

The effects of gamma-irradiation in sub-lethal doses in mice with different radiosensitivity

Yulia Medvedeva, Elena Arkhipova, Irina Alchinova

Institute of General Pathology and Pathophysiology, Moscow, Russia

Email address:

uliamed89@gmail.com (Y. Medvedeva)

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Abstract: Reactions to gamma-irradiation in an acute dose of 750 R in C57BL/6, 101/Hf and C3H/Sn mice at different physiological levels were analyzed. In C3H/Sn and 101/Hf mice, light scattering spectrum was shifted towards small particles, while in C57BL/6 mice increased contribution of large particles was observed. In 3 weeks, a shift towards larger particles was recorded in all three mouse strains, but its degree varied. By week 6, the histograms approached the control. Histological study showed that the incidence of severe injuries in the liver increased by the end of the experiment in C57BL/6 mice (but not in other mouse strains). The same shifts were observed in the pancreas and an opposite tendency was seen in the spleen. Thus, C57BL/6 mice responded to irradiation later than C3H/Sn and 101/Hf mice, but demonstrated high adaptation capacity. C3H/Sn mice best of all recovered from the radiation crisis.

Keywords: Irradiation, Strain-Specific Differences, Laser Correlation Spectroscopy, Radiosensitivity

1. Introduction

The progress in modern technologies put the focus of research on the individual response to extreme exposures. Any extreme exposure triggers activation of general adaptation systems, but the rate and degree of these processes and, most importantly, the degree of exhaustion and the development of compensatory responses can vary. These factors ultimately determine viability of the organism. Evaluation of individual adaptation of a human is difficult due to considerable genotypic diversity and is usually carried out mainly at the physiological level in exercise tests. However, these tests cannot adequately reflect the changes at the cellular and biochemical levels that, in turn, modify the limit of adaptation and compensatory reactions.

The aim of this study was evaluation of interstrain differences in adaptive capacities of mice exposed to extreme environmental factors.

2. Methods of the Study

The experiments were performed on 3 mouse strains, 101/Hf, C3H/Sn, and C57BL/6, obtained from the Collection of the Research Center of Biomedical Technologies, Federal

Biomedical Agency. Previous comparison of different mouse strains had demonstrated that BALB/cJ mice were most sensitive and 129/J mice were most resistant to the lethal effects of irradiation. C57BL mice are most frequently used for radiobiological research and are considered resistant. C3H/Sn mice are also considered radioresistant [1]. In experiments with chemical mutagens, 101/Hf strain was most sensitive to alkylating agents; this was most likely determined by excision repair system defects [2].

The mouse groups (C3H/Sn n=17; 101/Hf n=14; C57BL/6 n=18) were exposed to radiation on a Panorama cesium irradiator (K. I. Skryabin Moscow Veterinary Academy) in a total dose of 750 R. The control groups (C3H/Sn n=15; 101/Hf n=16; C57BL/6 n=12) were not irradiated.

The animals were weighed on a Mettler Toledo scales after 1-h food deprivation.

Blood serum was used for evaluation of shifts in serum homeostasis. The blood was taken from the small saphenous vein in 3 days and in 3 and 6 weeks after irradiation.

Subfraction composition of blood serum was analyzed by the method of laser correlation spectroscopy (LCS). The method allows evaluating the ratio relative content of particles of various sizes in biological fluids (blood serum, urine, etc.). For multicomponent samples, the analysis yields a curve with several peaks. Comparison of the areas under

the curves provides information on changes in the relative contribution of particles of different sizes to light scattering [3]. For description of shifts in the particle size, the total range of sizes was divided into intervals. For mouse blood serum, three major intervals were distinguished: 1 - from 0 to 20.58 nm, 2 - from 20.58 to 91.26 nm, 3 - >91.26 nm.

Changes in the quantitative parameters of blood cells were evaluated from differential leukocyte count (immersion microscopy of Pappenheim stained smears). Microscopy of the smears was performed by the Shilling technique. Subpopulation composition of blood lymphocytes (ratio of T and B cells) in mice of different strains was studied by flow cytometry using triple-labeled monoclonal antibodies.

Behavioral changes induced by the potent stress factor were studied using the open field test. Vertical and horizontal activities were measured over 3 min. Testing was performed in a square chamber (45×45 cm) at 40 lux illumination. In this test, behavioral phenomena caused by the conflict of fear motivation and exploratory activity were studied.

After the experiment, the animals were sacrificed by anesthetic overdose and the organs (liver and intestine) were taken for histological examination for evaluation of the damaging effects of irradiation. The organs were fixed in 4% neutral formalin. The preparations were prepared routinely.

To simplify sample processing, the severity of alterations was classified depending on the degree of morphological changes in cells and tissues [4, 5].

The parameters were analyzed by standard parametric ANOVA tests using Statistica 6.0 software.

All animal experiments and procedures were carried out in accordance with Laboratory Practice Standards in the Russian Federation (Ministry of Health Care of the Russian Federation, Order #267, June 19, 2003).

3. Results and Discussion

Ionizing radiation produces a nonspecific effect on living organisms. The energy of ionizing radiation is too low to be perceived; moreover, there are no specific receptors that could directly perceive it. In contrast to other influences, gamma-radiation directly and simultaneously affects all structural elements of the living system and modulates their functions. After irradiation, the high working capacity of cells, tissues, and organs is maintained primarily due to destruction of own vital structures and the gradual impairment in total resistance [6, 7].

We studied some parameters characterizing the response of a specific system to irradiation.

General status of mouse organism can be objectively characterized, among other parameters, by changes in body weight. Published data suggest that acute irradiation in high doses leads to sustained body weight loss due to radiation sickness development. In our experiments, a decrease in body weight gain percentage after acute irradiation was observed. However, there are data that radiation can induce the development of metabolic syndrome after exposure to high, but not lethal doses (Fig. 1).

In C3H/Sn mice, a decrease in body weight gain in the experimental group was recorded throughout 3 weeks of the experiment, which corresponded to the development of the acute phase of radiation sickness. By week 6 (recovery phase), this parameter increased.

In 101/Hf mice, body weight gain returned to normal by postirradiation week 3 and was similar to that in the control group.

By week 6, body weight gain in the experiment considerably surpassed that in the control, which can attest to either development of the metabolic syndrome, or sharp regain of body weight after radiation exposure [8].

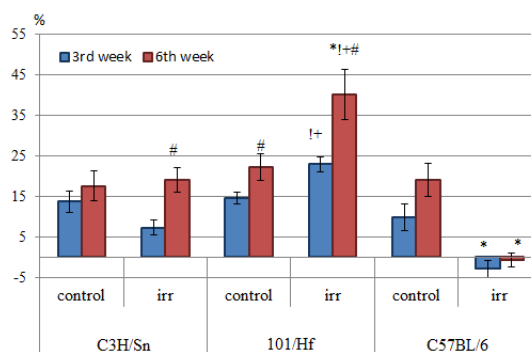


Figure 1. Weight gain in three mouse strains. ! – comparison with the C3H/Sn mice, + – comparison with the C57BL/6 mice, * – comparing control and experimental groups, $p < 0.05$, U-criteria; # – comparing 3rd and 6th weeks, $p < 0.05$, T-criteria.

In C57BL/6 mice, similar to C3H/Sn mice, body weight gain decreased throughout week 3 of the experiment, but was not restored by week 6, which attests to insufficiency of adaptive processes after irradiation.

Judging from changes in the body weight, the classical response to irradiation developed in C3H/Sn mice, while in 101/Hf and C57BL/6 mice irradiation induced the development of a chronic pathological process.

Changes in the body weight in the group can serve as a complex marker reflecting the rate of metabolic processes in laboratory animals, but unfortunately, it provides no information on the mechanisms of these changes.

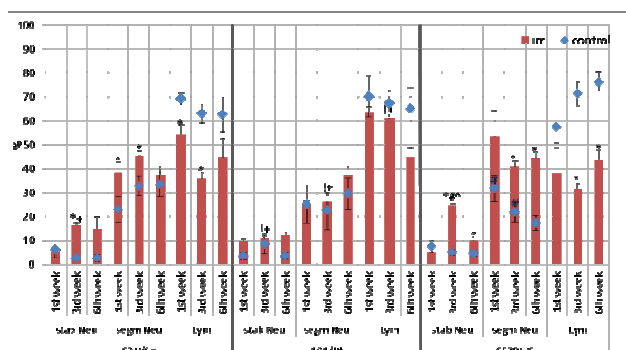


Figure 2. Comparison of blood cell content in three mouse strains after the irradiation. ! – comparison with the C3H/Sn mice, + – comparison with the C57BL/6 mice, * – comparison with the control group, $p < 0.05$, U-criteria; # – comparing 3rd and 6th weeks, $p < 0.05$, T-criteria.

Changes in the peripheral blood are most pronounced after exposure to gamma-radiation in high doses. Comparison of the blood cell content in mice of different strains after irradiation revealed most pronounced differences in the count of stab and segmented neutrophils and lymphocytes (Fig. 2).

Lymphopenia is the main indicator of radiation sickness. In C57BL/6 mice, the effect of irradiation developed as soon as one week after exposure, while by the 6th week of the experiment, leukocyte count started to increase. In 101/Hf mice, an opposite dynamics was observed and pronounced lymphopenia developed by the 6th week of the experiment. C3H/Sn mice were most resistant to irradiation: lymphocyte count only slightly decreased by the 3rd week and recovered by the 6th week.

Analysis of leukocyte subpopulations showed that the total leukocyte count remained practically unchanged throughout the experiment in all three mouse strains. At the same time, the count of both T and B cells decreased by the 6th week in C3H/Sn and 101/Hf mice (in C3H/Sn this decrease was more pronounced), while in C57BL/6 mice, an opposite shift towards a sharp increase in the content of both lymphocyte populations was noted. This attests to exhaustion of the pool of T and B cell precursors in C3H/Sn and 101/Hf mice and recovery of the hemopoietic function in C57BL/6 mice by the 6th week of the experiment after almost complete absence of T and B cells during the 3rd week (Fig. 3).

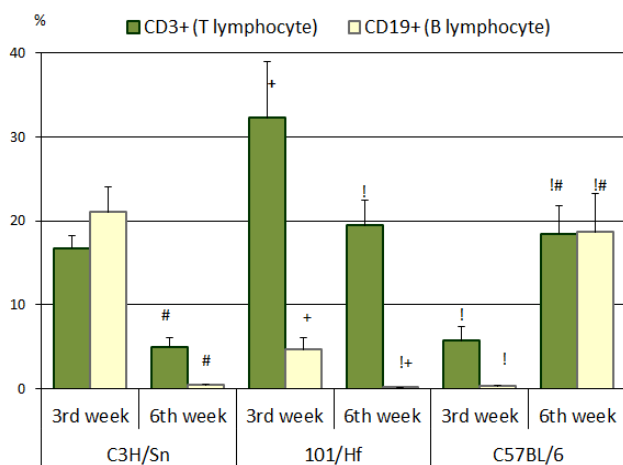


Figure 3. Lymphocyte subpopulations in three mouse strains after the irradiation. ! – comparison with the C3H/Sn mice, + – comparison with the C57BL/6 mice, $p < 0.05$, U-criteria. # – comparing 3rd and 6th weeks, $p < 0.05$, T-criteria.

Exposure to ionizing radiation exhausts the pool of stem cells, increases differentiated cell stress, and activated reparation and apoptosis processes. Laser correlation spectroscopy allows evaluation of changes in serum homeostasis [9]. In irradiated 101/Hf mice, an increase in the percent contribution of the third zone particles into light scattering during week 3 and a shift of the second zone towards larger particles in comparison with the control were observed. The contribution of zone 3 particles remained high up to week 6 (Fig. 4).

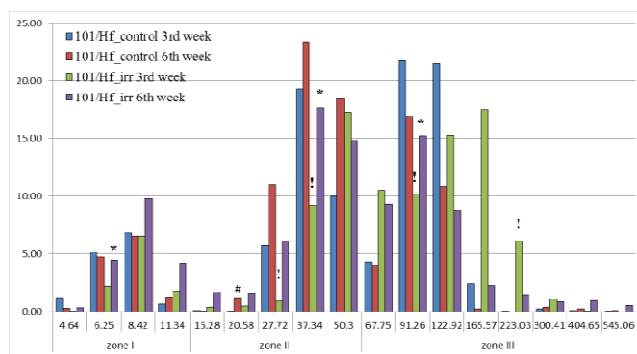


Figure 4. Laser correlation spectroscopy of blood serum of 101/Hf mice. Ordinate – contribution of particles of the corresponding zone into light scattering (%). Abscissa – particlesize (nm).! – comparing control and experimental groups, * – comparing 3rd and 6th weeks in control group, # – comparing 3rd and 6th weeks in group with radiation impact, $p < 0.05$, U-criteria.

In C3H/Sn mice, the contribution of large particles into light scattering significantly increased at the expense of reduced contribution of zone 1 particles. In 6 weeks, the contribution of zone 2 particles into light scattering increased and that of zone 3 particles decreased in comparison with the control serum (Fig. 5).

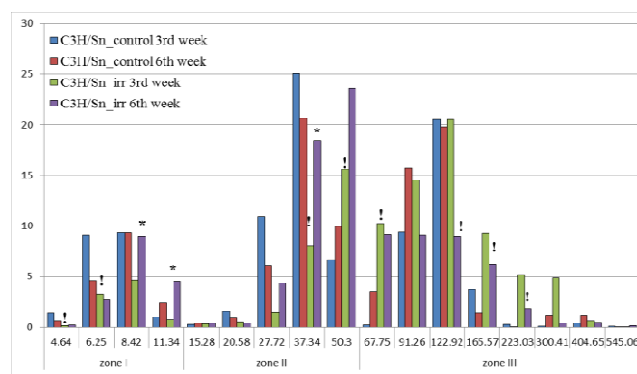


Figure 5. Laser correlation spectroscopy of blood serum of C3H/Sn mice. Ordinate – contribution of particles of the corresponding zone into light scattering (%). Abscissa – particlesize (nm).! – comparing control and experimental groups, * – comparing 3rd and 6th weeks in control group, # – comparing 3rd and 6th weeks in group with radiation impact, $p < 0.05$, U-criteria.

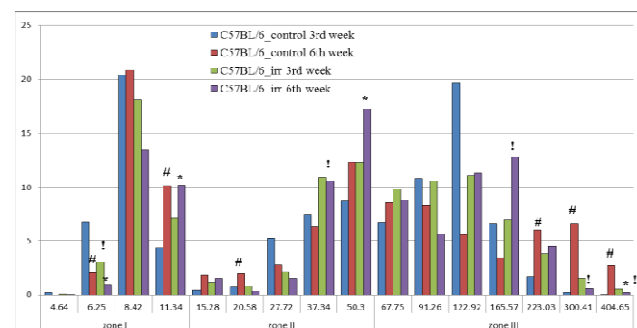


Figure 6. Laser correlation spectroscopy of blood serum of C57BL/6 mice. Ordinate – contribution of particles of the corresponding zone into light scattering (%). Abscissa – particlesize (nm).! – comparing control and experimental groups, * – comparing 3rd and 6th weeks in control group, # – comparing 3rd and 6th weeks in group with radiation impact, $p < 0.05$, U-criteria.

In C57Bl/6 mice, the contribution of zone 2 particles increased by week 3. By week 6, redistribution of particle spectra towards smaller particles was observed: the contribution of zone 2 particles increased and the contribution of zone 3 particles decreased (Fig. 6).

Increased contribution of large particles attests to the development of inflammatory process and accumulation of cell destruction products in blood serum [10]. Changes in LC-histograms in 101/Hf mice towards larger particles were most pronounced and long-lasting. In C57BL/6 mice, these shifts were minor, while in C3H/Sn mice, the contribution of the third zone particles into light scattering considerably increased by week 3 and returned to the initial values by week 6. The positive dynamics of the cell composition of the blood in C57BL/6 mice throughout the experiment agreed with minimum changes in the relative contribution of serum particles into light scattering.

Ionizing radiation affects the functional state of the nervous system and animal behavior directly and indirectly via modulation of reactivity of other body systems to radiation damage. Nervous reactivity can play even greater integrative physiological role than immediate damage to the nervous system caused by irradiation in sublethal doses. During week 3 of the experiment, locomotor activity remained unchanged in C57Bl/6 mice and slightly increased in 101/Hf mice. During the third week of the experiment, locomotor activity decreased in C57Bl/6 mice and slightly increased in 101/Hf and C3H/Sn mice (Fig. 7).

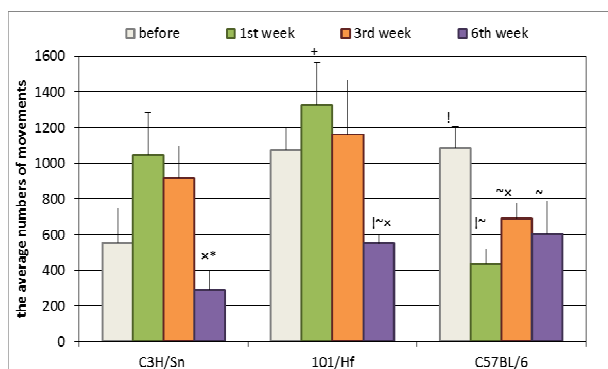


Figure 7. Change in horizontal locomotor activity in the three mouse strains. ! – comparison with the C3H/Sn mice, + – comparison with the C57BL/6 mice, $p < 0.05$, U-criteria; ~ – comparison before and after irradiation, × – comparison with the 1st week, * – comparison with the 6th week, $p < 0.05$, T-criteria.

During week 6 of the experiment, C57Bl/6 mice demonstrated maximum increase in horizontal locomotor activity among the studied mouse strains. However, in none of mouse groups, locomotor activity returned to the initial level.

In C3H/Sn mice, vertical motor activity remained unchanged over 3 weeks, but completely disappeared by week 6. The same tendency was observed in 101/Hf mice, but a sharp decrease in vertical activity was observed during week 3. C57BL/6 mice demonstrated maximum number of movements in comparison with other strains. By the end of

week 1, vertical locomotor activity decreased and became similar to that in other two strains, but then increased and did not statistically differ from the initial values (Fig. 8).

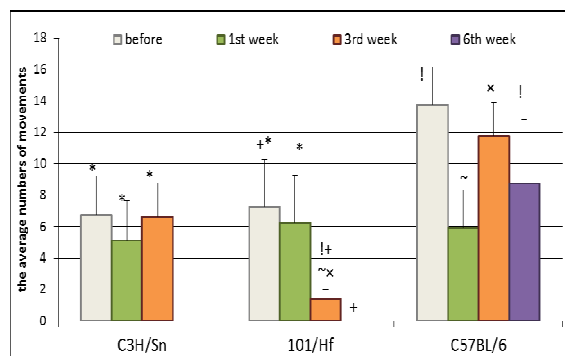


Figure 8. Change in the vertical locomotor activity in three mouse strains. ! – comparison with the C3H/Sn mice, + – comparison with the C57BL/6 mice, $p < 0.05$, U-criteria; ~ – comparison before and after irradiation, × – comparison with the 1st week, * – comparison with the 6th week, $p < 0.05$, T-criteria.

Table 1. Dynamics of measurable indicators in different mouse strains after irradiation. "+" – positive changes, "-" – negative dynamic

Parameters weeks	C3H/Sn		101/Hf		C57Bl	
	3rd	6th	3rd	6th	3rd	6th
Body weights	-	+	+	+	-	-
Blood counts	+	-	-	-	-	+
LCS	-	+	-	-	-	+
Locomotor activity	+	-	+	-	+	+

Open field testing revealed backward movements in mice 101/Hf, which is typical of neurological hot-foot mutants with structural and functional abnormalities of the cerebellum. The following changes in the motor activity were revealed in mice of different strains: in C57BL/6 mice it decreased during week 1 and increased by the end of the experiment; C3H/Sn mice demonstrated unchanged over 3 weeks, but later it sharply decreased by week 6; in 191/Hf mice, the decrease in locomotor activity was observed by week 3. These changes suggest that C3H/Sn mice have learned by week 6; in 101/Hf mice, the observed behavioral reactions were more likely explained by fear motivation and poor general health status after irradiation; in C57BL/6 mice, explorative activity predominated.

Irradiation in an acute dose of 750 R induces cell destruction both during exposure and at later terms due to generation of reactive oxygen species. In control mice (not exposed to gamma-irradiation) liver tissues were not damaged.

Mice of all lines after irradiation demonstrated severe liver damage, persistent throughout the experiment.

We found that despite the general reactions of tissues and organs were similar, the sensitivity of different mouse strains to gamma-irradiation in an acute dose of 750 R was different, which was determined by individual characteristics of these strains. It was found that C57BL/6 mice are characterized by high adaptation to learning, while C3H/Sn mice more easily recovered after radiation crisis in comparison with other

mouse strains. In 101/Hfno appreciable improvement after radiation sickness development was observed. The use of complex criteria helps to evaluate the total result of the pathological process and realization of adaptive mechanisms and allows choosing appropriate criterion for radiosensitivity assessment. The use of integral parameters will help to determine the direction and rate of the main pathological process.

Multi-level studies provide an assessment of the overall result of the pathological process and the effectiveness of adaptive mechanisms and promote development of appropriate criteria for evaluating of radiosensitivity of the organism when testing new strains or hybrids. Further study of individual radiosensitivity will enable to predict risks during nuclear accidents and terrorist attacks.

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