Mol. Mat., 1994, Vol. 4, pp. 339–343
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FORMATION OF OPTICALLY ANISOTROPIC TEXTURES IN BLOOD PLASMA AND SERUM SAMPLES FROM GAMMA-IRRADIATED MICE IN CONDITIONS OF CRYSTALLIZATION ON SOLID SUBSTRATE

S. A. SKOPINOV, I. P. ANTROPOVA and E. A. TARAKHTIJ*

Institute of Industrial Ecology,
**Institute of Plant and Animal Ecology of Russsian Academy of Sciences (Ural Section),*
Ekaterinburg 620219, Russia

(Received 3 March 1994; accepted 10 April 1994)

In experiments with γ-irradiated mice the optically anisotropic textures (AIT) in blood serum samples (7Gr) and in blood plasma (0.1Gr) were investigated. It has been shown the dependence of area of AIT in serum samples on the time interval after irradiation. Plasma samples without Σ-ACA create an insignificant number of AITs at crystallization in optic cells. Blood plasma with Σ-ACA demonstrated intense formation of birefringent textures. The area of these AITs depends on the time after irradiation and we propose that it can follow the dynamics of proteolytic processes in irradiated organism.

INTRODUCTION

In connection with the wide use of ionizing radiations in industry and medicine and also owing to radioactive contamination of territories in a number of regions, an assessment of earlier structural and functional alterations of γ-irradiated systems of organisms acquires great significance, in particular, a study of alterations in blood system playing an important part in homoeostasis maintenance. Blood serum-being a complex solution-contains components able to form birefringent textures upon crystallization on a solid substrate. Characteristics of such textures in blood serum samples change under the influence of weak physical fields and, in particular, of low intensity laser irradiation.[1,2] Our purpose was studying dynamics of changes of anisotropic textures in blood serum and plasma samples of mice subjected to γ-irradiation. Serum investigations were performed in parallel to quantitative blood system cells estimation used in radiobiology.

MATERIALS AND METHODS

Three-month-old BALB mice were used in experiments. Animals were once whole-body irradiated by γ-ray 137Cs ("Igur"-equipment), dose rate 102 sGr/min. 3-5 animals were used for each control and experimental point. For studying dynamics of changes

of anisotropic textures (AIT) in blood serum samples, animals were irradiated with the dose of 7 Gr (LD 50/30). Blood serum was prepared by the standard technique.

AITs in blood plasma samples were studied in mice irradiated with the dose of 0.1 Gr. Blood plasma for crystallization was prepared by two techniques: 1) without using a proteolysis inhibitor, and 2) by adding Σ-aminocaproic acid (Σ-ACA) as such an inhibitor. Plasma preparations were obtained by mixing blood with a solution containing 0.13 M natrium citrate (9:1),preparations of plasma with Σ-ACA-by mixing blood and a solution containing 0.13 M natrium citrate and 0.76 Σ-ACA (9:1); then, blood cells were sedimented by centrifugation (3000 R/min, 15 min) and the supernatant was investigated by method of crystallization on solid substrate.

Optic cells were prepared by dropping 10 μl serum or plasma between the glass plate and cover slip. Plane capillaries formed by natural surface tension were kept in dark at 37°C. During this exposure, a slow evaporation along the capillary perimeter took place which induced a concentrational phase lamination of the system. As a result, anisotropic textures were formed whose analysis was performed by means of a microscope MBI-15 with crossed polars and of the polarization photometer PP-1, (Ural Optic-Mechanical Works). Intensity of polarized light passing through the optic cell (photometric polarization index-PPI) was proportional to total area of anisotropic birefringent sample textures depolarizing partially a light beam passing through them. 6-9 optic cells of the same type were prepared all at once.

Counting of leukocytes was fulfilled by means of Goryaev camera and that of erythrocytes-by means of Celloscope-401. Total quantity of cells in suspensions of hemopoietic organs (femur bone marrow, spleen, lymph node) was determined according to[3].

RESULTS AND DISCUSSION

For studying formation of AITs in blood serum samples according to dynamics of radiation damage, mice were decapitated in 1.4 hour, 1,4,15 days after gamma-irradiation with the dose of 7 Gr. Polarization microscopy studies of serum samples showed availability of birefringent textures in all the optic cells. Examples of such textures are shown in Figure 1.

Figure 2 shows a PPI-expessed dependence of area of anisotropic textures on the time interval after irradiation. An average PPI value for a non-irradiated control corresponds to zero value on the abscissae axis and is accepted for 100%. It is shown that, relatively to the control, the PPI value is $48.3 \pm 6.9\%$ in an hour, $69.0 \pm 13.8\%$-in four hours and $62.1 \pm 10.3\%$-in a day after irradiation ($p < 0.001$, $p < 0.05$,$p < 0.01$, respectively). Relatively to controls, the PPI value is $89.7 \pm 24.1\%$ in four days and $110.0 \pm 6.9\%$ in 15 days. In both cases, discrepancy with controls is unreliable. Radiation damages were also detected by using quantitative determination of the total blood system cell number. Data are shown in Table I.

As it is seen from Table I, the blood-forming disturbances took place already on the first day after irradiation but maximum decrease of cell quantity in homogenates of bone marrow, spleen, lymph node and peripheral blood leukocytes took place up to the fourth day which is in accordance with the data[3] showing that at the "clinical dose"

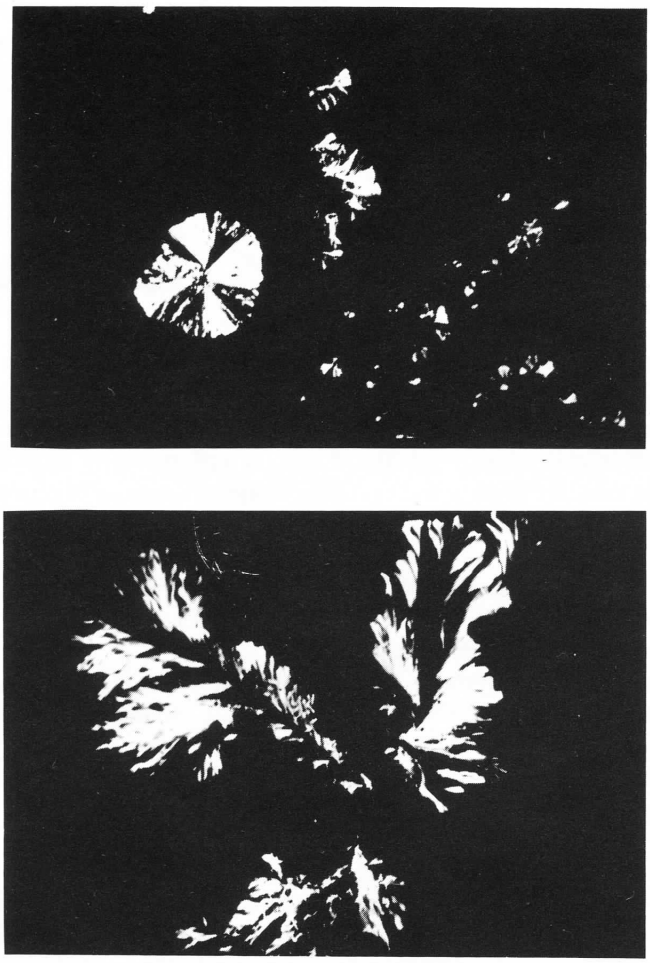

Figure 1 Optically anisotropic textures in blood serum samples upon the crystallization on solid substrate.

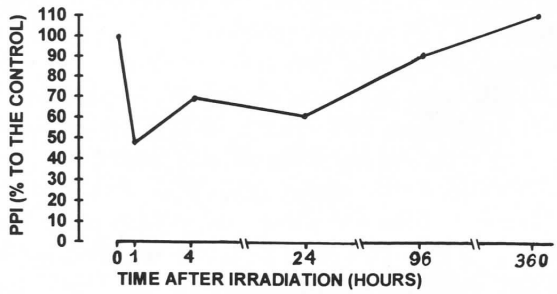

Figure 2 PPI of the blood serum samples at different time intervals following γ-irradiation at the dose of 7Gr. The results are given as % to the non-irradiated control (0 hours).

Table 1 Blood system cell number in dynamics of radiation damage. Dose 7 G.r

		Time	Interval	After	Irradiation
		0 days*	1 day	4 days	15 days
Erythrocytes	(mln/μl)	10,2 ± 0,1	9,0 ± 0,2	9,4 ± 0,3	5,2 ± 0,5
Leukocytes	(thds/μl)	7,2 ± 1,1	4,5 ± 0,3	2,9 ± 0,2	5,0 ± 0,6
Bone marrow	(mln)	14,5 ± 0,5	5,8 ± 0,3	1,6 ± 0,1	3,5 ± 1,5
Spleen	(mln)	249,8 ± 3,2	45,8 ± 1,7	31,1 ± 2,1	76,5 ± 11,8
Lymph node	(mln)	8,6 ± 1,2	1,8 ± 0,3	1,1 ± 0,2	1,2 ± 0,1

* 0 days-non-irradiated control

irradiation, the maximum cell quantity decrease falls on the fourth day. Decrease of cell quantity of peripheral blood erythrocytes fell on the 15th day. If to compare dynamics of AIT formation and change of blood system cell quantity after irradiation at half-lethal dose then, it can be noted that the change of quantity of serum components providing birefringent textures or the change of their ability to form such ones takes place on the earliest stages of the organism's response to irradiation and returns practically to the initial level up to the 4th day. While the blood system cell quantity is the most expressed at this term the recovery process is not yet finished to the 15th day (it is the maximum cell number decrease).

Thus, method of serum crystallization on solid glass reveals, apparently, homoeostasis disturbances which, according to[4] can stimulate the mobilization of organism's energetic and structural reserves and disappear gradually with developing reparation processes in the organism irradiated whose dynamics reflects a change of total blood system cell quantity.

It have been shown earlier that irradiation increases proteolysis.[5–7] Σ-ACA is a proteolysis inhibitor and as it was found, this substance shows an intense formation of birefringent textures of dendrite type at crystallization on solid substrate in optic cell[8]. We investigated blood plasma samples 1) with and 2) without Σ-ACA. Mice plasma was prepared in an hour, a day and four days after irradiation with the dose of 0.1 Gr. It is shown that plasma samples without Σ-ACA create an insignificant number of AITs on crystallization in optic cells which was a cause of zero values of PPI. Blood plasma contains, unlike serum, a coagulation complex consisting mainly of high-weight-molecular proteins; this, probably, influences its low ability to form AITs.

Figure 3 shows results of PPI measures of blood plasma samples with Σ-ACA from non-irradiated animals (0 hours) and ones taken in an hour, in a day, in 4 days after irradiation at 0.1 Gr. PPI value of control animals is considered as 100%. It is shown that in an hour after irradiation, PPI value is 46.8 ± 7.0%, relatively to the control ($p < 0.01$). In a day, PPI value did not differ reliably from that of the control and was 94 ± 25%. In 4 days, PPI decreases reliably again, being 70.8 ± 14.0%, relatively to the control ($p < 0.05$). The data obtained show that already in an hour after low dose irradiation, the decrease of the non-bound Σ-ACA is observed in plasma samples. To the end of the first day, a quantity of non-bound Σ-ACA approaches to the control value, and decreases, however, to the 4th day again. It can be supposed that Σ-ACA loses its ability to form AITs at bounding-up with proteases. Hence, proteolysis

Figure 3 PPI of the blood plasma with Σ-ACA at different time intervals following γ-irradiation at the dose of 0.1 Gr. The results are given as % to the non-irradiated control (0 hours).

intensity can be judged by decrease of quantity of non-bound Σ-ACA i.e. by decrease of the PPI value proportional to total area of AITs.

CONCLUSION

Thus, we consider it worthy to investigate the formation of optically anisotropic textures of blood serum and plasma upon the crystallization on the solid substrate for studying structural and functional alterations of the γ-irradiated organisms.

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