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Treatment of Radiation Lesions with Mesenchymal Stem Cells

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Acute radiation syndrome (ARS) is an acute illness caused by exposure to a high dose of ionizing radiation. ARS is the deterministic effect of radiation exposure of the whole body or a significant body volume (partial body irradiation) above a threshold dose of about 1 Gy (gray). Radiation accidents, such as those in Chernobyl (1986) and Fukushima (2011), or the possible use of nuclear weapons during the hostilities or terrorist attacks, can lead to the massive development of ARS in humans. The *aim of the work* is to introduce a new method of post-radiation treatment – the use of allogeneic mesenchymal stem cells (MSCs). *Materials and methods*. The information contained in specialized scientific journals that are freely available and accessible through the global Internet was studied. *Discussion of the results*. In the scenario of mass exposure of the population, when from several tens (hundreds) to millions of people can be irradiated, the transfusion of hematopoietic stem cells traditionally used in such cases would be impossible. MSCs can possibly differentiate into specialized cells, that is, turn into cells of various organs and tissues or induce such kind of regeneration. For practical use, there are two main sources of their isolation and reproduction *ex vivo* –bone marrow and adipose tissue. To date, it has been shown that MSCs derived from adipose tissue can be effective in mitigating the effects of acute radiation illness. Intravenously applied MSCs are migrating mainly to the bone marrow and are partially restoring its function. Deep anatomical structures are also involved in local radiation injuries: bone, muscles, nerves, blood and lymphatic vessels and skin. There is a strong body of evidence suggesting the «repair effect» of MSCs when used to treat such lesions. This is because MSCs can induce the repair and regeneration of the anatomical structures which they are locally applied, possibly by the paracrine effect. The main advantage of allogeneic MSCs over autologous ones is their logistical accessibility. They can be produced in advance in quantities and stored frozen. After thawing, the cells must be cultured for at least 48 hours in humidified incubators with the addition of 5% CO_2. *Findings*. Treatment of MSCs should be started as soon as possible after radiation exposure. Rescue of damaged hematopoiesis in the bone marrow can be achieved by multiple intravenous administration of up to 1 million (10^6) freshly prepared allogeneic MSCs/kg body weight. Locally (around and in the irradiation area), the dose of MSCs may be lower – 20 million cells. Repeated topical application should be carried out at intervals of two to four weeks. Subsequent surgical reconstruction should be performed by an experienced surgeon and in a specialized center with concomitant topical application of MSCs.

Keywords: *allogeneic adipose-derived stem cells; bone marrow cells; clinical praxis; mesenchymal stem cells; MSC treatment; radiation accident; radiation effects; radiation injuries; radiation lesions; radiation sickness.*

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Introduction

In the book «A slow death: 83 days of radiation sickness»[1] one, even a non-specialist can find the description of the effect of radiation on the human body. The incident happened on September 30, 1999, in a nuclear fuel processing facility in Tokaimura, Japan. Two men (Hisashi Ouchi and Masato Shinohara) were exposed to gamma and neutron beam radiation. According to the model, Ouchi has been exposed to a radiation level around 20 Sv (sievert). The lethal dose is an exposure 8 Sv. (1 Sv (sievert) = 1 joule/kilogram – a biological effect. The sievert represents the equivalent biological effect of the deposit of a joule of radiation energy in a kilogram of human tissue. The ratio to absorbed dose is denoted by Q. 1 Gy

[1] A Slow Death 83 Days Of Radiation. https://archive.org/details/ASlowDeath83DaysOfRadiation/page/n115/mode/2up (date: 20.02.2023).

(gray) = 1 joule/kilogram – a physical quantity. 1 Gy is the deposit of a joule of radiation energy per kilogram of matter or tissue [1]). It should be noted, that according to the nurse medical personnel his admission to the hospital was only temporally and he «would not be hospitalized» for a long time. Judging from his outward appearance it was hard to tell what was wrong with him and impossible to believe that he had received a supralethal dose of radiation. The biggest concern of the personnel was (possible) secondary radiation which finally was not justified. On day +1 his lymphocytes in peripheral blood decreased almost to zero values. On day +5 all chromosomes of the bone marrow were found to be destroyed into pieces. On day +6 Ouchi received peripheral blood stem cell transplant from his HLA identical sister as a donor. On day +9 was entirely forbidden to use tape on Ouchi's skin because the skin underneath started coming off with the tape. Like after burn blisters appeared on his right hand (the site of his body closest to the radiation). On day +10 he was intubated and put on mechanical (artificial) ventilation. On day +16 he his bone marrow showed a full chimerism. All examined cells were XX (female) positive. However, in 3 out of examined 30 cells chromosomal breaks were present. This chromosomal damage was a surprise. One theory was that the neutron beams hit atoms in the body, such as sodium (Na), phosphorus (P) and potassium (K) and these atoms become radioactive themselves. The isotope ^{24}Na has a half time of approximately 15 hours. This means that the 1/1000 (1/1024) of its radioactivity is achieved in 150 hours i.e., 6.25 days. Ouchi received the transplant on day +6 (he was irradiated on day 0) and the radiation could still be effective to cause at least some chromosomal breaks. On the other hand, another explanation comes from a so-called «bystander effect», an effect unique to neutron beam irradiation. The cells irradiated by neutron beams emitted reactive oxygen (oxygen radicals or free radicals), damaging nearby cells that had not been irradiated. On day +26 the intense diarrhea was observed. The damage of all gastrointestinal (GIT) membranes has been confirmed. Moreover, 30 times higher than the norm a level of myoglobin in Ouchi's blood serum has been detected. This was a sign of muscle necrosis. Remarkably, no sign of heart damage had been observed. On day +49 the skin of his front almost completely «disappeared». The team grew artificial pieces of skin *in vitro* (donor Ouchi's sister) and finally transplanted up to 70 pieces of them. No one regenerated, due to fluid leak all had fallen off. On day +82 Oguchi died. The autopsy of Ouchi's body showed the following results. His body was bright red, as he had been scalded. It was different as in thermally burnt corpses whose bodies were pitch black. No skin remained at the front site of his body. There were severe organ alternations. Every mucus membrane in the body had disappeared (GIT, trachea). The hematopoietic cells disappeared too. The bone marrow was «empty». Ouchi's muscle cells had lost most of their fiber and only the cell membrane remained. In contrast to «ordinary» muscles the vivid red muscle cells remained intact in the heart. In other words, only the muscle cells in the heart had not been destroyed by the radiation. The heart was the one internal organ in Ouchi's body which remained intact.

Main

At the beginning of this section the author would like to point out that the following material (Radiation, Radiation Injuries, Acute radiation syndrome) has been taken from the website[2].

Radiation. Radioactivity is the phenomenon whereby atoms undergo spontaneous disintegration, usually accompanied by the emission of radiation. Radiation is the transmission of energy through space and is of two types: ionizing and non-ionizing. Depending on the range in the electromagnetic energy spectrum, it is possible to characterize non-ionizing radiation such as heat, microwaves, visible light or others, and ionizing radiation such as X rays and gamma rays. These waves are characterized essentially by their energy, which varies inversely to the wavelength. Ionizing radiation may be emitted in the process of decay of unstable nuclei or by de-excitation of atoms and their nuclei from natural sources like the sun, the stars or cosmic radiation. It may also be produced by X ray machines, nuclear reactors, cyclotrons, and other devices. During radioactive decay, gamma (γ) rays are often produced alongside other types of radiation, such as alpha (α) or beta (β) particles. When a nucleus emits an alpha or beta particle, the daughter nucleus is sometimes left in an excited state which, after de-excitation, returns to a lower energy level by emitting a gamma ray in much the same way that an atomic electron can, in most cases, jump to a lower energy level by emitting visible light. Ionizing radiation can strip electrons from atoms and break the bonds between the atoms of a molecule. Ionizing radiation can be divided into low and high linear energy transfer radiation (as a guide to its relative biological effectiveness), or into strongly penetrating radiation and weakly penetrating radiation (as an indication of its ability to penetrate shielding or human body tissues).

The characteristics of the four major types of radiation emitted by radioactive material, namely,

[2] INTERNATIONAL ATOMIC ENERGY AGENCY, Medical Management of Radiation Injuries, Safety Reports Series No. 101, IAEA, Vienna. 2020. URL: https://www.iaea.org/publications/12370/medical-management-of-radiation-injuries (date: 25.12.2022).

alpha, beta, gamma, and neutron radiation, are as follows:

- Alpha radiation has a relatively short range, travelling only a few centimeters in air. It can be stopped by a sheet of paper and cannot penetrate the outer layers of intact human skin. For this reason, alpha radiation becomes a hazard only if an alpha-emitter radionuclide is taken into the body. Examples of alpha particle emitters are americium-241 (^{241}Am) and polonium-210 (^{210}Po).

- Beta radiation can travel several meters in air and can penetrate inadequately protected skin. Beta radiation emitters are considered primarily an internal hazard, but the deposition on the skin of radionuclides emitting beta particles of sufficient energy (such as caesium-137 (^{137}Cs)) can give rise to «skin burns».

- Gamma radiation is highly penetrating and can pass through most materials, including the human body. For this reason, gamma radiation is considered an external hazard as well as an internal hazard. Examples of gamma radiation emitters are iridium-192 (^{192}Ir) and cobalt-60 (^{60}Co).

- Neutrons are emitted in the processes of nuclear fission and reaction, or when some radioactive material undergoes spontaneous decay.

When ionizing radiation interacts with the human body, it deposits its energy in organs and tissues. The amount of energy absorbed per unit weight of the organ or tissue is called 'absorbed dose' and is expressed in units of gray (Gy). One Gy of absorbed dose is equivalent to one joule of radiation energy absorbed per kilogram of organ or tissue mass. Equal absorbed doses from different types of ionizing radiation are not equally harmful. Alpha particles produce greater harm than do beta particles, gamma rays and X rays for a given absorbed dose. To account for this difference, radiation dose is expressed as equivalent dose in units of sieverts (Sv). The equivalent dose in Sv is equal to absorbed dose multiplied by a so-called radiation weighting factor.

Radiation injuries. A nuclear or radiological emergency is defined as: An emergency in which there is, or is perceived to be, a hazard due to: (a) The energy resulting from a nuclear chain reaction or from the decay of the products of a chain reaction; (b) Radiation exposure. The hazard involves a sealed or unsealed radioactive source and may lead to an uncontrolled release of ionizing radiation or radioactive material into the environment or to individuals. Such radioactive sources include sealed sources of radioactive isotopes such as ^{60}Co, ^{137}Cs, or ^{192}Ir irradiators, used mostly in medicine and industry, and unsealed sources used in nuclear medicine and scientific research. Less frequently, X ray equipment, linear particle accelerators and other equipment have also been involved in the uncontrolled exposure of people. The second step is to identify those individuals possibly exposed or contaminated. The severity of radiation injuries depends on the radiation dose incurred, the

Table 1 – *Initial decision making for managing radiation injuries based on vomiting and erythema [5]*

Clinical manifestations		Estimated dose		Initial decision
WBE	LE	WBE	LE	
No vomiting	No erythema	<1 Gy	<3 Gy	Outpatient with five week surveillance (blood, skin).
Vomiting 2–3 h after exposure	Primary(early) erythema 12–24 h after exposure	1–2 Gy	>3~8 Gy	Monitoring in a general hospital.
Vomiting 1–2 h after exposure	Primary erythema 8–15 h after exposure	2–4 Gy	>15 Gy <25 Gy	Hospitalization in haematological or surgical (burn) department or specialized surgical department (ideally in a room with laminar air flow and air filtering, and with a plastic surgery team trained in radiation injuries).
Vomiting earlier than 1 h after exposure	Primary erythema within 3–6 h (or less) associated with itching, oedema and pain	>4 Gy	>25 Gy	Hospitalization in a haematological or specialized surgical department (ideally in a room with laminar air flow and air filtering, and with a plastic surgery team trained in radiation injuries). Specialized counselling is necessary.

Table 2 – *Methods for the early diagnosis of radiation injuries. The table presents the main methods for early diagnosis of whole body or partial body irradiation, including the procedures, manifestations, expected time of onset and minimum doses necessary for the appearance of the early symptoms and signs of radiation exposure (threshold)*[1]

Procedure	Manifestation	Time of onset*	Minimum exposure (Gy)
Clinical observations	Nausea, vomiting	Within 48 h	~1
	Erythema	Within hours to days	~3
	Epilation	Within 2–3 weeks	~3
Laboratory examinations: Blood cell count	Absolute lymphocyte count $<1\times10^9$ /L**	Within 2–72 h	~0.5
Cytogenetics***	Dicentrics/rings, micronuclei, translocations	Within hours	~0.1 (detection level)

Note. WBE – whole body exposure, LE – local exposure.

** The latency time is inversely dependent on radiation dose.*

*** The lymphocyte count may decrease within hours. Experts recommend that a baseline count be obtained as soon as possible and the counting be repeated every 4 h on the rst day and then daily.*

**** Results can be available in three to five days depending on the technique used.*

[1] INTERNATIONAL ATOMIC ENERGY AGENCY, Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies, EPR-Biodosimetry, IAEA, Vienna. 2011 (date: 22.12.2022).

dose rate, the radiosensitivity of affected tissues and organs, and the area and extent to which the body has been exposed. For the same absorbed dose, the health consequences of a partial body exposure are less severe than those of a whole-body exposure. A single absorbed dose of about 3.5 Gy to the whole body is generally expected to result in the death of 50% of the exposed population group within two months if there is no medical treatment ($LD_{50/60}$, meaning a lethal dose for 50% of the population in 60 days). The $LD_{50/60}$ can be increased to about 5.0–6.0 Gy with advanced mitigative treatment (e.g., bone marrow transplants, hematopoietic growth factors) or supportive treatment, as well as when the exposure is prolonged or fractionated. The survival probability of patients exposed to significantly higher doses is very limited. These patients require standard (best) supportive care.

Acute radiation syndrome

Acute radiation syndrome (ARS) is the acute illness caused by exposure to a high dose of ionizing radiation to the body. ARS is a deterministic effect of radiation exposure to the whole body or to a significant volume of the body (partial body irradiation) above a dose threshold of about 1 Gy. This deterministic effect induces a set of clinical and biological manifestations in the organs and tissues affected. To facilitate the understanding of clinical manifestations and how they overlap, ARS has typically been subdivided into three groups depending on the absorbed dose and the organs primarily involved (hematopoietic, gastrointestinal, and neurovascular types). However, the overlapping of these clinical manifestations reflects the expression of an inflammatory body response affecting all the organs and tissues, which in severe cases may lead to multiple organ failure. The hypothesis is that the organ system involvement is due not only to the radiation induced depletion of proliferating cells of rapid turnover tissues, but also to radiation induced changes in the vascular system, and specifically in the endothelial cells and the immune system, leading to the development of an uncontrolled systemic inflammatory response [2]. It appears that cytokines play a central role in mediating central nervous system response following irradiation. It has been shown that the radiation response of the central nervous system is characterized by local production of pro-inflammatory cytokines in different brain structures, causing a stimulation of inflammatory cascade, interaction with other inflammatory mediators and up-regulation of the inflammatory process that leads to neurotoxicity [3]. In the same way, radiation induced endothelial dysfunction can cause increased permeability, endothelial cell apoptosis, coagulation disorders, the expression of adhesion molecules, production of inflammatory cytokines and chemokines with transmigration of leukocytes and the release of proteases and reactive oxygen species that can contribute to tissue injury [2, 4].

An initial (rather orientational) judgment according vomiting and primary («early») erythema of a patient after radiation injury is shown in Table 1. A more advanced schedule is shown in Table 2. The

Table 3 – *Threshold doses and time of onset for different manifestations of local radiation injuries* [1]

Manifestation	Threshold dose, Gy	Time of onset*, days
Second phase erythema**	3	14–21
Temporary epilation	3	14–18
Definitive epilation	7	25–30
Dry desquamation (dry epithelitis)	10	20–28
Moist desquamation (exudative epithelitis)	15	15–25
Necrosis	25	>21

** Time of onset is a reference; it is influenced by actors such as the dose rate, duration o the exposure and individual radiosensitivity.*

*** Second phase erythema is a deterministic effect referring to an erythema that develops during the manifestation phase of a local radiation injury.*

[1] INTERNATIONAL ATOMIC ENERGY AGENCY, Medical Management of Radiation Injuries, Safety Reports Series No. 101, IAEA, Vienna. 2020. URL: https://www.iaea.org/publications/12370/medical-management-of-radiation-injuries (date: 23.12.2022).

diagnosis is more profound and is based on some laboratory tests. We are not planning to focus on the treatment of radiation injuries with «conventional» methods (growth factors, hematopoietic cell transplantation, supportive care, plastic surgery, etc.) Our aim is to introduce a new modality in the postradiation treatment – mesenchymal stem cells (MSC). These cells can be used in a systemic or local approach. The local injuries are in more detail characterized in Table 3. A damage of all deeper anatomical structures (bones, muscles, nerves, vessels) is earlier or later observed after high dose local irradiation. Here we would like to focus the reader's attention to an interesting fact. As shown in the Introduction the single organ which was not destroyed by the radiation (gamma rays and neutrons) was the heart. It was the one internal organ in Ouchi's body which remained intact. This «experimental fact» remains a mystery and is open for further laboratory studies.

An example of the skin changes after irradiation is shown in Figure 1.

Mesenchymal stem cells. In the text we will (mainly) use the original text of the author [6]. Mesenchymal stem cells (MSCs) can be isolated from almost all organs and tissues in the human body. For practical purposes, there are two main sources for their isolation and ex vivo expansion – the bone marrow and fat tissue. Based on their inherent plastic adherence properties, the ex vivo expansion of MSCs is a rather simple process. Nevertheless, the biological features (gained from decades of tissue culture experience) are contrary to bureaucratic rules, which govern the good laboratory practice. MSCs cannot be successfully used in the treatment of human diseases if they are not handled optimally akin to the conditions in their natural habitat. Moreover, extrapolation of the data obtained from animal studies (mainly

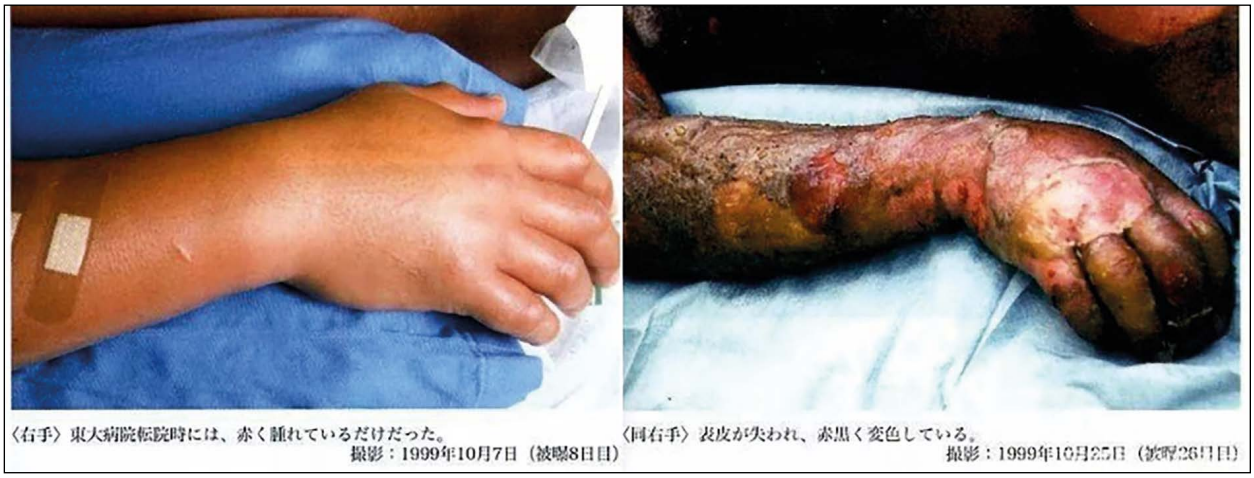

Figure 1 – *The right hand of the patient mentioned in the part Introduction. Left: day +7, Right: day +25 after the radiation accident (URL: https://archive.org/details/ASlowDeath83DaysOfRadiation/page/n95/mode/2up, p. 96; date: 23.12.2022)*

rodents) to humans is rather unfounded and with little relevance. The paper of Koç [7] symbolically «opened the door» for the mesenchymal stem cells (MSCs) in the third millennium. Here, the authors reported about the autologous blood stem cells and in tissue culture (*in vitro* or *ex vivo*) expanded bone marrow-derived MSCs in advanced breast cancer patients receiving high-dose chemotherapy. Since then, an enormous amount of material has been published [9]. Coming down to the molecular level, our knowledge each day is growing exponentially. Nevertheless, the primary question remains: What is the «therapeutic mechanism» of the applied MSCs? We call this effect as «posthypnagogic command». After the treatment with MSCs, the effect of healing is present for months, without the proven presence of MSCs. We are not coming in detail here; the reader could educate himself in the enormous amount of literature. In our opinion, it is useful to repeat the whole procedure of *ex vivo* expansion in detail as it has been described in part «*Ex Vivo* MSCs Culture» [8]: «Mononuclear cells (from bone marrow) were resuspended at 10^6 cells/mL in Dulbecco's modified Eagle medium, low glucose (DMEM-LG) with 10% fetal bovine serum and 30 mL of cell suspension was plated in a 175 cm^2 flask. MSCs were cultured in humidified incubators with 5% CO_2 and initially allowed to adhere for 72 h, followed by media change every 3–4 days. When cultures reached more than 90% confluence, adherent cells were detached with 0.05% trypsin-EDTA.» Later, additional characterizations and refinement added some regulatory rules. This ex vivo expanded MSCs fulfilled the criteria provided (later) by the International Society for Cellular Therapy [9]. Briefly, MSCs are defined by their plastic-adherent properties under standard culture conditions, by their ability to differentiate into osteocytes, adipocytes, and chondrocytes in vitro under a specific stimulus and by positive (CD105, CD73, and CD90) or negative (CD45, CD34, CD14, and HLA-DR) expression of specific surface markers. There are two main sources for their isolation and *ex vivo* expansion for practical purposes – the bone marrow and the fat tissue. This *ex vivo* expansion of MSCs is a rather simple process based on their inherent plastic adherence properties. The pilot paper [10] described the use of «third party» (here – haploidentical) MSCs for transplantation in a patient with severe treatment-resistant grade IV acute graft versus host disease (GVHD) of the gut and liver after allogeneic stem cell transplantation. For decades, two organs (or tissues), i.e., bone marrow and fatty tissue, were the primary sources for the isolation and ex vivo expansion of MSCs. MSCs cannot be successfully used to treat human diseases if they are not handled optimally akin to the conditions in their natural habitat. Let us discuss this in depth. As an example, we will consider the following research paper [11]. The authors claimed that «among patients with advanced heart failure, intramyocardial injection of mesenchymal precursor cells, as compared with the injections of a cryoprotective medium as sham treatment, did not improve successful temporary weaning from left ventricular assist device (LVAD) support at 6 months. These findings do not support the use of intramyocardial MSCs to promote cardiac recovery as measured by temporary weaning from device support» According to the authors, the patients were randomly assigned to cell therapy group who received intramyocardial injection of 150 million MSCs and a cryoprotective medium treatment group without cells for comparison. The allogeneic MSCs were obtained from healthy donors and expanded in a Good Manufacturing Practices (GMP) certified laboratory. It is evident that the cells were thawed directly before use («injections of mesenchymal precursor cells, compared to injections of a cryoprotective medium as sham treatment»). The cells were neither washed nor cultivated further for expansion before use. In the opinion of the authors that it is mandatory to use the MSCs that have been freshly prepared and not frozen or thawed immediately before use.

After decades of expanding the MSCs (and other cells) *ex vivo* (*in vitro*), we firmly stand behind this point of view. After thawing, the cells need to be cultured at least for 48 h in humidified incubators supplemented with 5% CO_2. Only after this wait period, one should start to consider further experimental (or therapeutic) work using these cells. On the other hand, one can consider growing the cells *ex vivo*, detaching them when 80% confluent and applying them to the patient in a short time (up to 3 h at room temperature). The practice to use freshly thawed cells (MSCs) makes the abovementioned study (and others in this fashion designed trials) from the biological point of view rather dubious and medically useless. In our opinion, it is necessary to return to the laboratory and to give the MSCs a «second chance» by consequently following the Good Biological Practice (GBP) developed during the decades of cell tissue culturing *in vitro*. We recommend returning to the praxis of small tissue culture centers associated with (or localized within) the hospitals. In coordination with the hospital departments, they could prepare fresh MSCs, which would be «on demand» prepared for use and treat the patients. Logistically, to prepare a total of $20–50 \times 10^6$ cells is not a difficult task. One skilled technician could obtain this amount under sterile conditions in 1–2 h. What about the tests for the differentiation and of sterility? Well, yes, one can ask a heretical, unorthodox question: Did anybody ever observe that the MSCs *in vitro* did not differentiate to osteoclasts, adipocytes, and chondrocytes during appropriate treatment?

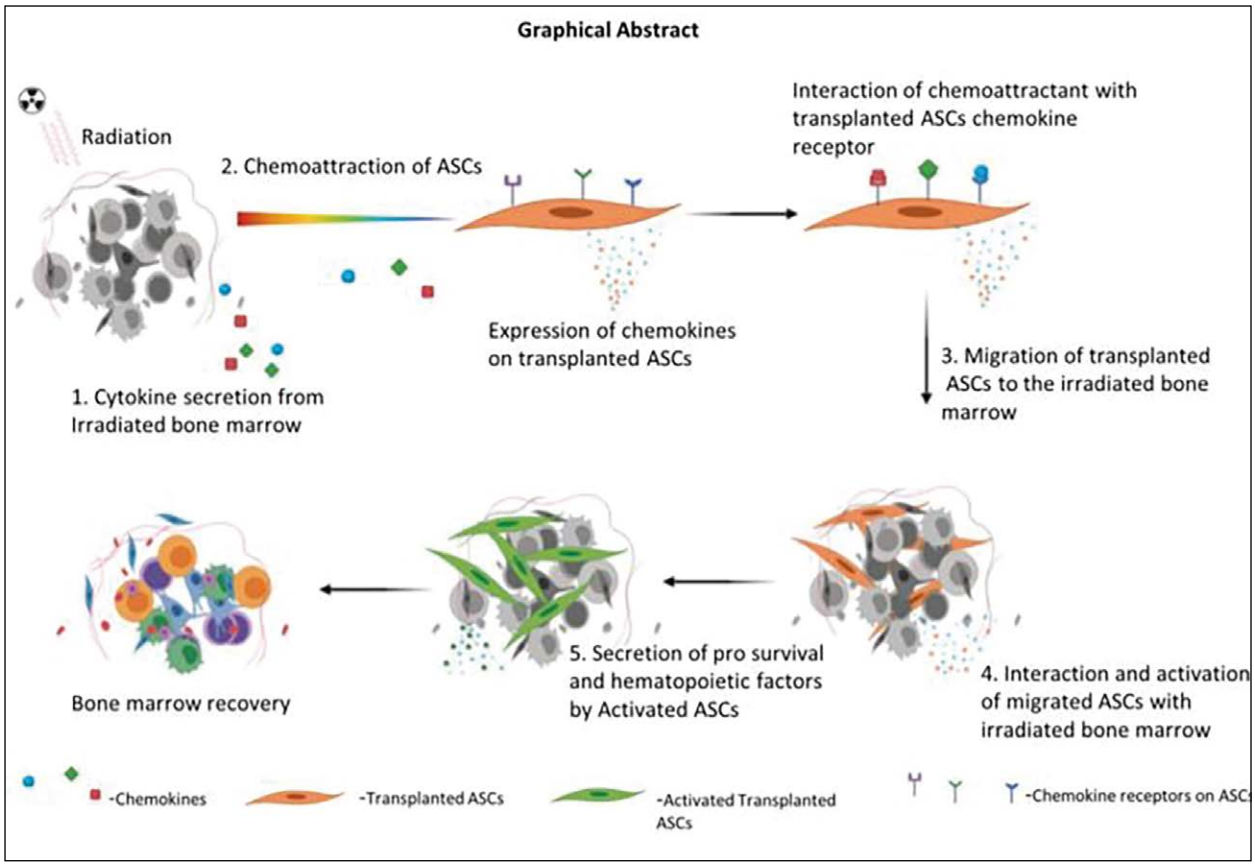

Figure 2 – *Allogeneic adipose-derived stem cells rescue irradiation bone marrow cells via secretion of pro-survival and hematopoietic factors. (URL: https://scitechdaily.com/in-case-of-nuclear-disaster-stem-cells-derived-from-fat-show-promise-as-a-treatment-for-mass-radiation-exposure/; date: 23.12.2022)*

Data emanating from the clinical studies have shown the safety and efficacy of allogeneic MSCs in adult and pediatric patients [12, 13]. Recent clinical application of allogeneic MSCs in ischemic cardiomyopathy patients vindicated these data and reported that allogeneic MSCs were as good as autologous MSCs in their functionality and efficacy, favorably affecting LV end-diastolic volumes, LVEF, and ventricular remodeling leading to improved quality of life [14, 15]. More importantly, these studies did not report any severe adverse reactions associated with the cell-based therapy with allogeneic cells, including immunologic responses. The safety profile of allogenic MSCs has also been substantiated during a systematic review and meta-analysis of 36 clinical studies, including 1012 participants [16]. Experimental studies assessing immunological profiling of MSCs have shown that although they are not immunopriviledged, allogeneic MSCs are weakly immunogenic, because they lack MHC class II and co-stimulatory molecules, i.e., CD40, CD80, and CD86, while they have weak MHC class I expression [17, 18]. Moreover, they do show immunomodulation by suppressing the activation and proliferation of immune cells [19, 20]. Their interesting immune profile tips them as a good candidate for cell-based therapy without the need for immunosuppression therapy and takes care of them not being «self» for the recipient [21]. These data about the allogeneic MSCs are a step forward towards the ongoing quest for «Universal donor cells,» which should be available off-the-shelf as a ready-to-use cell preparation [22]. One of the primary advantages of allogenic MSCs is their logistic superiority over autologous cells [23]. Unlike autologous MSCs, which need to be isolated, purified, and expanded in culture before use for each patient, allogenic cells are logistically feasible as they may be readily available off-the-shelf. This ready availability makes possible their use in urgent clinical situations, which is not possible with the autologous cells as it may take 3–4 weeks of isolation, purification, and expansion before they could be used for delivery. For any cell-based therapy to be of routine clinical significance as a therapeutic modality, it is imperative that the cells must be available off-the-shelf akin to any other conventional pharmacological agent. Additionally, allogeneic MSCs may allow repeated doses of the cells which may be more beneficial than one-time treatment [24].

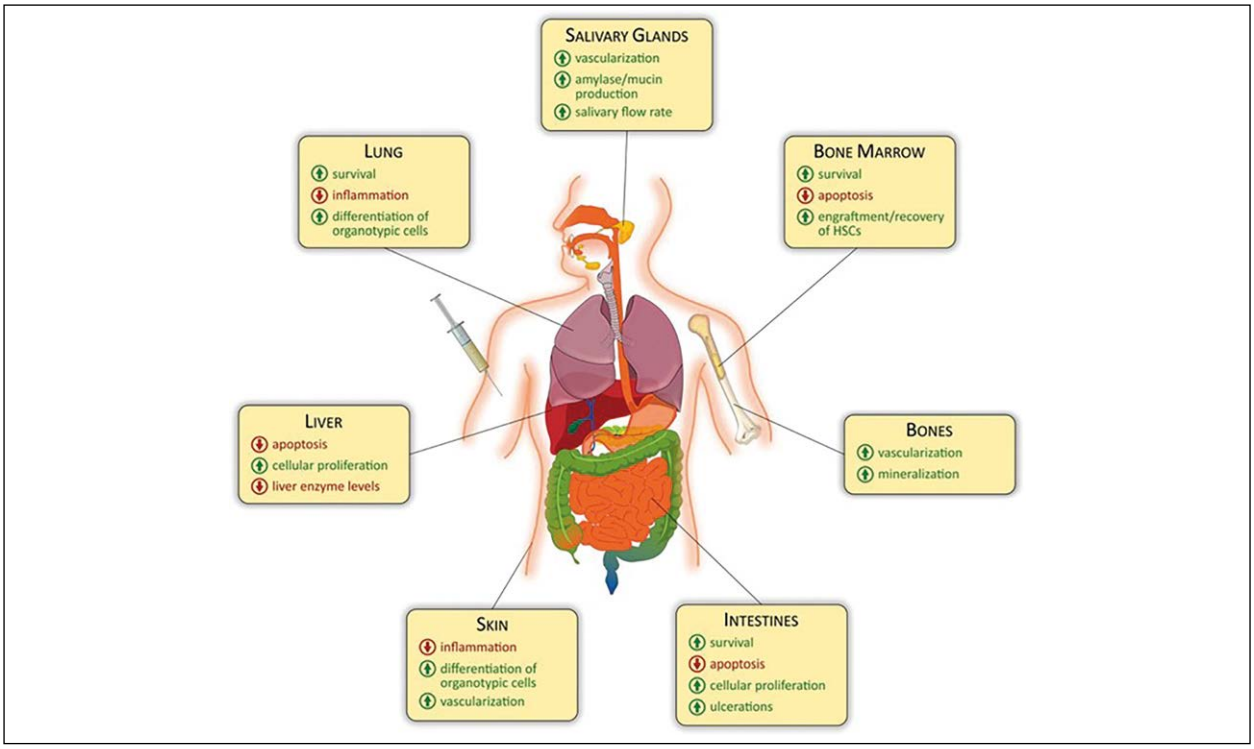

Figure 3 – *Organ tissue-specific beneficial effects of MSC treatment (the whole figure has been taken from [31])*

Treatment of radiation injuries. First, we will focus on the treatment of ARS. Events leading to the development of ARS can be a radiation accident like in Chernobyl (1986) or Fukushima (2011), a therapeutic misadventure, or nuclear weapon detonation during war or in a terroristic attack [25]. At present, few treatment options are available. Matched hematopoietic stem cell transplant is the therapy of choice. Although bone marrow/ peripheral blood stem cell transplantation is beneficial, maintenance of hematopoietic stem cells, radiation dose determination, and the lack of matched stem cell donor for allogeneic therapy applications are limiting factors. In a mass population exposure scenario, several tens (hundred) to millions of individuals can be exposed; from a practical standpoint, hematopoietic stem cell transfusion is an impossible way forward. In case such a scenario develops, it will be difficult to treat all individuals at the same time, and treatment will be delayed for many affected individuals, which may lead to an increased mortality rate. Other possible treatment options include administration of prohematopoietic cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte stimulating factor (G-CSF). In a recent paper «Allogeneic adipose-derived stem cells mitigate acute radiation syndrome by the rescue of damaged bone marrow cells from apoptosis» [26] the authors have shown that adipose tissue derived mesenchymal stem cells (ASC) could be effective in mitigating total body irradiation (TBI) induced ARS in mice and may be beneficial for clinical adaptation to treat TBI-induced toxicities. The possible explanation of the observed effect is shown in Figure 2.

In the author's opinion «the requisitioned data of the MSCs, mechanically taken from mice and rats, could negatively influence the trends of the research in the novel treatment(s) of human disease» [27]. However, on the other hand the author observed personally direct homing of i.v. applied MSC in the patient's bone marrow [28]. This experimental fact confirms the data obtained in mice: After systemic (i.e. intravenous) application the MSC in humans are (at least partially) homing in the bone marrow of the individual. Here we would like to suggest a possible way to treat the patient(s) with the ARS. The suggested approach is to apply intravenously up to 1 million (106) freshly prepared allogeneic MSC/kg body weight as soon as possible after the radiation injury (ARS). In our opinion this could rescue the damaged hematopoietic stem cells in the bone marrow. The treatment should be applied repeatedly in a span of few days, if possible. We understand that lack of experience can influence the results, nevertheless direct «trials» on a large cohort of patients are missing and it is not imaginable to perform them. Nevertheless, the accidental ARS could be used as «pilot studies». Considering the availability of allogeneic MSC, we repeat and are firmly standing

behind that «For any cell-based therapy, including the ASR treatment, it is imperative that the MSC must be available off-the-shelf akin to any other conventional pharmacological agent.»

The second possible use of MSC is treatment of the local radiation injuries. In [29] the authors present the treatment of four patients with chronically persistent chronic radiation injuries, who they suffer over a few decades, with autologous MSC. It should be noted that after local radiation (accidental) injury the deep anatomical structures are involved too: the bone, muscles, nerves, blood, and lymphatic vessels, and of course the complex structure – the skin. This makes the problem more complex.

In a publication «Cell technologies in the treatment of radiation burns: experience Burnasyan Federal Medical Biophysical Centre» [30] the authors demonstrated the treatment with the MSC of the patients with radiation burns. These patients received radiation therapy for their oncological diagnosis. The postradiational wounds («ulcers») were successfully treated with MSC in 3 patients. In a paper «Mesenchymal stem cells – A new hope for radiotherapy-induced tissue damage?» The authors suggest a possible beneficial role of the MSC in different organs of the human body (Figure 3) [31]. In other words, there is a convincing amount of data which show the effects of MSC in the treatment of radiation injuries. Moreover, the use of MSC in a «classical» wound healing is well documented. (For review see ref. [32]).

So, repeatedly: First – when should we to start the treatment? Second – should the treatment be local or systemic? Third – how often should the treatment be repeated? Our suggestions are as follows. Due to beneficial effects of MSC (Figure 3) one should apply the MSC intravenously as soon as possible after the local radiation injury. The recommended dose should be up to 1 million (106) freshly prepared allogeneic MSC/kg body weight. The other doses should be applied locally (around and in the irradiated area) rather early. The dose of MSC can be lower (we suggest 20 million cells). The repeated local application should occur in the two to four weeks interval(s). Due to the rather unconvincing effects of the MSC application in the radiological accident in Yanango, Peru[3], we are suggesting two points. The MSC should be applied rather early (as suggested above), and they must be prepared in the «spirit» of Good Biological Practice (GBP). We believe that the early («as soon as possible») and repeatable application of MSC can minimize the radiation damage. Of course, the later surgical reconstruction should be performed by an experienced surgeon and in the specialized center with a concomitant local MSC application.

[3] URL: https://www.iaea.org/publications/6090/the-radiological-accident-in-yanango (date: 22.12.2022).

Authors Contribution

Elaboration of the concept of the paper; collection, analysis, and systematization of scientific literature; writing and edition of paper / Разработка концепции статьи; сбор, анализ и систематизация научной литературы; написание статьи.

Conflict of interest statement

I am declaring that I prepared the article from sources freely available on the Internet and free available publications, figures, and other possible legal sources. I, as a sole author declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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References

1. Ferrari C., Manenti G., Malizia A. Sievert or Gray: Dose Quantities and Protection Levels in Emergency Exposure // Sensors (Basel). 2023. V. 23(4). P. 1918. https://doi.org/10.3390/s23041918

2. Macià I., Garau M., Lucas Calduch A., López E.C. Radiobiology of the acute radiation syndrome // Rep Pract Oncol Radiother. 2011. V. 16. № 4. P. 123–130. https://doi.org/10.1016/j.rpor.2011.06.001

3. Gourmelon P., Marquette C., Agay D. et al. Involvement of the central nervous system in radiation-induced multi-organ dysfunction and/or failure // Br. J. Radiol. 2005. Suppl. 27. P. 62–68. https://doi.org/10.1259/bjr/77700378

4. Gaugler M.-H. A unifying system: Does the vascular endothelium have a role to play in multi-organ failure following radiation exposure? // Br. J. Radiol. 2005. Suppl. 27. P. 100–105. https://doi.org/10.1259/bjr/24511652

5. Medical Management of Radiation Accidents / Eds Gusev I.A., Guskova A.K., Mettler F.A., Barabanova A.V. 2nd edn, Boca Raton: CRC Press, FL, 2001. 225 p.

6. Lakota J., Dubrovcakova M., Haider KH. Human Mesenchymal Stem Cells: The Art to Use Them in the Treatment of Previously Untreatable // In: Handbook of Stem Cell Therapy / Ed. Haider K.H. Springer, Singapore. 2022. https://doi.org/10.1007/978-981-19-2655-6_1

7. Koç O.N., Gerson S.L., Cooper B.W., et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy // J. Clin. Oncol. 2000. V. 18. № 2. P. 307–316. https://doi.org/10.1200/JCO.2000.18.2.307.

8. Musiał-Wysocka A., Kot M., Majka M. The pros and cons of mesenchymal stem cell-based therapies // Cell Transplant. 2019. V. 28. № 7. P. 801–812. https://doi.org/10.1177/0963689719837897

9. Horwitz E.M., Le Blanc K., Dominici M. et al. International Society for Cellular Therapy. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement // Cytotherapy. 2005. V. 7. № 5. P. 393–395. https://doi.org/10.1080/14653240500319234

10. Le Blanc K., Rasmusson I., Sundberg B. et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells // Lancet. 2004. V. 363. № 9419. P. 1439–1441. https://doi.org/10.1016/S0140-6736(04)16104-7

11. Yau T.M., Pagani F.D., Mancini D.M. et al. Cardiothoracic Surgical Trials Network. Intramyocardial injection of mesenchymal precursor cells and successful temporary weaning from left ventricular assist device support in patients with advanced heart failure: A Randomized Clinical Trial // JAMA. 2019. V. 321. № 12. P. 1176–1186. https://doi.org/10.1001/jama.2019.2341

12. Koç O.N., Day J., Nieder M., et al. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH) // Bone Marrow Transplant. 2002. V. 30. № 4. P. 215–222. https://doi.org/10.1038/sj.bmt.1703650

13. Horwitz E.M., Gordon P.L., Koo W.K. et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone // Proc Natl Acad Sci U S A. 2002. V. 99. № 13. P.8932–8937. https://doi.org/10.1073/pnas.132252399

14. Hare J.M., Fishman J.E., Gerstenblith G. et al. Comparison of allogeneic vs autologous bone marrow–derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial // JAMA. 2012. V. 308. № 22. P. 2369–2379. https://doi.org/10.1001/jama.2012.25321

15. Hare J.M., DiFede D.L., Rieger A.C. et al. Randomized comparison of allogeneic versus autologous mesenchymal stem cells for nonischemic dilated cardiomyopathy: POSEIDON-DCM Trial // J. Am. Coll. Cardiol. 2017. V. 69. № 5. P. 526–537. https://doi.org/10.1016/j.jacc.2016.11.009

16. Lalu M.M., McIntyre L., Pugliese C. et al. Canadian Critical Care Trials Group. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials // PLoS One. 2012. V. 7. № 10. P. e47559. https://doi.org/10.1371/journal.pone.0047559

17. Machado Cde V., Telles P.D., Nascimento I.L. Immunological characteristics of mesenchymal stem cells // Rev. Bras. Hematol. Hemoter. 2013. V. 35. № 1. P. 62–67. https://doi.org/10.5581/1516-8484.20130017

18. Lohan P., Coleman C.M., Murphy J.M. et al. Changes in immunological profile of allogeneic mesenchymal stem cells after differentiation: should we be concerned? // Stem Cell Res. Ther. 2014. V. 5. № 4. P. 99. https://doi.org/10.1186/scrt488

19. Asari S., Itakura S., Ferreri K. et al. Mesenchymal stem cells suppress B-cell terminal differentiation // Exp. Hematol. 2009. V 37. № 5. P. 604–615. https://doi.org/10.1016/j.exphem.2009.01.005

20. Corcione A., Benvenuto F., Ferretti E. et al. Human mesenchymal stem cells modulate B-cell functions // Blood. 2006. V. 107. № 1. P. 367–372. https://doi.org/10.1182/blood-2005-07-2657

21. Kariminekoo S., Movassaghpour A., Rahimzadeh A. et al. Implications of mesenchymal stem cells in regenerative medicine / Artif. Cells Nanomed. Biotechnol. 2016. V. 44. № 3. P. 749–757. https://doi.org/10.3109/21691401.2015.1129620

22. Kinkaid H.Y., Huang X.P., Li R.K., Weisel R.D. What's new in cardiac cell therapy? Allogeneic bone marrow stromal cells as "universal donor cells" // J. Card. Surg. 2010. V. 25. № 3. P. 359–366. https://doi.org/10.1111/j.1540-8191.2009.00984.x

23. Zhang J., Huang X., Wang H. et al. The challenges and promises of allogeneic mesenchymal stem cells for use as a cell-based therapy // Stem Cell Res. Ther. 2015. V. 6. P. 234. https://doi.org/10.1186/s13287-015-0240-9

24. Poh K.K., Sperry E., Young R.G., Freyman T. et al. Repeated direct endomyocardial transplantation of allogeneic mesenchymal stem cells: safety of a high dose, "off-the-shelf", cellular cardiomyoplasty strategy // Int. J. Cardiol. 2007. V. 117. № 3. P. 360–364. https://doi.org/10.1016/j.ijcard.2006.04.092

25. Dainiak N., Albanese J. Medical management of acute radiation syndrome // J. Radiol. Prot. 2022. V. 42. № 3. https://doi.org/10.1088/1361-6498/ac7d18

26. Chinnapaka S., Yang K.S., Samadi Y. et al. Allogeneic adipose-derived stem cells mitigate acute radiation syndrome by the rescue of damaged bone marrow cells from apoptosis // Stem Cells Transl. Med. 2021. V. 10. № 7.

P. 1095–1114. https://doi.org/10.1002/sctm.20-0455

27. Lakota J. Fate of human mesenchymal stem cells (MSCs) in humans and rodents-Is the current paradigm obtained on rodents applicable to humans? // J. Cell Mol. Med. 2018. V. 22. № 4. P. 2523–2524. https://doi.org/10.1111/jcmm.13561

28. Lakota J., Gocarova K., Spanik S. Treatment of metastatic head and neck cancer with mesenchymal stem cells combined with prodrug gene therapy // Exp. Oncol. 2015. V. 37. № 4. P. 298. PMID: 26710845.

29. Akita S., Yoshimoto H., Ohtsuru A. et al. Autologous adipose-derived regenerative cells are effective for chronic intractable radiation injuries // Radiat. Prot. Dosimetry. 2012. V. 151. № 4. P. 656–660. https://doi.org/10.1093/rpd/ncs176

30. Kotenko K.V., Eremin I.I., Moroz B.B. et al. Cell technologies in the treatment of radiation burns: experience Burnasyan Federal Medical Biophysical Centre // Genes & Cells. 2012. V. 7. № 2. P. 97–102. https://doi.org/10.23868/gc121646

31. Nicolay N.H., Lopez Perez R., Debus J., Huber P.E. Mesenchymal stem cells – a new hope for radiotherapy-induced tissue damage? // Cancer Lett. 2015. V. 366. № 2. P. 133–140. https://doi.org/10.1016/j.canlet.2015.06.012. Epub 2015 Jul 9. PMID: 26166559.

32. Haider K.H. Handbook of Stem Cell Therapy, Springer Nature Singapore, 1st ed., 2022. ISBN 978-981-19-2654-9. https://doi.org/10.1007/978-981-19-2655-6

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Лечение лучевых поражений мезенхимальными стволовыми клетками

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Острый лучевой синдром (ОЛС) – острое заболевание, вызванное воздействием на организм высокой дозы ионизирующего излучения. ОЛС представляет собой детерминированный эффект радиационного облучения всего тела или значительного объема тела (частичное облучение тела) выше пороговой дозы около 1 Гр (грей). К массовому развитию ОЛС у людей могут привести радиационные аварии, такие как произошли в Чернобыле (1986 г.) и Фукусиме (2011 г.), и, что сегодня уже нельзя игнорировать – применение ядерного оружия в ходе боевых действий или в результате теракта. *Цель работы* – внедрить новый метод постлучевого лечения – использование аллогенных мезенхимальных стволовых клеток (МСК). *Материалы и методы.* Исследовалась информация, содержащаяся в специализированных научных журналах, находящихся в свободном доступе и доступных через глобальную сеть «Интернет». *Обсуждение результатов.* В сценарии массового облучения населения, когда облученными могут оказаться от нескольких десятков (сотен) до миллионов человек, традиционно используемое в таких случаях переливание гемопоэтических стволовых клеток окажется невозможным. МСК способны дифференцироваться в специализированные клетки, то есть превращаться в клетки различных органов и тканей. Для практических применения существует два основных источника их выделения и размножения *ex vivo* – костный мозг и жировая ткань. К настоящему времени показано, что МСК, полученные из жировой ткани, могут быть эффективными в смягчении последствий острой лучевой

болезни. МСК способны наводиться в костный мозг и частично восстанавливать его функцию. В локальные лучевые поражения вовлекаются и глубокие анатомические структуры: кость, мышцы, нервы, кровеносные и лимфатические сосуды и кожа. Имеется убедительный объем данных, свидетельствующих об эффектах МСК при их применении для лечения таких поражений. Это объясняется тем, что МСК способны дифференцироваться в те анатомические структуры, в которые они попадают. Основное преимущество аллогенных МСК перед аутологичными – логистическая доступность Их можно наработать заранее количествах и хранить в замороженном виде. После оттаивания клетки необходимо культивировать не менее 48 ч во влажных инкубаторах с добавлением 5 % CO_2. *Выводы.* Лечение МСК необходимо начинать как можно раньше после лучевого воздействия. Спасение поврежденных гемопоэтических стволовых клеток в костном мозге может быть достигнуто многократным введением внутривенно до 1 млн (10^6) свежеприготовленных аллогенных МСК/кг массы тела. Локально (вокруг и в области облучения) доза МСК может быть ниже – 20 млн клеток. Повторное местное применение следует проводить с интервалом от двух до четырех недель. Последующая хирургическая реконструкция должна выполняться опытным хирургом и в специализированном центре с сопутствующим местным применением МСК.

Ключевые слова: клетки костного мозга; клиническая практика; лечение мезенхимальными стволовыми клетками; лучевая болезнь; лучевые поражения; мезенхимальные стволовые клетки; радиационная авария; радиационные эффекты.

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Информация о конфликте интересов

Я заявляю, что подготовил статью из источников, находящихся в свободном доступе в Интернете, а также свободно доступных публикаций, рисунков и других возможных легальных источников. Я, как единственный автор, заявляю, что исследование проводилось при отсутствии каких-либо коммерческих или финансовых отношений, которые могли бы быть истолкованы как потенциальный конфликт интересов.

Сведения о рецензировании

Статья была рецензирована двумя экспертами в соответствующей области. Рецензии доступны в редакции и в базе данных Российского индекса научного цитирования.

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