediate position between the intact control and irradiated mice by this parameter. In the blood, neutrophil (stab and segmented) and lymphocyte blood counts in the experimental group occupy an intermediate position between the corresponding values in the intact control and irradiated mice. Leukocyte count and count of T and B cells in the experimental group were lower than in the control groups (intact and irradiated) (figure 1). Exhaustion of the pool of mature B and T cells is observed 3 weeks after irradiation. Precursors of these cells in the bone marrow are destructed by radiation. By the 6th week, the release of young lymphocytes into the blood is noted. It can be hypothesized that injection of the studied cells in the experimental group delays T cell maturation. LCS showed that the contribution of large particles into light scatter increased; these shifts are opposite to typical changes induced by irradiation (accumulation of small particles due to cell destruction and release of
nucleoproteins and other components into the medium) (figure 2). In the experimental group, the histological picture of the spleen was considerably worsened with predominance of the 4th degree alterations. Round fluorescent cells were detected in cryosections of the bone marrow from mice receiving green protein-labeled cells.

Figure 2. Changes in LC spectra of mice blood serum. Particle size (nm) is along the X axis; contribution of particles with different hydrodynamic radius to light scattering in % is along the Y axis. * - $P_{U} < 0.05$ – intact/irradiated groups; + - $P_{U} < 0.05$ – intact/experimental groups; ! - $P_{U} < 0.05$ – irradiated/experimental.

After 3 months: lymphocyte count returned to normal. The distribution of subcellular components in blood serum remained unchanged. Severe disturbances are less incident in the liver and spleen, but more incident in the pancreas, although the morphological picture of the pancreas in the experimental group is better than in the group of irradiated control. Round fluorescent cells were detected in cryosections of the bone marrow and liver of mice receiving green protein-labeled cells.

4. Conclusion
Detection of labeled cells in the bone marrow and liver suggests that the observed effects are caused by the experimental influence. The general trend towards intermediate position of the parameters observed in the experimental group between those in intact and irradiated controls attests to partial protective/restorative effects of the injected cells. This is also confirmed by amelioration of the histological picture of the liver and pancreas after 3 weeks. Deterioration of the histological picture of the spleen by the 6th week can be determined by the reaction to lymphocyte release after a period of radiation-induced suppression. The absence of animal deaths at the final stage of the experiment (from the 6th week to the 3th month), normalization of lymphocyte count, stability of the subfraction composition of the blood, lower incidence of severe liver and spleen damages attest to long-term nature of the observed effects. The obtained results agree with data of other authors about the prospects of using of stem cells after irradiation in high doses [5, 6, 7].

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