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CYTOLOGICAL EFFECTS IN BONE MARROW CELLS IN EXPERIMENTAL ANIMALS UNDER CHRONIC LOW-INTENSITY γ -IRRADIATION

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Owing to irradiation in cells occur damage in the genome, resulting into the cell death, or, in the case of intracellular repair system activation and a development of adaptation - to survive. A result of exposure mainly depends on the state of prooxidant-antioxidant homeostasis. We found that chronic low-intensity γ–irradiation causes an increasing in the number of abnormal cell and expand the range of abnormalities in the granulocyte lineage of bone marrow. In erythroid lineage spectrum of abnormal cells decreased, but in this case increased frequency of abnormal cell shape.

Keywords: chronic low-intensity γ-rays, bone marrow, granulocyte lineage, erythroid lineage.

Under conditions of exposure of different species of animals to low doses of ionizing radiation, the stimulation effects have been detected, which lie in the phenomenon of compensatory intensification of physiological functions and serve as the indirect evidence of the induction of changes in the genetic apparatus [3]. Hence, such effects caused by low doses, expressed in the form of adaptive responses of cells, may be triggered by the activation of the inducible reparation systems. Based on such changes in the irradiated cells the probability of fixation of DNA damages in true mutation is altered. According to S.A. Heras'kyn [2], this approach does not require postulation of new cellular reactions for its justification and can uncontroversially explain all phenomena, which take place after the exposure to low doses of ionizing radiation.

It is suggested that among the heterogeneous pool of cells, that comprise an evolutionary reserve, there is a programmed subpopulation, the death of which is preceded by genetically determined cellular responses - auto-induction of chromosomal abnormalities or other structural genome realignments. This leads to the emergence of new genetic variants and makes possible the selection of variants, which will be the most adapted to the alterations in the environment or to the potentially expected new stimuli. The number of cells derived from this population, which are in the process of genetic adaptation and are exposed to low dose of ionizing radiation, will depend on the population size [10, 11].

Due to radiation-associated genomic damages, the cells can go through one of the following two ways: 1) cell death, including apoptosis, and 2) their survival, combined with a) the emergence of mutations in the cell progeny, b) the adaptation and return of the cells to their initial state, and c) with functional changes, caused by adaptation processes, which make the cells

differ from those that were prior to the radiation exposure. Selection of the possible consequences of the exposure to radiation essentially depends on the state of the key elements of the redox homeostasis [1].

The objective of this study was a cytological characterization and estimation of the spectrum of abnormalities in bone marrow cells in CBA line mice under chronic γ-radiation exposure at a dose-rate of 1 cGy/day.

Materials and methods

The experiment was held on 80 mice (line CBA), whose weight was in the range of 24–26g and who aged 90 days prior to the beginning of the research. The laboratory experiment was conducted in the Urals Research Center for Radiation Medicine (Chelyabinsk).

The source of gamma-rays was a modified OCK-400 device with a charge of ^{137}Cs [8]. Exposure of the experimental animals was carried during the day and at night, except for the time for cleaning of cages and examination of the animals (which did not exceed 1 hour). The total tissue-absorbed doses in the period of the study (30, 90, 180 and 270 days) of chronic external γ-radiation with a dose-rate of 1 cGy/day varied from 0.3 to 2.7 Gy.

Cytological methods of bone marrow cells research. Fixation and staining of bone marrow specimens were carried out by generally accepted Romanovsky-Giemsa method (1968). Differential ratio of cells, the mitotic index and the index of maturation in the myeloid and erythroid lineage were analyzed [5, 7]. The various pathological changes in the bone marrow cells, which are associated with mitosis (chromosome fragments, chromosome and cytoplasmic bridges, polynuclear and giant cells were taken into account.

Statistical analysis of the research outcomes. For comparison of the average values in the analysis of hematological data Student's t-test was applied. The authenticity of differences of cytogenetic indices in bone marrow cells were assessed by the chi-squared and Fisher criteria [4, 6].

Results and discussion

Results of cytological indices research of bone marrow cells in intact CBA line mice (Table 1, Table 2) indicated that the mitotic index of normocytes was not changed with age, and the mitotic index of neutrophils decreased 5 times by the time the experimental animals reached 12 months. Chronic γ-radiation at a dose-rate of 1 cGy/day was accompanied by a three-time decrease in the mitotic activity of neutrophils on the 180th day of exposure to radiation, and the mitotic index of normocytes dropped twice: on the 30th and 270th day of the experiment by 2,6 and 6,6 times respectively.

Maturation index of normocytes in irradiated mice does not differ from the same age control values during all periods of the experiment, and the maturation of neutrophil index increased 1,5–2,5 times under the total absorbed doses 1,8–2,7 Gy.

Table 1

Cytological indices of bone marrow cells in CBA mice under chronic g-radiation at a dose-rate of 1 cGy/day

Duration of exposure to radiation, days		Mitotic index of neutrofils (%)	Mitotic index of normocytes (%)	Maturation index of neutrofils	Maturation index of normocytes
30	Control	1,45±0,56	3,24±0,86	1,76±0,40	0,80±0,02
	1 cGy/day	0,62±0,61	1,22±0,93*	2,59±0,35	0,76±0,00
90	Control	0,81±0,42	2,60±0,93	2,78±0,60	0,82±0,03
	1 cGy/day	0,60±0,32	1,89±0,53	3,77±1,38	0,80±0,03
180	Control	1,25±0,34	2,19±0,51	3,63±0,40	0,78±0,02
	1 cGy/day	0,38±0,09*	2,01±1,09	5,74±0,08*	0,82±0,02
270	Control	0,29±0,21	2,78±0,43	2,85±0,29	0,77±0,01
	1 cGy/day	0,71±0,24	0,60±0,53*	7,24±1,77*	0,82±0,03

Notes: Data are means M±m; *– significantly different from control (P<0.05).

It can be assumed, when the index of neutrophils maturation is increased and the mitotic indices of neutrophils and normocytes (in the period during 180–270th day of exposure to radiation) are decreased on the background of control level of karyocytes of bone marrow [9], the compensatory reactions occurred in the neutrophil series. These reactions are directed on maintaining of the repopulation process of granulocyte lineage development in bone marrow of irradiated animals.

Irradiation with a dose-rate of 1 cGy/day had no effect on leuko/erythroid correlation in bone marrow (Table 2): during the experiment, the fraction of erythroid and granulocyte blast cells was almost unchanged in comparison to control values. Based on the state of leuko/erythroblastic correlation, the kinetics of hematopoietic cells of these lineages repopulation can be evaluated. The obtained results show the lack of competitive interconnection between the erythroid and granulocyte lineages under the total absorbed dose in the range of 0,3 to 2,7 Gy.

Table 2

Cytological indices of bone marrow cells in CBA mice
under chronic g-radiation at a dose-rate of 1 cGy/day (continuation)

Duration of exposure to radiation, days		Leuko/erythroblastic correlation	Fraction of leukocytes in general amount of cells
30	Control	0,75±0,03	3,24±0,86
	1 cGy/day	0,71±0,04	1,22±0,93*
90	Control	0,73±0,04	2,60±0,93
	1 cGy/day	0,77±0,04	1,89±0,53
180	Control	0,76±0,02	2,19±0,51
	1 cGy/day	0,75±0,02	2,01±1,09
270	Control	0,73±0,01	2,78±0,43
	1 cGy/day	0,72±0,01	0,60±0,53*

Notes: Data are means M±m; *– significantly different from control (P<0.05).

Cytological analysis detected a statistically reliable increase in the fraction of blast cells in granulocytic series in the bone marrow by 80% on the 30th day of chronic γ -radiation under a total cumulative dose of 0,3 Gy (Table 3). Herewith, the fraction of abnormal neutrophils decreased 2,6 times, in comparison to the same age control. Increase in the number of abnormal cells in the catena of neutrophils by 1,5–2 times was observed starting from the 180th day of the experiment (the total absorbed dose made 1,8–2,7 Gy).

Table 3

The fraction of cells with abnormalities and number of blast cells in the bone marrow
of CBA mice under chronic g-irradiation at the dose-rate of 1 cGy/day

Duration of exposure to radiation, days		Fraction of cell abnormalities in myeloid series (neutrophils), %	Fraction of cell abnormalities in erythroid series (normocytes), %	Number of blast cells of myeloid series, %	Number of blast cells of erythroid series, %
30	Control	0,88±0,15	0,55±0,11	1,43±0,17	0,78±0,06
	1 cGy/day	0,34±0,07*	0,85±0,32	2,61±0,21*	1,38±0,40
90	Control	0,55±0,11	0,66±0,23	1,81±0,54	0,60±0,17
	1 cGy/day	0,73±0,08	0,46±0,12	2,39±0,35	0,95±0,23
180	Control	0,41±0,14	0,38±0,26	3,28±0,55	1,22±0,19
	1 cGy/day	0,83±0,11*	0,53±0,08	2,46±0,44	1,01±0,17
270	Control	0,60±0,08	0,71±0,19	3,03±0,32	1,45±0,12
	1 cGy/day	0,93±0,13*	0,94±0,57	3,86±0,16	1,31±0,28

Notes: Data are means M±m; *– significantly different from control (P<0.05).

The spectrum of cytological abnormalities in granulocyte and erythroid lineages of the bone marrow in intact CBA mice is presented in Tables 3 and 5. Analysis of cytological abnormalities in ***granulocyte lineage*** of the bone marrow in control animals revealed no age devia-

tions. The data in Table 4 show that in the proliferative pool of granulocytic lineage the most frequently detected forms of affected cells are promyelocytes and myelocytes – namely binucleated and giant forms. For the aging pool are typical giant and hypersegmented cells. In the population of stab neutrophils no cells with dysplastic changes were detected.

Table 4

The fraction of cells with abnormalities in granulocyte lineage in the bone marrow of intact mice (line CBA) (absolute number of cells/10^3 karyocyte)

Pool of cells	Micronucleus	Concatenated metaphase	Binuclear cells	Giant cells	Hypersegmentation
Proliferative:	0,25±0,02	0,57±0,09	1,83±0,28	0,76±0,21	--
- myeloblasts	0,06±0,01	--	0,06±0,04	0,125±0,09	--
- promyelocytes	0,06±0,01	0,06±0,03	0,42±0,12	0,38±0,13	--
- myelocytes	0,13±0,02	0,51±0,05	1,35±0,11	0,25±0,04	--
Metamyelocytes	0,06±0,01	--	--	0,38±0,07	--
Stab neutrophils	--	--	--	--	--
Segmented neutrophils	0,06±0,01	--	0,06±0,02	--	0,19±0,03

Notes: Data are means M±m.

In irradiated CBA mice significant changes in the number of abnormal cells of granulocyte lineage in bone marrow were not detected. Similary to the control group, abnormal cell forms were predominantly detected among promyelocytes and myelocytes, however the range of cell abnormalities increased: cells with the presence of cytoplasmic bridge, the telophase bridge, and karyorrhexis appeared (Table 5). Quantitative and qualitative analysis of abnormal cells of neutrophil group depending on the total absorbed dose allows to state the following. With the increasing of absorbed dose (1,8–2,7 Gy) the number of cells with chromosomal aberrations in the proliferating pool increase, herewith the spectrum of abnormal cells expands. In the maturing pool of irradiated mice cytological pathologies in the form of giant forms and hypersegmented neutrophils were identified primarily on the 180–270 days of radiation exposure.

Table 5

The fraction of cells with abnormalities in granulocyte lineage in the bone marrow of CBA mice under chronic γ - irradiation at a dose-rate 1 cGy/day (absolute number of cells/10^3 karyocyte)

Pool of cells	Micro-nucleus	Concatenated metaphase		Kario-reksys	Binucleated cells				Giant cells				Hypersegmented		
	Duration of exposure, month														
	3	3	9	6	1	3	6	9	1	3	6	9	3	6	9
Proliferative pool, among the,:	0,70± 0,13	0,70± 0,19	0,52± 0,09	0,87± 0,26	2,07± 0,39	3,49± 0,61	3,93± 0,82	2,08± 0,23	0,69± 0,17	1,05± 0,17	0,87± 0,11	5,21± 0,83	–	–	–
- myelo-blasts	–	–	–	–	–	–	–	0,52± 0,21	0,69± 0,17	–	–	1,57± 0,27	–	–	–
- promyelo-cytes	0,70± 0,13	0,35± 0,12	0,52± 0,09	0,44± 0,11	0,69± 0,13	2,79± 0,33	0,87± 0,09	0,52± 0,02	–	0,35± 0,08	0,87± 0,11	1,57± 0,21	–	–	–
- myelo-cytes	–	0,35± 0,10	–	0,44± 0,15	1,38± 0,21	0,70± 0,24	3,05± 0,64	1,04± 0,11	–	0,70± 0,04	–	2,07± 0,22	–	–	–
Metamyelo-cytes	–	–	–	–	–	–	–	0,52± 0,21	–	0,69± 0,05	–	0,52± 0,05	–	–	–
Stab neutrophils	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Segmented neutrophils	–	–	–	–	–	–	–	–	–	–	–	–	1,39± 0,32	2,62± 0,41	0,52± 0,04

Notes: Data are means M±m.

Analysis of ***erythroid lineage*** cells of the bone marrow in the control group animals (Table 6) showed that the number of abnormal cells compared to the cells of myeloid lineage are 1,5 times lower in the aging pool and 5 times lower in the proliferative pool. Cellular abnormalities are mainly present as binuclear forms and by the presence of cells with the concatenated metaphase.

Table 6

The fraction of cells with abnormalities in erythroid lineage in the bone marrow of intact mice (line CBA) (absolute number of cells/10^3 karyocyte)

Pool of cells	Micronucleus	Concatenated metaphase	Cytoplasmic bridge	Karyorrhexis	Giant cells	Binuclear cells	Dissociation of the cytoplasm
Normoblast	–	0,06±0,01	–	–	–	–	–
Pronormocyte	–	–	–	–	–	0,06±0,02	–
Basophilic normocyte	–	0,19±0,03	0,06±0,03	–	–	0,19±0,04	–
Polychromatophilic normocyte	0,06±0,01	0,125±0,01	–	0,06±0,01	0,06±0,02	0,19±0,03	–
Oxyphilic normocyte	–	–	–	–	–	0,57±0,04	0,06±0,01

Notes: Data are means M±m.

In irradiated experimental animals the spectrum of cellular abnormalities in the erythroid lineage of the bone marrow decreased due to the absence of cells with cytoplasmic bridge, karyorrhexis and giant cells, if compared to the control. The number of pathological form of cells increased to the maximum when the accumulation of total absorbed doses reached 2–4 Gy (6–9 month of the exposure), herewith anomalies of erythroid cells were observed mainly on the final stages of maturation, i.e. among the polychromatic and oxyphilic normocytes (Table 7). These facts may testify that under the exposure to low-intensity radiation (to the accumulation of total absorbed doses of about 1 Gy) DNA repair systems are activated and effectively operate in poorly differentiated erythroid cells. Our data may indicate also acceleration in the process of maturation of these cells.

Table 7

The fraction of cells with abnormalities in erythroid lineage in the bone marrow of CBA mice under chronic γ - irradiation at a dose-rate of 1 cGy/day (absolute number of cells/10^3 karyocyte)

Pool of cells	Micronucleus		Concatenated metaphase	Dissociation of the cytoplasm		Binuclear cells			
	Duration of exposure, month								
	6	9	1	1	3	1	3	6	9
Normoblast	–	–	–	–	–	–	–	–	–
Pronormocyte	–	–	–	–	–	–	–	–	1,34±0,12
Basophilic normocyte	1,34±0,15	–	–	–	–	1,22±0,29	–	–	–
Polychromatophilic normocyte	–	–	1,22±0,07	–	–	–	1,15±0,31	4,00±0,42	1,34±0,26
Oxyphilic normocyte	–	1,34±0,22	–	1,22±0,04	1,15±0,19	4,88±0,63	2,30±0,34	–	5,35±0,62

Notes: Data are means M±m.

Thus, in the course of chronic low-intensity external γ-irradiation of CBA mice the following processes took place:

- increase in the number of pathological forms of cells of granulocyte lineage in the bone marrow assosiated with an increase in the total cumulative dose of radiation;
- expansion of the range of pathological changes in the granulocyte lineage (due to the appearance of cells with cytoplasmic bridge, a bridge in telophase and karyorrhexis) and, conversely, reduced range of abnormal cells in the erythroid lineage, in which there were no cells with such abnormalities as cytoplasmic bridge, karyorrhexis and giant cells;
- increase in the frequency of abnormal form of cells in the granulocyte proliferative pool and the maturing pool of the erythroid lineage.

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ЦИТОЛОГІЧНІ ЕФЕКТИ У КЛІТИНАХ КІСТКОВОГО МОЗКУ ПРИ ХРОНІЧНОМУ НИЗЬКОІНТЕНСИВНОМУ γ –ОПРОМІНЕННІ ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН

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Унаслідок дії іонізуючого випромінювання у клітинах виникають пошкодження в геномі, в результаті яких клітина може загинути. Виживання клітини можливе лише у разі активації внутрішньоклітинних систем репарації та, як наслідок, розвитку адаптації. Результат опромінення головним чином залежить від стану антиоксидантного-прооксидантного гомеостазу. Виявлено, що хронічне низькоінтенсивне γ-опромінення призводить до збільшення кількості патологічних форм клітин і розширення спектра патологій у гранулоцитарному ростку кісткового мозку. У еритроцитарному ростку спектр аномальних клітин скорочувався, проте зростала частота аномальних форм клітин.

Ключові слова: хронічне низькоінтенсивне γ-випромінювання, кістковий мозок, гранулоцитарний росток, еритроїдний росток.

ЦИТОЛОГИЧЕСКИЕ ЭФФЕКТЫ В КЛЕТКАХ КОСТНОГО МОЗГА ПРИ ХРОНИЧЕСКОМ НИЗКОИНТЕНСИВНОМ γ-ОБЛУЧЕНИИ ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ

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Вследствие действия ионизирующего излучения в клетках возникают повреждения в геноме, в результате которых клетка может погибнуть. Выживание

клетки возможно в случае активации внутриклеточных систем репарации и, как следствие, развития адаптации. Результат облучения главным образом зависит от состояния антиоксидантно-прооксидантного гомеостаза. Нами обнаружено, что хроническое низкоинтенсивное γ-облучение приводит к увеличению количества патологических форм клеток и расширению спектра патологий в гранулоцитарном ростке костного мозга. В эритроцитарном ростке спектр аномальных клеток сокращался, однако увеличивалась частота аномальных форм клеток.

Ключевые слова: хроническое низкоинтенсивное γ-излучение, костный мозг, гранулоцитарный росток, эритроидный росток.