STUDY OF MUTATION PROCESSES IN BONE MARROW AND BLOOD CELLS AFTER SEPARATE AND COMBINED EXTERNAL AND INTERNAL γ-IRRADIATION OF ORGANISM

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The general risk of radiation for man is one of problems of radiation genetics. The cytogenetic effects in bone marrow and blood cells of animals of different age were studied under the conditions of separate and combined acute external (^{137}Cs, dose rate: 5.4 cGy/h, dose: 1 Gy), internal (incorporation of ^{137}Cs as 160 kBq and 320 kBq) γ-irradiation and administration of enterosorbent Vaulen to white rats. Besides of cytological, radiological methods and cytofluorimetry, new cytogenetic approach was used, which was based on complex analysis of marker cells (by karyotype) of stem cells differentiation of bone marrow and chromosome aberrations rate in them. It was shown that the marker cells of three lines of blood formation (leucocytal, erythroid and megakaryocytic) in bone marrow of rats were characterized by different radiosensitivity. In 2-month rats the single external (0.25 and 0.5 Gy doses), combined acute external (1 Gy)/prolonged (1 Gy) and the internal irradiation caused the inhibition of leukocytal line of blood formation, and the separate acute external (1 Gy) and internal irradiation inhibited the erythroid one. The cytogenetic effect in bone marrow of these animals was more expressed at the external prolonged (1 Gy) and internal (^{137}Cs, 320 kBq) irradiation (3.5-fold increase of chromosome aberrations rate in cells on the 10th and 5-fold - on the 90th day). On the contrary, in 6-month rats, the high level of mutations was observed under the conditions of combined acute external (1 Gy)/ internal (^{137}Cs, 320 kBq) irradiation. Administration of Vaulen to irradiated 6-mouth rats did not decrease the level of mutations (chromosome aberrations) in bone marrow cells. However, decrease of aberrant cells rate in the blood of these animals was observed after Vaulen administration, especially after separate internal and external irradiation.

Introduction

One of the problems of radiation genetics is study of genetic effects of γ-radiation. Low dose irradiation of organism causes early and remote effects in cells of different organism systems. These effects are mainly connected with cell mutation damage (chromosome, genome and gene) and epigenetic changes of regulator genes.

Some authors (1,2) showed that remote cytogenetic effects in different mammalian tissues vary by degree of mutagenic effect manifestation and spectrum of chromosome aberration types. Uncertainty of dose-rate effects for low dose radiation, that is observed, make difficulties for the solutions of the series of fundamental (radiation mutagenesis and carcinogenesis) and applied (risk estimation, health regulatory actions, biodosimetry, etc.) problems. External and internal irradiation effects on organism are not studied fully. At the same time, these questions are actual for estimation and development of measures for decreasing of ecological factor effects on people's health.

The purpose of the present work was to study early and remote cytogenetic effects in bone marrow and blood cells of rats under the conditions of separate and combined internal and external low dose irradiation.

Methods

The following experiments were carried out: 2-month rats were exposed at the IGUR (radiation source - ^{137}Cs, dose rate - 5.4 cGy/min) to acute external irradiation at doses of 0.25 Gy; 0.5 Gy and 1.0 Gy; to internal irradiation by incorporation of ^{137}Cs, 320 kBq/rat during 16 days (20 kBq/rat daily; combined acute external 1.0 Gy and internal (^{137}Cs, 320 kBq) irradiation; internal (^{137}Cs, 160 kBq/rat during 16

days; acute external 1.0 Gy and internal (^{137}Cs, 160 kBq) irradiation; prolonged external 1.0 Gy irradiation (GAMMARID, 129/120, 87 days, dose rate - 47,5 cGy/hour) and incorporation of radionuclide ^{137}Cs (320 kBq/rat) during 16 days after prolonged exposure to 1 Gy.

6-month rats were exposed to acute external γ-radiation at doses of 1.0 Gy; internal (^{137}Cs, 320 kBq) irradiation; combined acute external 1.0 Gy and internal (^{137}Cs, 320 kBq) irradiation; combined acute external 1.0 Gy irradiation and administration of enterosorbent Vaulen; internal (^{137}Cs, 160 kBq) irradiation with simultaneous administration of Vaulen and separate administration of Vaulen. 25 mg/day of Vaulen was given to each rat during 16 days. Vaulen was synthesized in the Institute of General and Inorganic Chemistry, ASB (Reg. B-1-LS №95/127/131). Control and experimental groups included 20 rats. Bone marrow and blood cells were studied.

Rate of chromosome aberrations, chromosome sets (type I, II, III) and rate of cells with micronuclei were examined. 3 types of chromosome sets in bone marrow population characterize structure and function chromosome organization in three main marrow stem cells lines of differentiation: megakaryocytic - I type, leukocytal - II type, erythroid - III type (3). They were used as stem cells differentiation markers.

Cytogenetic method and method of flow cytofluorimetry were used. Cytogenetic method included registration of chromosome aberrations in marrow lymphoid cells at metaphase stage with optical microscope (Jevanal, Germany). Selection of metaphase plates, classification and chromosome aberrations registration methods were standard (4). 50-100 cells were analyzed for each rat. Number of micronuclei blood cells was detected according to DNA quantity in nuclei by flow cytometry.

Results

Cytogenetic research of chromosome aberration rate in bone marrow of exposed to 0.25, 0.5 and 1.0 Gy rats showed, that aberration cells rate increased 1 day after irradiation in comparison with the control group (7-fold increase at dose of 0.25 Gy, 6-fold **-** at 0.5 Gy, 8-fold - at 1.0 Gy). Single fragments rate increased 1.5-2-fold after 0.25 and 0.5 Gy irradiation and didn't exceed control level after 1.0 Gy. Chromatid and chromosome interchange rates rose with the increasing of doses. Such aberrations as dicentric chromosomes, inversions, acentric and centric rings were registered in bone marrow of 0.5 and 1.0 Gy irradiated rats.

Observed mutation processes in bone marrow cell population were accompanied by disorder of stem cells differentiation. Inhibition of leucocytal line of blood formation was detected 1 day after external acute irradiation at doses of 0.25, 0.5 and 1.0 Gy. Doses of 0.25 and 0.5 Gy caused acceleration of differentiation in megakaryocytic line, 1.0 Gy - in erythroid one.

Also, early (on the 10th day) and remote (on the 90th day) cytogenetic effects in bone marrow of rats, exposed to external acute (1.0 Gy), internal (^{137}Cs, 320 kBq) and combined irradiation, were examined (table 1). Aberration cells rate increased in rat bone marrow on the 10th day after external (1.0 Gy) and internal (^{137}Cs, absorbed dose - 0.1 Gy) irradiation. Internal irradiation with ^{137}Cs led to 7.5-fold increasing of cells with dicentric chromosomes and acentric rings, combined action of external and internal irradiation (1.0 Gy) caused 2.2-fold increasing of cells with chromatid interchanges and 1.6-fold increasing of pair fragments. On the 90th day cytogenetic effects in bone marrow were more expressed under the conditions of internal irradiation (^{137}Cs, 320 kBq) - 2.4-fold increasing of pair fragments, 4.2-fold increasing of cells with chromatid interchanges. Combined action of external acute and internal continuous irradiation at remote period caused increasing of cells with chromosome interchanges and decreasing of aberration cells rate because of elimination of dicentric chromosome cells. Besides, it was found out there was 2-fold increase of aberration cells on 90th day in comparison with that on the 10th day. Cytogenetic effects in bone marrow cells were more marked at the remote period - increase of cells with single and pair fragments after internal irradiation and increase of chromatid interchanges after combined irradiation. Number of cells with chromosome interchanges was 4-7 times less than on the 10th day in the control group.

Besides, low dose radiation effects on processes of stem cells differentiation were investigated (table 2). Inhibition of leucocytal and megakaryocytic lines of blood formation accompanied with increasing of proliferation of erythroid line cells was observed after combined irradiation in early and remote periods.

It was revealed that micronuclei cells rate in blood of 2-month rats, exposed to external continuous radiation (1.0 Gy), decreased rapidly on the 10^{th} day and increased on the 90^{th} day. Number of cells with micronuclei increased on the 10^{th} day after internal (^{137}Cs, 302 kBq) irradiation and such level saved for a long term. Micronuclei cells rate was increasing after combined (external prolonged,1.0 Gy; and internal, ^{137}Cs, 302 kBq) irradiation during the whole observation period. On the contrary, micronuclei cells rate of the 6-month rats increased after 1.0 Gy external irradiation on 92.9% (10^{th} day) and 382.9% (90^{th} day). Enlarged number of micronuclei cells after combined irradiation in the early period reduced down to control level in the remote period.

Administration of Vaulen to irradiated 6-mouth rats did not decrease the level of chromosome aberrations in bone marrow cells. However, micronuclei cells rate after Vaulen administration decreased rapidly on the 10^{th} day after external (1.0 Gy), internal (^{137}Cs, 302 kBq) and combined irradiation and then increased to the 90^{th} day. This fact witness radioprotective effect of Vaulen in the early period.

Discussion

Obtained data as the results of other researches (3) testifies to the high radiosensitivity of precursor cells and their low capacity of genome reparation in early post-irradiation period. Deficiency of stem cells in haemopoiesis organs creates emergency situation in processes of post-irradiation regeneration (5). 6-7 hours after irradiation, rapid decrease of young and immature cells rate of different lines, especially of erythroid and myeloid, was observed (6).

This results adjust with the data of other authors (7) that studied effects of 0.2-0.9 Gy radiation on dog CFU-GM. Dose of 0.2 Gy led to short-term decreasing of CFU-GM in dog bone marrow during 7 days after irradiation. Higher doses (more than 1.0 Gy) caused rapid decrease of CFU-GM number in early period with regeneration during next 3 months. Decrease of thrombocyte, lymphocyte and neutrophil number in peripheral blood that correlated with injury level of different stem cells after 0.4 and 0.9 Gy irradiation was also observed.

Conclusion

New cytogenetic method of study of low dose effects at different irradiation conditions on bone marrow haemopoiesis was used in the present work. It was based on comprehensive analysis of markers (karyotype) of rat marrow stem cells differentiation and their aberration rate. It was shown that marker cells of three lines of blood formation (leucocytal, erythroid and megakaryocytic) in bone marrow of rats were characterized by different radiosensitivity. External acute irradiation at dose of 1.0 Gy caused injury of megakaryocytic and leucocytal lines cells, internal (^{137}Cs, 302 kBq) irradiation - injury of erythroid line cells, combined - leucocytal line cells. Changes of mutation (chromosome aberration) rate depended on the irradiation conditions, dose, level of differentiation and proliferation activity of cells.

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Table 1. Chromosome rate in bone marrow cells of male rats in different conditions of whole-body γ-irradiation

Groups	Cytogenetic rates, %									
	Aberration cells		Single fragments		Chromatid interchanges		Pair fragments		Chromosome interchanges	
	10^{th} day	90^{th} day	10^{th} day	90^{th} day	10^{th} day	90^{th} day	10^{th} day	90^{th} day	10^{th} day	90^{th} day
2-month rats										
C[1]	8.04±0.1	5.4±0.6	6.0±0.61	6.1±0.45	0.12±0.01	0.45±0.08	3.18±0.63	1.9±0.31	0.72±0.02	0.3±0.01
1 Gy[2]	15.32±0.24**	14.8±0.26	4.08±0.1	4.6±0.46	1.8±0.72	1.5±0.8*	4.83±0.67	3.2±0.86	4.4±1.02*	4.2±0.5**
1 Gy[3]	15.38±1.84	11.01±0.08	2.75±0.11*	1.5±0.6*	0.0	0.0	8.25±1.65	1.2±0.04	5.88±0.72**	8.11±0.8***
^{137}Cs, 160 kBq[4]	6.8±0.24	11.5±1.67*	3.8±0.11	6.0±0.92	0.8±0.06	1.5±0.64	2.2±0.07	3.5±0.08	0.0	0.5±0.03
^{137}Cs, 320 kBq[5]	20.6±0.18	17.65±0.22	7.15±0.25*	5.75±0.87	1.15±0.36	1.65±0.05***	4.5±0.82*	4.3±0.74	7.25±0.11***	4.24±0.32*
1 Gy+^{137}Cs, 160 kBq[6]	15.8±0.22	14.0±0.99	3.8±0.07	3.8±0.4	1.0±0.05	1.0±0.44	6.2±0.06	3.2±0.09	0.8±0.04	5.0±0.06***
1 Gy+^{137}Cs, 320 kBq[7]	15.0±1.4*	13.4±1.96**	4.8±0.48	5.6±0.07	2.2±0.37	2.6±0.68	5.4±1.02	2.4±0.24	3.33±0.9*	2.8±0.66**
1 Gy+^{137}Cs, 320 kBq[8]	25.91±1.21***	24.1±0.11***	8.1±0.8*	6.2±0.03	4.2±0.1	5.0±0.3***	6.0±0.05**	9.0±0.12***	11.0±0.18***	10.91±0.21***
6-month rats										
C[2]	5.64±0.9	6.1±0.05	2.65±0.07	2.6±0.4	0.0	0.0	2.8±0.35	2.65±0.51	0.58±0.04	0.41±0.02
1 Gy[10]	43.83±0,64**	61.31±0,48***	5.0±0.54	5.94±0.11	36.67±0.16	34.82±0.31	8.0±0.49***	10.1±0.59***	6,8±0,91***	6.83±0.52***
^{137}Cs, 320 kBq[11]	24.91±1.57***	39.47±0.35***	18.88±0.1		2.83±0.54	2.19±0.7	10.95±0.9***	10.77±0.3***	6.04±0.14**	5.25±0.16
1 Gy+^{137}Cs, 320 kBq[12]	58.61±0.03***	63.82±0.12***	19.3±0.2***	21.3±0.03***	32.92±0.09	32.9±0.93	16.0±0.05***	12.3±0.04**	11.0±0.32***	10.3±0.99**
Vaulen[13]	3.8±0.07	3.85±0.09***	1.2±0.02	0.9±0.03***	0.02±0.02	0.0	1.5±0.06	1.4±0.08	0.0	0.38±0.004
1 Gy + Vaulen[14]	31.6±0.08***	50.9±0.78***	4.8±0.09***	6.2±0.31***	28.5±0.93	21.4±0.45	12.83±0.11***	12.0±0.61	8.5±0.77***	5.9±0.04***
^{137}Cs, 320kBq + Vaulen[15]	29.0±0.07***	27.93±0.09***	16.0±0.14***	15.9±0.93***	3.2±0.04	2.1±0.35	11.9±0.07***	7.3±0.14	7.0±0.53***	6.3±0.05***

[1] Control group; [2] acute external 1 Gy γ-irradiation; [3] prolonged external 1.0 Gy irradiation; [4] internal irradiation (^{137}Cs, 160 kBq/rat); [5] internal irradiation (^{137}Cs, 320 kBq/rat); [6] acute external 1.0 Gy and internal (^{137}Cs, 160 kBq) irradiation; [7] acute external 1.0 Gy and internal (^{137}Cs, 320 kBq) irradiation; [8] prolonged external 1.0 Gy and internal (^{137}Cs, 320 kBq); [9] control (6-month rats); [10] acute external 1 Gy irradiation; [11] internal irradiation (^{137}Cs, 320 kBq/rat); [12] acute external 1.0 Gy and internal (^{137}Cs, 320 kBq) irradiation; [13] administration of Vaulen; [14] acute external 1 Gy irradiation and 16 day administration of Vaulen; [15] internal irradiation (^{137}Cs, 320 kBq/rat) and 16 day administration of Vaulen.

* $p < 0.05$; ** $p<0.01$; ***$p<0.001$

Table 2. Assessment of marrow haemopoiesis lines in male rats in different conditions of whole-body γ-irradiation

Experimental conditions	Days after irradiation					
	10			90		
	Haemopoiesis lines			Haemopoiesis lines		
	megakaryocytic	leucocytal	erythroid	megakaryocytic	leucocytal	erythroid
2-month rats						
C[1]	20.74±5.78	57.44±4.44	14.0±2.85	14.14±2.47	63.59±2.65	21.7±3.8
1 Gy[2]	8.79±0.32**	23.41±0.5***	69.09±6.69***	6.55±2.4**	63.55±2.65***	67.41±3.97***
1 Gy[3]	30.5±16.67	57.75±3.75	26.25±5.17	31.0±8.0	55.93±1.24***	13.9±0.04**
^{137}Cs, 160 kBq[4]	17.0±1.73	76.2±4.0**	8.8±3.02**	13.75±1.31	67.75±1.93	18.5±2.6
^{137}Cs, 320 kBq[5]	70.0±0.25	56.06±4.0	41.36±4.68***	9.51±2.09	39.7±6.59***	50.35±4.88***
1 Gy+^{137}Cs, 160 kBq[6]	15.8±3.65	18.0±2.68***	65.6±4.9***	13.0±2.17	22.4±1.69***	64.6±2.82***
1 Gy+^{137}Cs, 320 kBq[7]	8.54±1.38	62.06±9.86	29.0±9.96	6.0±1.92	59.4±3.82	34.6±2.75
1 Gy+^{137}Cs, 320 kBq[8]	34.2±2.8*	60.08±2.11	5.1±0.69***	32.08±3.1*	58.68±0.03	9.91±0.12**
6-month rats						
C[2]	15.77±0.18	50.97±0.55	30.16±0.15	15.21±0.26	52.62±0.88	27.1±0.64
1 Gy[10]	26.68±0.8***	38.86±0.49***	34.37±0.7***	26.67±0.23***	38.34±0.83***	40.58±0.42***
^{137}Cs, 320 kBq[11]	61.7±0.75***	24.71±0.81***	10.87±0.18***	67.44±0.5***	22.98±0.5***	11.27±0.44***
1 Gy+^{137}Cs, 320 kBq[12]	70±1.18***	12.8±0.09***	11.98±0.38***	65.3±0.1	15.8±0.9***	9.48±0.73
Vaulen[13]	18.3±0.2***	45.11±0.85***	32.2±0.63**	10.99±0.05***	48.1±0.11***	35.0±0.11*
1 Gy + Vaulen[14]	26.7±0.09***	38.12±0.11***	46.81±0.02***	28.11±0.9***	45.0±0.81	42.5±0.09***
^{137}Cs, 320kBq + Vaulen[15]	57.3±0.16***	20.33±0.13***	12.3±0.77***	69.3±1.4***	17.81±0.77***	10.4±0.52***

[1] Control group; [2] acute external 1 Gy γ-irradiation; [3] prolonged external 1.0 Gy irradiation; [4] internal irradiation (^{137}Cs, 160 kBq/rat); [5] internal irradiation (^{137}Cs, 320 kBq/rat); [6] acute external 1.0 Gy and internal (^{137}Cs, 160 kBq) irradiation; [7] acute external 1.0 Gy and internal (^{137}Cs, 320 kBq) irradiation; [8] prolonged external 1.0 Gy and internal (^{137}Cs, 320 kBq); [9] control (6-month rats); [10] acute external 1 Gy irradiation; [11] internal irradiation (^{137}Cs, 320 kBq/rat); [12] acute external 1.0 Gy and internal (^{137}Cs, 320 kBq) irradiation; [13] administration of Vaulen; [14] acute external 1 Gy irradiation and 16 day administration of Vaulen; [15] internal irradiation (^{137}Cs, 320 kBq/rat) and 16 day administration of Vaulen.

* p< 0.05; ** p<0.01; ***p<0.001