Nonmonotonous Changes in Metabolic Parameters of Tissues and Cells under Action of Ionizing Radiation on Animals

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Abstract. A nonmonotonous relationship between changes of metabolic parameters of tissues and cells of animal and radiation dose were discussed. Under acute irradiation of animals the nonmonotonous dose-response curve for metabolic parameters of tissues and cells were found. The nonmonotonous dose-response curves of metabolic and functional tissues and cells parameters were also revealed upon chronic irradiation of animals at a low dose-rate. The nonmonotonous shape of dose-response curves may be explained on the basis of nonmonotonous kind of the time-course of metabolic response after irradiation. Living cells were supposed to possess a fundamental property in response to action of different stress agents by nonmonotonous changes of cell metabolism. This response was damping in time oscillation of the value of metabolic parameters around the normal level. Amplitudes and periods of oscillations in these changes of metabolic parameters could be observed. In case of chronic irradiation at a low dose-rate the metabolic and functional parameters showed some modified oscillation during irradiation. The nonmonotonous type of changes in metabolic and functional parameters of tissue and cell by chronic low dose-rate irradiation threw some new light on the peculiarities of biological effects of chronic irradiation.

Key words: Catecholamine, dose-response, hypothalamus, ionizing irradiation, lipids, lung, nonmonotonous metabolic response, ornithine decarboxylase, spleen, synaptosomal membrane, thymus

1. Introduction

The relationship between organism survival and radiation dosage was known to be monotonous. The monotonous type of dose-response curve was well studied and described. However, the nonmonotonous type of dose-response curve in some instances was exhibited by metabolic and functional parameters under action of ionizing irradiation on animal and mammalian cells in the culture. This phenomenon was never discussed. We analysed the nonmonotonous type of dose-response curves of metabolic and functional parameters and came to the conclusion that the nonmonotonous time-course response of metabolic and functional cell and tissue parameters played an important role in achieving dose-response curve nonmonotony.

2. The Time-Course Changes of Metabolic Parameters of Mammalian Tissues and Cells by Action of Ionizing Radiation on Animals

The nonmonotonous shape of time-course curve of metabolic changes after irradiation of animals was well known and widely investigated. Bacq and Alexander [1] and Kuzin [2] were the first who discussed this phenomenon. Kuzin focused his attention on time-course changes of metabolic and functional tissue and cell parameters after irradiation and designated them as 'development of radiation damage in time after irradiation' [2].

The typical pattern of time-course changes of a metabolic parameter of mammalian tissues is shown in Figure 1. It depicts the changes of ornithine decarboxylase (ODC) enzyme activity in thymus of rats exposed to single total whole-body γ -irradiation at minimal lethal dose 6 Gy [3]. ODC is the first and rate-limiting step in the biosynthesis of natural polyamines in animal. Polyamines are included in regulation of cell growth, division and differentiation [4].

The time-course changes of ODC activity after irradiation at a dose 6 Gy were nonmonotonous. On day 1 after irradiation the ODC activity was shown to decrease reaching 6% of control. On day 7 the ODC activity was about 400% of control. On day 30 the ODC activity dropped in value again and comprised 6% of control at that time. The likely period of ODC activity oscillation is within 30 days. The time-course changes of ODC activity in thymus of irradiated rats were obviously nonmonotonous.

In many cases after action of ionizing radiation on animal and mammalian cells in culture the time-course curves of metabolic parameters were nonmonotonous [1–3, 6–8].

A new notion of the time-course changes of metabolic cell and tissue parameters in irradiated animals was proposed. So, the concept of a nonmonotonous metabolism response to acute action of ionizing radiation on an organism was introduced. The concentrations of different cell constituents maintained on constant level by constant synthesis in the cell and constant disappearance of cell constituents. If irradiation or another agent changes this level, the systems keeping up homeostasis by direct and feed-back regulation return it to the norm. Since the system is inert, the value of metabolic parameter could be higher than normal level. Thus, it may lead to a stage of hypercompensation. Thereafter, the changes of constituent level repeat, but in a less swing. In this case the time-course curve of metabolic changes runs as oscillations damping in time.

The neuroendocrine and cytokine factors are involved in the metabolic changes after acute whole-body irradiation and the effect of these factors may be very essential for time-course curve shape.

Figure 1. Time-course changes of ornithine decarboxylase (ODC) activity in thymus of rats exposed to single total γ -irradiation at a dose of 6 Gy (^{60}Co , dose-rate $0.55 \text{ cGy min}^{-1}$). Each point is mean $\pm$ SE of 5 to 11 animals. ODC activity was evaluated by measuring the rate of $^{14}\text{CO}_2$ formation from L-(1- ^{14}C)-ornithine as described in [5]. The initial value of ODC activity in thymus of control rats was $202 \pm 31 \text{ pmol CO}_2 \text{ mg}^{-1} \text{ protein h}^{-1}$. Data are expressed as the percentage of ODC activity from the control rates of the same ontogenesis period. Statistical significance was calculated by Student's *t*-test.

3. Dose-Response Nonmonotony

At present in certain experiments, nonmonotony of the dose-response curve for metabolic cell and tissue parameters was observed [6–8] after acute irradiation at a low and minimal lethal doses in animals and in cell culture.

This phenomenon was never discussed previously.

The nonmonotony of dose-response curve for metabolic cell and tissue parameters by acute irradiation was supposed to result from the nonmonotony of the time-course curves. The dose-response nonmonotony appeared to occur: if the

Figure 2. Dose-response for ODC activity in thymus on day 7 after single whole-body γ -irradiation of rats. Each point is the mean $\pm$ SE of 5 to 11 animals. Details are as in Figure 1.

dose of irradiation was changed, the rate of metabolic response also changed. The position of time-course curve maximum and minimum on time axis varied correspondingly. Similarly, the variations of radiation doses modified amplitudes and the period of the oscillations of metabolic parameters. Depending upon a dose, the value of metabolic parameters at some time-point could be registered below or above that of the control. Thus, due to nonmonotonicity of dose-response curve the radiobiological effects of acute irradiation are inversely related to dose at some time-point after irradiation. Figure 2 presents the dose-response curve of thymus ODC activity on day 7 after the whole-body irradiation of rats with the doses of 0.1, 1, 2, and 6 Gy. ODC activity in thymus of rats exposed to dose 0.1 Gy was below the control, while in thymus of rats exposed to dose of 1, 2 and 6 Gy it was enhanced. The recovery of thymus cellularity occurred on the 7th day after irradiation. That day the dose-response curve of thymus ODC activity was non-

Figure 3. Effect of chronic irradiation at a dose-rate of 1.1 cGy day^{-1} on ODC activity in lung, spleen and thymus of rats. In this and following figures the number of irradiated rats is indicated at experimental points. Control values are shown as a hatched band of the standard error of the mean. The initial values of ODC activity in lung, spleen and thymus of the control rats were 21.3 ± 3.3 , 24.6 ± 3.6 and $202 \pm 31 \text{ pmol CO}_2 \text{ mg}^{-1} \text{ protein h}^{-1}$, respectively. Other details are as in Figure 1.

monotonous. The low value of ODC activity on day 7 after irradiation with a dose of 0.1 Gy may be explained by the low rate of returning to control level.

4. Nonmonotonous Time-Course and Dose-Response Changes of Metabolic and Functional Cell and Tissue Parameters of Animals upon Irradiation at Low Dose-Rates

Signal transduction pathway takes part in metabolic response of living cells to ionizing radiation. It may explain the high radiosensitivity of cell metabolism to low doses of radiation. Under chronic irradiation a time-course curve coincides with a dose-response one. The first portion of irradiation initiates the metabolism changes and triggers the systems which keep up homeostasis. Then the first metabolic response is modified by constant action of low dose-rate irradiation. As a result the metabolic oscillation may be increased or diminished, and in any event is certainly modified. Our experiments demonstrated the nonmonotonous metabolism response to chronic irradiation of animals. The specificity of time-course response for each metabolic parameter and its dependence on dose-rate value were shown. Rats were exposed to low dose-rate γ -irradiation in a special room with γ -source [9]. ODC activity in radiosensitive (thymus, spleen) and radioresistant (lung) organs were studied. The dose-rates used were 1.1 (Figure 3) and $12.9 \text{ cGy day}^{-1}$ (Figure 4). In all cases ODC activity in different organs dropped in early terms (on day 8,

Figure 4. Effect of chronic irradiation at a dose-rate of $12.9 \text{ cGy day}^{-1}$ on ODC activity in lung, spleen, and thymus of rats. Details are as in Figure 3.

Figure 5. Changes in mass of body, thymus, and spleen of rats upon chronic irradiation at a dose-rate of $12.9 \text{ cGy day}^{-1}$. Data represent the mean $\pm$ SE. Figures near points are the number of irradiated rats. Statistical significance was calculated by Student's *t*-test.

Figure 6. Time-dependence of noradrenaline level in hypothalamus by chronic irradiation of rats. Each point is the mean $\pm$ SE of 6 to 12 animals. The amount of noradrenaline in hypothalamus of control rats is 421 ± 20 ng per g of wet tissue weight. Details are as in Figure 3.

15) and normalized in distant ones. Tenfold increase in dose-rate (12.9 cGy per day instead of 1.1 cGy per day) caused increasing radiobiological effect in some time points. However, in case of exposure at a dose-rate of 12.9 cGy per day, slow recovery of ODC activity to control level was observed. Moreover, inverse relation to dose-rate is shown. The inverse relationship between the decrease in ODC activity and irradiation dose was noticed in the spleen on day 15 of the exposure. The ODC activity in the spleen after 15 days of irradiation at a dose-rate 1.1 cGy day⁻¹ and 12.9 cGy day⁻¹ was 25.2 ± 1.4 (Figure 3) and 42.1 ± 4.4 percentage (Figure 4) of control, respectively. Difference is significant by $P < 0.01$. Since we analyzed ODC activity at a fixed time point (15 day), it is not improbable that the greatest radiobiological effect at a dose-rate 12.9 cGy day⁻¹ could occur early (in the interval between 8 and 15 days) as compared with dose-rate 1.1 cGy day⁻¹. This phenomenon is explained in terms of nonmonotony concept. Thus, the rats adaptation to low dose-rate irradiation in most cases is attended with returning of ODC activity to normal level. On day 45 of irradiation, the body weight and the organ mass also returned to normal level (Figure 5). Studying the response of the central nervous systems metabolism to low dose-rate chronic irradiation is of great interest. The metabolism of catecholamines in hypothalamus was investigated. The catecholamine values were measured spectrofluorimetrically in hypothalamus of control and irradiated rats [10]. These compounds play an important role in animal stress response. Hypothalamus is a central regulatory area of the sympathetic adrenal system. Drastic changes in the level of catecholamines (dopamine and noradrenaline) were found in hypothalamus of rats exposed to continuous ionizing irradiation at a dose-rate of 1.1 and 2.1 cGy per day. Figure 6 shows the changes of the amount of noradrenaline in hypothalamus of rats under conditions of irradiation described above. The noradrenaline amount in hypothalamus was shown to

decrease on day 4 of irradiation at a dose-rate 2.1 cGy day^{-1} , then it increased to 220% of control value on day 8 and greatly exceeded control on day 90 of exposure. The dose-response curve of the catecholamine amount in the hypothalamus of irradiated rats was nonmonotonous. During the irradiation a swing of oscillations in catecholamine level became larger. Significant sensitivity of CNS metabolism to low dose-rate irradiation can be assumed. Adaptation of animals to low dose-rate irradiation is accompanied by increase of the catecholamine amount in the hypothalamus of irradiated rats.

5. Changes of Membranous and Other Structural Cellular Components Under Chronic Irradiation of Animals at Low Dose-Rates

Under chronic irradiation of rats at a dose-rate $12.9 \text{ cGy day}^{-1}$ on day 90 (cumulated dose 10 Gy) thymus mass and the ODC activity normalized. It was noted that incorporation of ^{14}C -acetate in proteins and lipids of thymus of irradiated rats increased slightly [11]. These observations imply that thymus cells metabolism is essentially normalized. At the same time the deep changes of important cellular components were observed. The profound alterations in thymus nuclear and chromatin lipid metabolism were shown. In thymus nuclei from irradiated rats the amount of phosphatidylserine (PS) increased and in chromatin the value of PS and phosphatidylinositol (PI) enhanced (Table I). These lipids play an important role in signal system pathways. The data suggest the involvement of nuclei and chromatin lipids in cell response to the action of dose-rate irradiation. The lipid metabolism in thymus nuclei from rats exposed to γ -irradiation at a low dose rate of 3 cGy day^{-1} was investigated. In early terms (on day 8, 15), when ODC activity in thymus cells decreased, the synthesis of thymus nuclear lipids was activated, especially that of cholesterol (Figure 7). At a later period (on day 45 and 90) thymus ODC activity normalized, and on day 65 of irradiation lipid synthesis also recovered to normal level. At the same time deep changes of nuclear lipid composition took place. Although in early terms the phospholipid amount did not change, at a later period (on day 65) the significant sphingomyelin and cardiolipin decrease was shown (Table II).

Therefore, thymus cell response to chronic low dose-rate irradiation included the important nuclear lipids, and of special interest was the role of nuclear sphingomyelin as being involved in sphingomyelin cycle [12]. Probably, the nonmonotonous changes of metabolic parameters were integrated in nonmonotonous changes of metabolic-dependent functional parameters. Thus, the responses of immune functions of spleen lymphocytes in continuously irradiated rats were nonmonotonous [9]. The behaviour reactions of rats as an important test of CNS function were studied. The behaviour reactions of rats subjected to low-dose rate γ -irradiation were shown to be damaged [13]. The behaviour reactions of rats under chronic irradiation at a dose-rate 12.9 Gy day^{-1} fell below the control level on receiving 4–6 Gy and returned to normal level on receiving 8 Gy. At a dose 10 Gy the behaviour

Figure 7. Dose-response curves of incorporation of $2\text{-}^{14}\text{C}$ -acetate in cholesterol, total lipids (TL), free fatty acids (FFA), and diacylglycerol (DAG) of thymus nuclei under chronic irradiation of rats at a dose-rate of 3 cGy day^{-1} . Thymocytes were incubated in a solution containing 0.4 MBq of $2\text{-}^{14}\text{C}$ acetate per ml for 45 min, nuclei were isolated, lipids were extracted and separated by method of TLC using silica gel L in the system hexan:diethyl ether:glacial acetic acid (73:25:2, v/v) [11]. Each point is the mean $\pm$ SE of three experiments. The values of specific radioactivity of nuclear lipids are expressed as a percentage of control. For each experiment 3–5 animals were used. Statistical significance was calculated by Student's t -test.

Table I. The amount of phospholipids in thymus cells, nuclei and chromatin after chronic γ -irradiation of rats at a dose-rate of 12.9 cGy day⁻¹ up to a dose of 10 Gy (μ g lipid per mg protein)&

Lipids	Thymocytes		Nuclei		Chromatin	
	control	irradiation [#]	control	irradiation [#]	control	irradiation [#]
Sphingomyelin	1.1 $\pm$ 0.1	119 $\pm$ 12	1.4 $\pm$ 0.3	107 $\pm$ 8	1.1 $\pm$ 0.3	93 $\pm$ 11
Phosphatidylcholine	22.7 $\pm$ 0.8	105 $\pm$ 12	15.1 $\pm$ 4.8	116 $\pm$ 12	17.1 $\pm$ 3.0	134 $\pm$ 17
Phosphatidylserine	3.7 $\pm$ 0.1	114 $\pm$ 27	1.8 $\pm$ 0.5	141 $\pm$ 2*	1.9 $\pm$ 0.4	156 $\pm$ 2*
Phosphatidylinositol	3.1 $\pm$ 0.1	110 $\pm$ 10	2.0 $\pm$ 0.4	139 $\pm$ 20	1.6 $\pm$ 0.4	171 $\pm$ 9*
Phosphatidylethanolamine	7.5 $\pm$ 0.4	103 $\pm$ 9	4.5 $\pm$ 1.4	110 $\pm$ 13	5.4 $\pm$ 1.3	111 $\pm$ 16
Cardiolipin	1.7 $\pm$ 0.2	92 $\pm$ 6	1.0 $\pm$ 0.2	112 $\pm$ 11	1.0 $\pm$ 0.1	88 $\pm$ 7

& Each point is the mean $\pm$ SE of three experiments. For each experiment 3–5 animals were used.

* Difference is significant by $P < 0.02$.

[#] The values are expressed as percentage of control under paired comparison.

The methods used for cell, nuclei and chromatin isolation are as described in [11]. Lipids were extracted by chloroform: methanol (2:1, v/v) solution, purified and then separated using TLC. A system methylacetate: *n*-propanol: chloroform: methanol: 0.025% KCl (25:25:25:10:9, v/v) was used to separate phospholipids in silica gel H. Their amount was determined by the method for assay of lipid phosphorus and was expressed as μ g lipid per mg protein of cells or organelles [11]. Statistical significance was calculated by Student's *t*-test.

Table II. The amount of phospholipids in thymus nuclei under chronic γ -irradiation of rats at a dose-rate of 3 cGy day⁻¹ (μ g lipid per mg protein)&

Lipids	25 rad		50 rad		150 rad		200 rad	
	control	irradiation [#]	control	irradiation	control	irradiation	control	irradiation
Sphingomyelin	1.5 $\pm$ 0.2	82 $\pm$ 24	1.8 $\pm$ 0.2	87 $\pm$ 6	1.1 $\pm$ 0.2	117 $\pm$ 18	2.0 $\pm$ 0.1	59 $\pm$ 10*
Phosphatidylcholine	23.5 $\pm$ 0.6	98 $\pm$ 10	19.0 $\pm$ 0.6	98 $\pm$ 21	14.6 $\pm$ 1.5	105 $\pm$ 1	24.2 $\pm$ 0.4	104 $\pm$ 7
Phosphatidylserine + Phosphatidylinositol	4.5 $\pm$ 1.2	60 $\pm$ 16	3.8 $\pm$ 0.5	142 $\pm$ 33	5.9 $\pm$ 1.4	88 $\pm$ 4	6.0 $\pm$ 0.1	96 $\pm$ 9
Phosphatidylethanolamine	11.2 $\pm$ 0.8	101 $\pm$ 16	6.0 $\pm$ 0.6	139 $\pm$ 17	6.5 $\pm$ 1.5	95 $\pm$ 8	8.9 $\pm$ 0.8	93 $\pm$ 8
Cardiolipin	1.7 $\pm$ 0.4	122 $\pm$ 6	1.7 $\pm$ 0.3	98 $\pm$ 15	1.1 $\pm$ 0.3	133 $\pm$ 9	2.53 $\pm$ 0.4	74 $\pm$ 4*

& Each point is the mean $\pm$ SE of four experiments. Other details are as in Table I.

Table III. The lipid amount (μg lipid per mg protein) in synaptosomal membrane of brain cortex after chronic irradiation of rats at a dose-rate of $12.9 \text{ cGy day}^{-1}$ up to dose of 20 Gy (155 days)[&]

Lipids	Control	Irradiation [*]
Total phospholipids	419 ± 88	$75.5 \pm 7.8^*$
Lysophosphatidylcholine	20.8 ± 3.0	$71.3 \pm 5.6^*$
Phosphatidylcholine	33.3 ± 14.2	$68.3 \pm 4.5^*$
Phosphatidylethanolamine	110.0 ± 17.9	$77.5 \pm 6.6^*$
Phosphatidylinositol	25.9 ± 2.0	93.8 ± 4.1
Phosphatidylserine	69.0 ± 12	94.2 ± 20
Sphingomyelin	52.8 ± 7.6	97.7 ± 32.8
Cholesterol	100.0 ± 21	$75.0 \pm 4.9^*$

[&] Each point is the mean $\pm$ SE of three experiments.

[#] The values are expressed as percentage of control under paired comparison.

^{*} Difference is significant by $P < 0.05$.

Brain cortex synaptosomal fraction were prepared according to [14]. Then synaptosomal fraction was shocked in hypotonic solution and synaptosomal membrane were isolated by centrifugation [13]. Details are as in Table I.

reactions also dropped. The pronounced changes of the structure of the synaptosomal membrane from the brain cortex were preceded by the nonmonotonous changes of rat attention and exploratory activity. Synaptosomal membrane fraction was isolated from the brain cortex of the rats subjected to chronic irradiation at a dose-rate of $12.9 \text{ cGy day}^{-1}$ up to dose of 20 Gy on day 155. Behaviour reactions at this stage of irradiation were diminished. At that time in synaptosomal brain cortex membrane the amount of phospholipids and cholesterol also decreased. The amount of total phospholipids, lysophosphatidylcholine, phosphatidylcholine and phosphatidylethanolamine deep diminished. Thus, it was seen, that irradiation changed the metabolism of brain choline-related phospholipids. The content of cholesterol also decreased and proportion cholesterol/phospholipids did not change under irradiation (Table III). Thus the metabolic response to low dose-rate irradiation was observed in the structural component of such important membrane as synaptosomal brain cortex membrane. In the course of the animal adapting to low dose-rate irradiation, the behaviour reactions dropped accompanied by deep changes of lipid composition of synaptosomal membrane. The lipid amount in synaptosomal membrane was shown to be highly radiosensitive (Table III). Therefore, under chronic low dose-rate irradiation of the animal, the adaptation is attended by changes of value of different structural-metabolic parameters. These observations are important in understanding the mechanism of animal adaptation to continuous stress condition via metabolic nonmonotonous response.

6. Discussion

We made some qualitative conclusions concerning time-course and dose-response relations of metabolic and functional parameters in cells and tissues of an animal exposed to radiation. The nonmonotony of the time-course curve is assumed to play a significant role in occurrence of nonmonotony of relationship between dose and response of metabolic and functional parameters in irradiated animals.

The mathematical model was proposed to explain the nonmonotonous shape of dose-response curve for initiation of genetical defects in human lymphoid cells after low doses of irradiation [15]. According to this concept, lymphoid cell population is heterogeneous, and part of cell population consists of very radiosensitive cells. This part of the population dies after irradiation at low doses and in this way the dose-response relation is modified and the nonmonotony of dose-response curve occurs. Burlakova and co-authors have investigated biochemical, biophysical and functional properties of the cell and tissues of mice exposed to radiation in the wide range of dose-rates from 0.6 cGy day^{-1} to 6 cG day^{-1} . It was shown that the dose-response of changes in investigated properties is of nonlinear (bimodal) nature. The explanation is given in terms of the changes in the relations between the quantity of damages and activity of repair systems induced by action of different doses of radiation [16]. The above mentioned concepts are aimed at an explanation of a bimodal character of the first peak on the curve of dose-response observed by acute low dose irradiation and by chronic irradiation at a low dose-rate. These concepts are hardly applicable to our data. The concept of nonmonotony of metabolic and functional cell responses to damage is open for discussion. Our concept deals with oscillations observed during prolonged period after acute irradiation and in the course of chronic irradiation. The concept may be useful for discussion of experimental data obtained by continuous irradiation of animals. Thus, elevated levels of heat shock protein 70 mRNA in cells of lung, spleen, and small intestine are observed upon chronic in vivo γ -irradiation of mice at a dose-rate of 3 cGy day^{-1} for 30 days. On the other hand, this phenomenon was not observed upon chronic irradiation of mice at a dose-rate of 1 and 12 cGy day^{-1} at the same time [17]. Authors wrote that it was unclear whether the differences in dose-rate or the time of treatment were responsible for the absence of the stress response upon chronic irradiation at the dose-rates of 1 and 12 cGy day^{-1} . By our concept, both factors are responsible for this effect, since the differences in dose-rate values lead to differences in times of gene induction. Now the quantitative approach is necessary to clear up a biological role of nonmonotonous metabolic response.

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