Treatment of late radiation-induced cytopenia with hematopoietic stem cells

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Abstract. Hematopoiesis is one of the most radiosensitive system of the human organism. The intensity of effects induced by chronic radiation exposure depends on dose and dose rate. Completeness and duration of hematopoiesis recovery are determined by the intensity of changes occurring during the maximal radiation exposure and on individual radiosensitivity. Long-term (months, years) irradiation even during low dose rate exposure may lead to decrease of compensation and adjustment mechanisms and failure of adaptation. Exhaustion of abilities to adaptation and cumulation of radiation injuries in tissue cells comes when processes of alteration prevail in conditions of chronic radiation exposure. This effect is supposed to be a consequence of reduction of reparatory DNA synthesis during long-term exposure and exhaustion of anti oxydative potential of the cell. Physiological loss of mature cells takes place in normal circumstances of organism functioning. This process could be compensated insufficiently under chronic radiation exposure as a result of reduction of highly radiosensitive stem and progenitor cells potential. Thus, in late period after the onset of chronic irradiation disturbance of organ (tissue) functioning can occur. Not organic changes (i.e. RBM hypoplasia, vascular insufficiency) lay in the basis of these processes but limited capacities of physiological regeneration of tissues as in consequence of stem cell pool exhaustion. Currently, the use of hematopoietic stem cells for treatment of the above-mentioned pathologic conditions is very perspective. Clinical trials approbation of the methods for hematopoietic stem cell therapy of hematologic disorders in chronically exposed population of Techa riverside villages is conducted at the URCRM.

KEYWORDS chronic radiation exposure, late effects, hemopoiesis, cytokines, growth factors, hematopoietic stem cell, treatment

Hematopoietic system is well adapted to chronic radiation exposure at low doses [1, 2]. People in some areas of Ramsar, a city in northern Iran, receive an annual radiation absorbed dose from background radiation that is up to 260 mSv, have no changes in blood count, Hemoglobin level and other parameters of hematopoiesis in comparison with unexposed people [3]. “Mayak” PA workers who underwent chronic γ-irradiation had no decrease in blood cell counts at dose rate less then 0.25 Gy/year. Thrombocytopenia and unsteady leucopenia were registered among them at dose rates of 0.25-0.5 Gy/year and total dose 1.5-2.0 Gy. Persistent thrombocytopenia and leucopenia were marked at dose rates more than 0.5 Gy/year [4]. Lymphopenia was registered at annual doses of over 2 Gy and total doses over 6 Gy [5].

Recovery of hematopoiesis in “Mayak” PA workers was going on for several decades [5]. In 20% of cases moderate leucopenia was still observed in blood 35-40 years after exposure at total doses of 2.0–9.33 Gy (annual dose more then 1 Gy) [6]. Red bone marrow (RBM) hypoplasia accompanied by depression of all hematopoietic lineages and lethal outcomes developed at dose rates over 4.5 Gy/year and total dose over 8 Gy [6].

The decrease in thrombocytes, neutrophiles and lymphocytes in people who underwent long term external γ- and internal irradiation on the Techa River was marked at RBM doses 0.3-0.5 Gy/year an higher [7] and continued for several years [8].

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Complex rearrangement of hematopoiesis with involvement of different mechanisms of adaptation take place during chronic radiation exposure. Under chronic exposure the hematopoietic system can maintain a sufficient level of blood cells through their increased production as a result of cell cycle and maturation time shortening [9], reinforcement of early precursors (stem and progenitor cells), proliferation activity [10], extra-medullary hematopoiesis development [2]. Besides, increased repair of sublethal injuries of bone marrow precursors is registered in radio-adapted experimental animals [1].

The quantity and quality of surviving hematopoietic stem cells (HSC) after irradiation are critical for hematopoiesis recovery. Recoverable injury (i.e. spontaneous hematopoietic recovery is possible) is observed if more than 5% of stem and progenitor cells remain intact for replication and differentiation. If their level after irradiation decreases below critical, the hematopoietic system becomes depleted due to insufficient proliferation and differentiation [2].

The key factor which stimulates proliferation of resting HSC is decrease in their amount. Hematopoietic microenvironment plays an important role in regulation of polypotent hematopoietic precursors. It interacts with HSC and supports stability of their quantity in normal conditions and provides its restoration in case of injury. Intensification of stem cells proliferation is revealed starting from doses 0.2-0.3 Gy [10]. The mechanisms of the influence of microenvironment on hematopoiesis under chronic radiation exposure has not been adequately investigated.

The experimental findings established that dose rate determines the depth of stem cells pool depletion and influences greatly the speed and completeness of its recovery. Proliferating, maturing and functional pools as well as HSC were recovering slower after irradiation at lower daily doses, than at higher dose rates at comparable levels of total doses [10]. This phenomenon is associated with the formation of great amount of disintegration products at high dose rates. These products are composed of stimulators of hematopoiesis which provoke an increase in the concentration of erythropoietin, leucopoietin, thrombopoietin in blood. They contribute to acceleration of differentiation of committed cells and proliferation of stem cells [9]. However, after chronic radiation exposure as a rule their amount does not recover to normal levels up to the end of life of experimental animal.

T.M.Fliedner et al. (1994) showed that doses below 2 sGy/day do not induce granulocytopenia in dogs during 8 years of exposure, at the same time the increase of dose rate up to 10 sGy/day causes hematopoietic insufficiency for a short period. Experiments with beagles testify that prolonged exposure at dose over 7.5 sGy/day leads to death of 60% of exposed dogs from aplastic anemia. The level of leucocytes, thrombocytes and erythrocytes suppression which could disturb vital functions of immunity, hemostasis and oxygen supply of tissues was not observed at doses less than 3.75 sGy/day. Leucocytes and thrombocytes suppress linearly (without a threshold), at the same time the erythrocyte response was nonlinear with a rather wide threshold [1]. Correlation between level of HSC suppression, its recovery and following pathology of hematopoiesis and individual survival of animals was established. Adaptation to chronic radiation exposure is determined by changes of radiosensitivity of RBM precursors. Its concentration in short lived dogs susceptible to aplastic anemia progressively declined in the course of irradiation to the level incompatible with resumption of myelopoiesis functions [12].

Other adaptation mechanism is development of extramedullar hematopoiesis connected with HSC migration from RBM to spleen, liver and lymph nodes. During the irradiation, RBM could be gradually replaced by fibrous tissue. It leads to deficiency of hematopoietic cells and stromal elements (reticular and endothelial cells) which support replication, proliferation and differentiation of HSC [2].

Internal irradiation of RBM with $^{90}$Sr (37 kBc/day and more intake) during lifetime of white outbred rats leads to progressive decrease in blood leucocytes counts. The radioresistance of erythrocytes was manifest relative to other hematopoietic lineages [13].
Mechanisms of adaptation to chronic radiation exposure are still insufficiently studied in spite of sizeable amount of publications [14]. It is suggested that the complex of processes at different levels of organism (cell, tissue, organ, organism) increases radioresistance of cells and tissues and takes part in adaptation. Two stages are supposed to develop during adaptation: initial stage (urgent, but incomplete adaptation) and long-term stage of adaptation. Urgent stage of adaptation begins directly after the irradiation and can only be realized on the basis of physiological mechanisms developed earlier (e.g. essential level of antioxidants, physiological reserve of functional cells). The basis of long-term adaptation is laid by repair, regeneration and compensatory processes, which are realized through cell (tissue) responses [15]. The latter include: inactivation of active forms of oxygen, induction of DNA repair, increase in cell proliferation, shortening of cell cycle period, increase in radioresistance of cell populations, elimination of damaged cells by means of apoptosis and immune reactions.

Basic mechanisms of cells adaptation to chronic radiation are determined by: stimulation of DNA repair systems [16]; new proteins synthesis induction (gene expression effect is mediated through protein kinase C and nuclear factor κB) [14, 17] in cells of critical organs; activation of radio protective systems (endogenous stress proteins synthesis - HSP70, HSP72; metallothioneins; antioxidants – glutathione, superoxiddismutase, catalase and others), which lead to increase in cell radioresistance (including stem cells) [18-20].

It is shown that selection of radioresistant cells is very important for maintenance of cell homeostasis [20], arrest of apoptosis, increase in cell proliferative activity (including stem and progenitor cells pool) which allows replacement of damaged and dead cells. Induction of adaptive reactions in cells, excluding apoptosis, decreases with dose of low-LET ionizing radiation [14].

Reactions of the organism as a whole to chronic radiation exposure correspond to classical conceptions about general adaptation syndrome (GAS). In response to chronic exposure, changes typical of GAS develop in the hypophysis – adrenal cortex system. That leads to an increase in ACTG and steroids synthesis. A special role in development of GAS during chronic radiation exposure is played by the central nervous system [21].

Long-term (months, years) irradiation even at low dose rates may lead to a decrease in compensation-adjustment mechanisms and failure of adaptation. Reduced abilities to adaptation and cumulation of radiation injuries in tissue cells is observed when the processes of alteration prevail in the conditions of chronic radiation exposure. In people chronically exposed on the Techa River in wide range of doses (RBM doses 0.2-2.05 Gy) a decreased capacity of lymphocytes to induce adaptive response was registered 48-52 years after the onset of the exposure [22]. This effect is supposed to be a consequence of reduction of reparatory DNA synthesis during long-term exposure [23] and exhaustion of antioxidative potential of the cell [24].

Physiological loss of mature cells takes place under normal conditions of organism functioning. This process can be insufficiently compensated under chronic radiation exposure as a result of a reduction in highly radiosensitive stem and progenitor cells potential. Thus, in late periods after the onset of chronic irradiation disturbance of organ (tissue) functioning can occur for which the basis is laid not so much by organic changes (i.e. RBM hypoplasia, vascular insufficiency) as by limited capacities of physiological regeneration of tissues due to depletion of the stem cell pool.

At present, one of the actively developing fields of radiation medicine involves a search of therapeutic methods for stimulating hemopoiesis regeneration at different times after radiation exposure [25-27]. The results of experimental works [28] show that injected grows factors of microenvironment and HSC can promote maintenance vitality of HSC by suppression of gene p53 expression. Radiosensitive tissues are characterized by substantial level of p53 gene expression [29]. This gene is a key unit in apoptosis induction after radiation induced DNA damage [30]. The universality of this mechanism is confirmed in [31], where the role of p53 in hematopoietic tissue recovery after 5-fluorouracil is estimated. Neutralization of p53 expression promotes hematopoietic recovery (more able to
repopulation and clonogenically active HSC are registered) prohibits from the exhaustion of HSC pool, reduces sensitivity of HSC to apoptosis induction and intensifies proliferative response to growth factors acting in situ.

One of those factors is thrombopoetin – physiological regulator of thrombopoiesis. At the early stages of hematopoiesis it influences growth of multipotent progenitor and stem cells [32-34]. Another growth factor – c-kit manifested its activity in suppressing apoptosis of hematopoietic cells in cases of absence of their proliferative activity, by reducing their ability to differentiate. Also, this cytokine supports the viability and self renewal of hematopoietic precursor cells. Moreover, a great reduction in peripheral blood CD34-cells apoptosis under the influence of a combination of thrombopoetin and c-kit is registered [28]. The application of thrombopoetin and flt-3 composition maintain an appropriate level of the quantity of CD34+Thy1+ phenotype cells [35]. Material presented in [28, 36-38] shows that regulation through receptors to thrombopoetin and flt-3 is of great significance at the early stages of hematopoiesis as it influences viability, self renewal and proliferation of HSC.

In addition to antiapoptotic effect, growth factors have other properties (angiogenic, antioxidant, mitogenic), which contribute to hematopoietic recovery [27, 39].

Search for the approaches to investigation of the mechanisms of action of different grows factors and their therapeutic effectiveness for correction of late effects of radiation exposure is a very promising line of research [25, 26].

One of the tasks in the context of this tendency is getting a source of cytokines and growth factors for therapeutic application. One of the essential sources of such kind of substances is donor’s cells and tissues of different origin [40-43]. Nowadays, the syngenic RBM of genetically identical twin, allogenic RBM of HLA compatible donor, autologous RBM are widely used. More and more reports of using alternative sources (peripheral HSC, placental/umbilical cord blood) are reported [43, 44].

An ideal donor of RBM is an identical twin. However such an opportunity is a very rare occasion. If the relatives have no HLA identical donor, transplantation from unrelated HLA-matched donor could be performed. However, due to the polymorphicity of HLA (MHC) system the probability to find an HLA compatible unrelated donor is rather low. The probability to find an HLA-matched donor accounts for about 30-40% of recipients. An average period of searching for a donor is about 135 days (USA, Western Europe), and sometimes the patient does not live up to transplantation. For ethnical minorities the probability of finding of HLA-matched donor is reduced to a minimum [44].

One of the common methods is the application of the patient’s autologous RBM and peripheral blood stem cells. The major advantage of this method is the absence of incompatibility of donor and recipient, but it has some difficulties associated with necessity to increase the number of stem and progenitor cells by growing them ex vivo following reinfusion of the obtained cell suspension. This procedure is rather labor-consuming. There is also risk of transformation of cells during cultivation on media [45]. The problems mentioned above dictate the necessity to continue the search for HSC source.

In the recent years placental/umbilical cord blood HSC are widely applied in hematological practice. The content of early committed progenitor hematopoietic cells of all lineages and their proliferative potential is significantly higher than in peripheral blood or in RBM of an adult. At the same time the use of HSC of this origin is accompanied by different schemes of myeloablative conditioning for preventing rejection of transplantat, its grafting, since immunological characteristic of placental/umbilical cord blood HSC does not differ from an adult HSC. In most cases, such HSC have mature antigens of major histocompatibility complex and are well recognized by immunocompetent cells of recipient. HSC of placental/umbilical cord blood fit ideally for autotransplantation [46, 47].

One of the directions of the search for HSC source is investigation of the possible use of embryonic and fetal stem cells as a transplantation material. It is known that embryonic stem cells (ESC) have a capacity for long term reproducibility in culture on a feeder layer and according to defined physiological and/or experimental conditions can differentiate in any type of cells. In connection with
the perspectives for its application are very wide, but there are some difficulties in their cultivating and practical using [48]. ESC could be obtained from epiblast of embryo or by the somatic cell nucleus transfer into the cytoplasm of ovary cell. The hematopoietic differentiation of ESC should be induced before their application, otherwise there exists a risk of incorrect differentiation or development of teratomas. These procedures are labor-consuming and, consequently, expensive [49, 50].

Fetal derived hematopoietic cells have some properties which allow us to consider them as a perspective source of hematopoietic and mesenchymal stromal progenitor cells MSC. First, majority of fetal cells have poorly expressed antigens of MHC which reduces dramatically decrease the level of posttransplantation complications [51-55]. Second, 95% of fetal liver hematopoietic stem cells are represented by differentiated and premature elements; considerable amount of them are non-differentiated blasts of myeloid progenitors [56]. Relative content of committed stem cells of myeloid line in fetal liver is higher than in RBM of an adult [57]. After the injection into the blood fetal liver HSC can exert a regulatory influence on the processes of hematopoiesis. Fetal hematopoietic cells are able to enhance the immature hematopoietic cell pool and increase the proliferative potential of hematopoiesis [58]. Third, fetal liver derived cells work out a wide spectrum of cytokines and growth factors (hemopoietins) which ensure full-scale stimulation of adult organism hematopoietic activity [59]. Application of HSC and MSC as a microenvironment can form a coordinated network – so-called “niche”, where nourishment, differentiation and proliferation of hematopoietic cells. Intercellular communication inside the niche and with the surrounding tissues is implemented by means of signals of appropriate growth factors and cytokines [60]. These signals also can activate hematopoiesis of the recipient.

Fetal liver derived cells can greatly improve adaptive capacities in patients suffering from cytopenia by reinforcement of the processes of regeneration of hematopoiesis. Fetal liver tissue of 17-24 weeks of gestation consists entirely of progenitor cells with high potential of migration and repopulation [56, 57, 61].

The approaches to the therapy of late radiation-induced cytopenia with HSC in people chronically exposed to radiation on the Techa River is currently being developed and tested.

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