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EXPERIMENTAL ARTICLES

The Effect of Ionizing Radiation on the Creatine–Creatine Kinase System in the Rat Brain and the Radioprotective Effect of Creatine

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Abstract—Creatine kinase (CK) and its substrates creatine (Cr) and creatine phosphate (CrP) form the Cr–CrP–CK system, which, along with its buffer and transport roles in the energy metabolism of the cell, also performs the function of maintenance of the stability of mitochondrial membranes, which together determines the neuroprotective role of Cr. Considering the anti-apoptotic and antioxidant properties of Cr, as well as the fact that oxidative stress is the basis of radiation damage, we studied (1) the effect of ionizing radiation on the dynamics of post-radiation changes in CK activity and Cr content in outbred rats after their irradiation with a sublethal dose of 4.5 Gy in the presence and absence of Cr and (2) the radioprotective efficacy of Cr for the Cr–CK system of the brain and the survival rate of rats after irradiation at a $LD_{70/30}$ dose equal to 6.3 Gy. The data we obtained showed a high degree of radiosensitivity and adaptability of the Cr–CK system of the brain, as well as a significant radioprotective efficacy of Cr, both in relation to the Cr–CK system of the brain and the survival rate of rats. The radioprotective effect of Cr calculated using the Kaplan–Meier statistical survival model was 38.6% for the group that received the Cr in 0.9% glucose solution compared to the control group that received water instead of Cr and 30.3% compared to the control group treated with 0.9% glucose. For the group that received the aqueous solution of Cr, the effect was smaller, 20.5% compared with the corresponding control group, which is obviously related to the relatively worse availability of the Cr to cells from the aqueous solution.

Keywords: creatine kinase, creatine, brain, blood serum, rats, ionizing radiation, creatine radioprotective efficiency

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INTRODUCTION

Creatine kinase (EC 2.7.3.2.), which catalyzes the reversible transfer of the phosphoryl residue from ATP to creatine (Cr), plays a key role in the energy homeostasis of cells with high energy demands, in particular, brain cells. Together with its substrates Cr and creatine phosphate (CrP), creatine kinase (CK) forms the Cr–CrP–CK system, which, according to recent studies [1–3], performs not only buffer and transport roles in cell energy metabolism but also a number of other functions, including the maintenance of the stability of mitochondrial membranes, which is related to the protective role of Cr. In the brain, dimeric cytoplasmic CK is present both in the cytoplasm and in subcellular structures, such as the endoplasmic reticulum and the plasma membrane of neurons, as well as in membranes that are rich in acetylcholine receptors [1]. It was shown that BB-CK is involved in the maintenance of calcium homeostasis and neurotransmitter processing, as well as axonal and dendritic transport [4]. With the help of the ATP produced in a mitochondria octameric brain mitochondrial CK in proteolipid complex completes the process of direct phosphorylation of the Cr into CrP.

In addition, mitochondrial CK has a stabilizing and protective effect on the opening of permeable mitochondrial pores, and, therefore, affects the processes of apoptosis and necrosis [1]. The main part of the necessary Cr comes into the human body with food, while the rest is synthesized in the body. Cr from the bloodstream easily passes through the blood–brain barrier using a special Na-dependent transport protein, after which it is actively extracted from the extracellular brain fluid by neurons and oligodendrocytes, whereas astrocytes that lack the transport protein can synthesize Cr by themselves [1, 3].

Ionizing radiation (IR) has a direct effect on cells, causing breaks in chemical bonds in macromolecules, including enzyme proteins, and indirect effect, which is determined by the formation of free radicals that aggressively interact with macromolecules [5]. As a result, there are changes in both the activity and the biosynthesis of enzymes, which serves as the basis for

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the use of analysis of the activity of enzymes for evaluation of the effect of ionizing radiation and various radioprotectors [6–8]. Previously, we studied the effect of X-ray radiation on the activity of a number of key enzymes of cellular metabolism, including CK, in various organs and serum; CK was the most radiosensitive enzyme [9].

We note that, until recently, the focus of the research was on muscle CK. Interest in the brain CK has increased in the last decade due to the discovery of its role in the pathogenesis of neurodegenerative diseases and aging processes, which are based on oxidative stress, as well as the protective effect of Cr on these processes. Cr contributes to noticeable neuroprotection in experimental animal models of amyotrophic lateral sclerosis, Huntington's disease, parkinsonism, traumatic injuries of the brain and spinal cord, and cerebral ischemia [10–13]. Using various methodological approaches numerous studies have shown that the protective effect of Cr is mainly due to its antiapoptotic and antioxidant properties. The basis of the etiopathogenesis of radiation damage is also oxidative stress; therefore, in this paper we investigated the effect of ionizing radiation on the Cr–CK system of rat brain and the anti-radiation effect of Cr.

MATERIALS AND METHODS

In the two series of experiments we used 60 and 72 white outbred male rats weighing 180–210 g, respectively; they were kept in standard conditions, including the standard diet. A total single irradiation of rats was performed in an isolated room designed for these experiments on a RUM-17 X-ray unit (voltage 200 kV, current 20 mA, Cu-Al filter; skin focal distance 50 cm, irradiation dose 1.78 Gy/min).

In the first series of experiments animals that were exposed to radiation at a dose of 4.5 Gy were divided into one experimental and two control groups of 15 individuals each: the experimental group 2 weeks before and 2 weeks after irradiation was given creatine per os at a dose of 1 g/kg weight, which was dissolved in 0.9% glucose solution, and 2 control groups, 0.9% glucose solution and water, respectively. In addition, a group of intact animals equivalent in the number of rats was used as a source control. Post-radiation effects were investigated on the 1st, 7th, and 15th day after radiation exposure by choosing five rats for each period from the experimental and control groups. In the second series of experiments on the survival of rats after irradiating them at a dose of $LD_{70/30}$ equal to 6.3 Gy in the presence and absence of creatine, the animals were divided into six groups of 12 individuals each. Rats of the first and second experimental groups were given creatine dissolved in 0.9% glucose solution and in water per os at a dose of 1 g/kg weight, respectively; the two irradiated control groups were given the 0.9% glucose solution and water, respectively. In addition, as a control, two groups of intact animals were used that received or did not receive creatine solution in 0.9% glucose, respectively. The radioprotective properties of the radioprotector were assessed by the survival rate of animals for 30 days after irradiation according to the Kaplan–Meier statistical method of the SPSS 16 software [14]. The levels of CK activity and the content of Cr were determined in the extracts of the brain and serum of rats that survived in the experimental groups 30 days after irradiation, as well as in the above control groups,.

To determine the activity of CK and the content of creatine in the brain and serum, decapitation of animals was performed after ether anesthesia. Blood was collected in tubes without an anticoagulant; after it was coagulated, it was centrifuged in a refrigerated centrifuge at 800 *g* for 20 minutes and the resulting serum was used on the same day to determine the enzyme activity. The brain was washed from the blood with cooled saline and homogenized in an extracting buffer solution with a pH of 7.2 (0.1 mol/L Tris-HCl, 5 mmol/L dithiothreitol (DTT), and 1 mmol/L ethylene diamine tetraacetate (EDTA)). Extracts obtained after centrifugation of the homogenates at 23000 *g* for 30 minutes were used to determine the enzyme activity. CK activity was determined spectrophotometrically by the accumulation of the creatine reaction product [15]. Enzyme activity was expressed in μmol/g wet tissue per minute for brain tissue and μmol/L min for serum.

The creatine content in μg/g wet brain weight and in μg/mL serum was determined spectrophotometrically according to the method of Ennor and Rosenberg [16].

The reagents we used were creatine monohydrate, creatine phosphate disodium salt (tetrahydrate), ADP sodium salt, 1-naphthol, diacetyl(2,3-butanediol), dithiothreitol, and EDTA from Sigma Aldrich (Germany), as well as 40% glucose (OAO Yerevanskaya HFF).

For visual images, the calculated means and their standard deviations for the CK activity levels and the Cr content on the plots are expressed as the percent relative to the control level, which were the corresponding data obtained for intact animals (the results of three independent experiments).

The SPSS (Statistical Package for Social Science) package was used for statistical data processing. The nature of the distribution of the data obtained was determined by the Kolmogorov–Smirnov method. A comparative analysis was performed using the non-parametric Mann–Whitney test. Differences were considered significant at $p < 0.05$. Correlation analysis was performed using the non-parametric Spearman test.

RESULTS

The biochemical criterion of radiation damage to enzymes is the loss of their inherent activity. The final manifestation of a biological lesion of enzyme molecules in vivo may be delayed for days [5]. Previously, when studying the effect of sublethal X-ray doses on the activity of a number of enzymes, including CK, in various organs and serum of rats, we showed that: (1) exposure causes oscillatory changes in the level of CK activity over time, which, in a number of cases, are biphasic in nature; (2) CK is one of the most radiosensitive and adaptable enzymes [9]. Considering this, as well as the neuroprotective properties of Cr, it was of interest to investigate the effect of X-ray radiation on the dynamics of post-radiation changes in the levels of CK activity and Cr content in the brain and serum of rats in the presence and absence of the natural adaptogen Cr.

Figure 1 presents data on the dynamics of changes in the activity of CK (A) and creatine content (B) in the brain and serum of rats after their total irradiation at a dose of 4.5 Gy and the effect of creatine supplements on these changes. These data suggest that on the first postradiation days in the brain of rats of control groups 2 and 3 that received water and glucose solution, a statistically significant drop in CK activity occurred (by 40–50%), whereas in the experimental group of rats that received Cr in glucose solution the enzyme activity decreased by only 20% (Fig. 1, A, (a), 1). At the same time, in these control groups, an increase in the content of endogenous Cr in the brain (by almost 30%) was observed, which, however, is not statistically significant. The increased Cr content in the brain of rats of the experimental group 1 (by 27%) is obviously related to the enrichment of the body of these animals with a creatine supplement (Fig. 1, B, (a), 1), since correlation analysis did not reveal any possible relationship between this increase and the aforementioned drop in the activity of the cerebral CK of these animals ($r_1 = 0.017$, $p = 0.983$). In this regard, we note that according to the literature data the content of creatine and its derivatives may increase in the cell by up to 30% with prolonged use of creatine as an ergogenic agent [17]. The small fluctuations observed in the first post-radiation day in the levels of CK activity and in the Cr content in the blood serum of animals of all three groups are statistically insignificant (Figs. 1A and 1B, (a)).

On the 7th postradiation day in the experimental group of animals the decrease in the activity of the cerebral CK on the first day was replaced by a statistically significant increase in the activity of the enzyme, which is most likely compensatory in nature; by the 15th day stabilization of the CK activity at the control level occurred in the brain as well and in serum. The Cr content in the brain of these rats on this day is kept at the level of control. In control groups 2 and 3 there was a tendency to restore the level of cerebral CK activity to the control level, which, however, on the 15th postradiation day was replaced by a significant drop in the level of enzyme activity (by 20–40%), apparently, due to the exhaustion of the native adaptive capabilities of the Cr–CK-system of the brain of these animals. At the same time, the levels of Cr content in the brain and serum of rats of these groups at specified times fluctuate around the control level. Thus, it follows from the above data that Cr has a significant anti-radiation effect on the Cr–CK system of the brain, thus providing the adaptation of the energy metabolism of the tissue to radiation exposure.

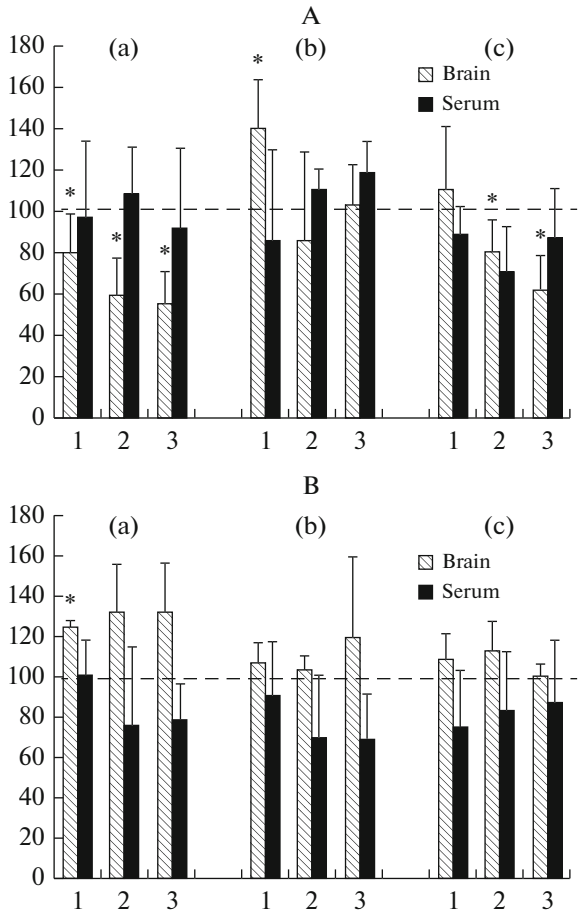

Fig. 1. The dynamics of changes in CK activity (A,%) and creatine content (B,%) in rat brain and serum after total exposure at a dose of 4.5 Gy in the presence and absence of creatine. $n = 5$ for each group of animals: (1) the group treated with creatine at a dose of 1 g/kg body weight in a 0.9% glucose solution; (2 and 3) the control groups that received, respectively, water and the 0.9% glucose solution. Post-radiation periods: (a) 1st, (b) 7th, (c) 15th day. The dashed line is the control level (intact rats) which was taken as 100%. *The difference from control is significant at $p < 0.05$–0.001.

The radioprotective effect of Cr was also evaluated using the model that is commonly accepted in this type of research, that is the survival of rats after their total irradiation at a dose of 6.3 Gy, which is equal to

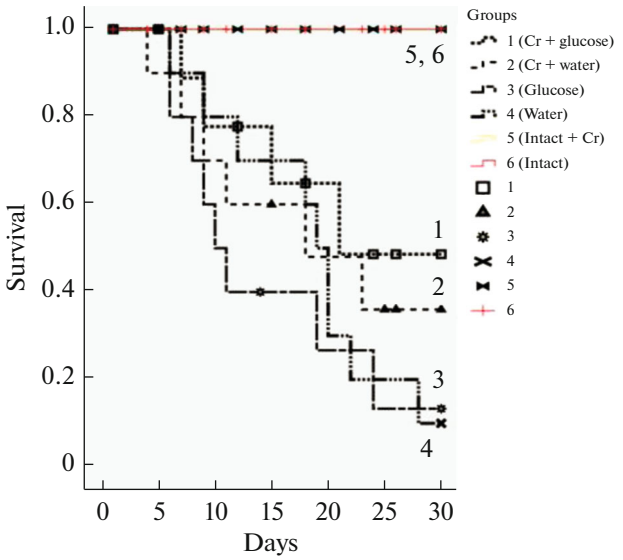

Fig. 2. The survival curves of rats subjected to total irradiation at a dose of $LD_{70/30}$ = 6.3 Gy when using creatine at a dose of 1 g/kg body weight 2 weeks before and 2 weeks after irradiation. Experimental groups 1 and 2, (creatine + 0.9% glucose) and (creatine + water), respectively; control groups 3 and 4, 0.9% glucose and water, respectively; group 5, intact rats treated with creatine + 0.9% glucose; group 6, intact rats. n = 12 for each group.

the $LD_{70/30}$. Taking the literature data on the increase in the absorption of creatine per os in the presence of sugars into account [18], in the experimental groups we used a Cr solution in both water and 0.9% glucose. Preliminary experiments showed that (1) the most effective dose of creatine supplement is 1 g/kg rat weight, (2) the drug we used is non-toxic, (3) the most effective route of administration is 2 weeks before and 2 weeks after irradiation, and (4) an increase in Cr in the brains of experimental animals is directly proportional to the dose received, which indicates the permeability of the blood–brain barrier for our Cr. Figure 2 shows the survival curves of rats in the control and experimental groups subjected to total irradiation at a dose of $LD_{70/30}$ = 6.3Gy using creatine at a dose of 1 g/kg body weight 2 weeks before and 2 weeks after irradiation. Comparative analysis of these data shows that the death of animals in experimental group 1, which was treated with Cr in glucose solution, began from the 7th day after irradiation, whereas in control groups 3 and 4 this occurred 2 days earlier, which indicates the greater resistance of the first group of animals to the effects of IR; moreover, in the control groups, the death of animals lasted to the 25–28th days, whereas in experimental group 1, their death stopped on the 21st day after irradiation; this occurred despite the fact that already from the 16th day after irradiation the physical condition of the rats in this group, in general, was significantly better than in the controls. Finally, the mortality of animals in the control group 4, where only 3 out of 12 rats survived, significantly exceeds the mortality of rats in experimental group 1, in which 8 of 12 animals survived. A somewhat more modest anti-radiation effect was found in experimental group 2 treated with an aqueous solution of Cr: the death of rats in this case began from day 6 and ended on day 23; 6 of 12 rats survived as opposed to the corresponding control group 4, in which, as we mentioned above, only 3 out of 12 rats survived. The radioprotective effect of Cr calculated using the Kaplan–Meier statistical survival model for group 1 relative to the control group 4 is 38.6%; in relation to control group 3 it was 30.3%. As expected, given the relatively poor absorption of Cr from an aqueous solution [18], the radioprotective efficiency of Cr for experimental group 2 was somewhat lower compared to the corresponding control group 4; the radioprotective effect was 20.5%. Thus, this series of experiments unambiguously indicates the radioprotective efficacy of Cr, which increased the resistance and adaptability of the body to IR.

Changes in the levels of CK activity and Cr content in their brain and blood serum were determined in order to assess the adaptation properties of the cerebral Cr–CK system of rats that survived 30 days after irradiation, both in the experimental and control groups. Figure 3 suggests that in experimental groups 1 and 2, which were treated with Cr in glucose solution and aqueous Cr solution, respectively, the rat brain CK activity and creatine content are almost the same as the control level, which indicates a complete recovery of the physical condition of the rats to the norm due to the radioprotective efficiency of Cr and the significant adaptation capabilities of the Cr–CK system. Thus, these data are fully consistent with the above data on survival. However, an increased level of CK activity and an equivalent increase in the Cr content were detected in the blood serum of these animals. It may be assumed that during this period Cr obtained in the experiment as a protector is released in blood from organs and tissues.

The data on the control irradiated rats (group 3) that received water instead of Cr solution, in which 3 rats survived from 12 animals 30 days after irradiation, are of particular interest. In these surviving animals, the activity of cerebral CK was significantly elevated (by more than 2 times compared to intact rats) with a normal level of Cr in it, which clearly indicates that the increased natural resistance of these animals to IR involves the brain Cr–CK system. At the same time, in the blood serum of these animals the level of CK activity was significantly lower than in the control and the Cr content was two times higher with a high degree of correlation of these parameters ($r_3 = 0.865$, $p_3 = 0.05$). The following assumption may provide an explanation: a compensatory increase in the level of CK activity during the adaptation of cell energy metabolism to IR in the brain and also, probably, in other organs, requires additional synthesis of endogenous Cr, which

is known to occur in the liver, kidney, and spleen, and is transported to other organs and tissues through the blood [13]; this results in an increase in the Cr content in the serum and substrate inhibition of CK activity by high Cr concentrations [19]. The data on control group 4, which received an aqueous Cr solution, show that in the absence of sugars in the brain, there is an accumulation of Cr (it is almost 1.5 times higher than the control level).

DISCUSSION

It is known that ionizing radiation induces the formation of free oxygen radicals in cells, which causes changes in the antioxidant system, leading to oxidative stress and, as a result, damage to cell components, such as enzymes, DNA, and lipids. The effect of oxidative stress depends on its severity. Cells can return to their original state with minor impairments, while more pronounced oxidative stress causes cell death. The biochemical criterion of damage to enzymes is the loss of their inherent activity, which serves as the basis for the use of analysis of the enzyme activity to assess the effect of ionizing radiation and to study the effectiveness of various radioprotectors [5]. CK exhibits high sensitivity to oxidative stress at both the post-transcriptional and gene levels [3, 20, 21]. It is involved in the immediate response of the cell to the effects of stress, which lead it to energy depletion. In this regard, the activity of this enzyme, as well as the CrP/ATP and CrP/Cr ratios, are commonly regarded as an indicator of the energy status of the cell [1, 3]. In this context, it should be noted, the basis for interest in the study of the radioprotective activity of Cr was the discovery of its protective role in neurodegenerative diseases, aging, and the action of stress factors, such as ultraviolet radiation [22], which is based on the pathophysiology of similar molecular mechanisms of oxidative stress [1, 3, 13]. Based on numerous studies using various methodological approaches, the following mechanisms of the protective action of Cr are discussed: (1) the mechanism of maintenance of energy homeostasis, which helps to maintain high ATP/ADP and CrP/ATP ratios in a cell in stressful situations; (2) the mechanism of maintenance of Ca^{2+} homeostasis; (3) the mechanism of its anti-apoptotic action, which is caused by the joint participation of Cr and mitochondrial CK in inhibition of the opening of permeable mitochondrial pores, which is an early trigger for apoptosis; and (4) the mechanism of antioxidant action due to the joint inducing effect of Cr and F1-ATPase on the efficiency of coupling of the respiratory chain and the ATP production, which results in lower production of reactive oxygen species [1, 22–24]. Thus, it may be argued that the Cr–CrP–CK system improves the resistance of cells to stress, making them less susceptible to damage. These facts and the data we obtained allow discussing the effect of IR on the Cr–CK system and the anti-radiation properties of the natural adaptogen Cr.

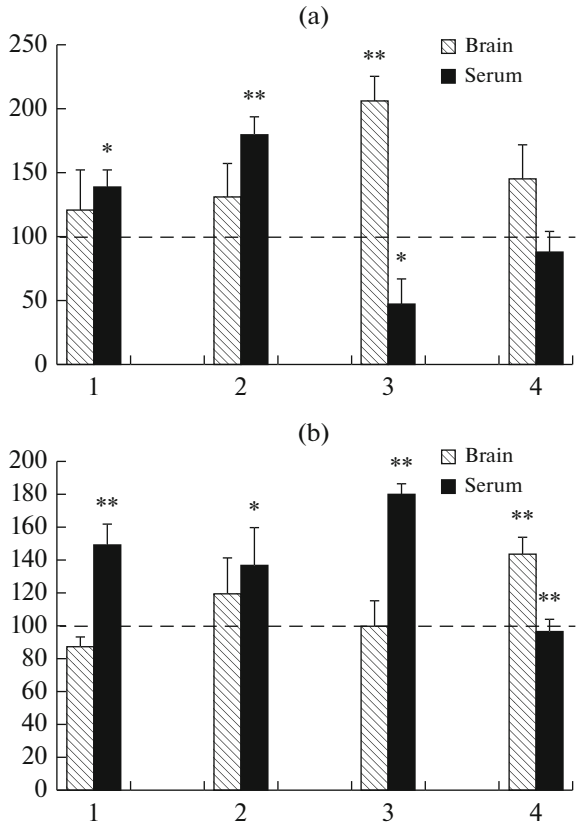

Fig. 3. The CK activity (%) (a) and creatine content (%) (b) in the rat brain and serum after treatment with creatine in a dose of 1 g/kg body weight of rats 2 weeks before and 2 weeks after irradiation on the 30th day after total irradiation at a dose of 6.3 Gy. $n = 5$ for each group of rats, with the exception of group 3. 1, the irradiated group treated with creatine at a dose of 1 g/kg body weight in a glucose solution; 2, the irradiated group treated with creatine dissolved in water at a dose of 1 g/kg of weight; 3, the control irradiated group ($n = 3$) treated with water; 4, the intact group of animals treated with creatine dissolved in water. The dashed line is the control level (intact rats), which was taken as 100%. *Significant difference compared to the control, $p < 0.05$–0.001.

An analysis of the post-radiation changes in the levels of activity of the cerebral CK under the action of a sublethal dose of IR equal to 4.5 Gy in the presence and absence of Cr (Fig. 1) showed that:

(1) These changes are oppositely directed in time and oscillatory in nature, which is apparently due to triggering of different adaptation mechanisms during different post-radiation periods; it should be noted that the post-radiation oscillations of the activity levels, as well as isoenzyme spectra and kinetic parameters, were shown for many enzymes and, in general, are characteristic of post-radiation biological effects [6, 7, 9, 25];

(2) The high radiosensitivity of CK, whose level of activity in control groups 2 and 3, which did not receive Cr, dropped by 40–50% on the first day; in this context, it should be noted that the inactivation by free radicals shown for CK is due to the presence of easily modifiable SH-groups in the active center of the enzyme [21, 26]; further, despite the subsequent trend to an increase, the CK activity in these groups decreased again by the final observation period, apparently, as a result of the exhaustion of the native adaptation potential of the cerebral Cr–CK system;

(3) Addition of Cr had a protective effect on the Cr–CK system (experimental group 1) by reducing the damaging effect of radiation in the first days of radiation and stimulating the adaptation of the Cr–CK system during subsequent periods, thus providing full restoration of the energy metabolism of the brain by the end of the study;

(4) The 0.9% glucose solution does not have antiradiation activity with respect to the Cr–CK system (control group 3) and, in this sense, as a Cr solvent does not affect the radioprotective data obtained with respect to Cr.

It is known that the resistance of organisms to the effects of extreme factors, which are based on oxidative stress, is largely determined by the effectiveness of the regulatory mechanisms for maintenance of cell energy [27]. It should be noted that the dynamics of these changes in the Cr–CK system in the presence and absence of Cr, as a biological additive that helps to maintain the energy metabolism of the brain tissue, largely coincides with the rat survival dynamics we studied. As shown by a comparative analysis of data obtained using the model of survival of rats after their irradiation at a dose of $LD_{70/30}$ = 6.3 Gy (Fig. 2) the death of animals in the experimental groups treated with Cr began 2 days later and ended 5–7 days earlier than in the control groups that did not receive Cr, which indicates a significant increase in the resistance of rats enriched in Cr to the effects of radiation. At the same time, from the 13th postradiation day, the mortality of animals in these groups dropped sharply (one and two cases in groups 1 and 2, respectively), which coincides with the stabilization of the levels of CK activity and the Cr content in the brain by the 15th radiation day in the first series of experiments. The radioprotective effect of Cr, which was calculated using the Kaplan–Meier statistical survival model, was 38.6% for group 1 that received a Cr solution in 0.9% glucose compared to the control group that received water instead of Cr, and 30.3% compared to the control group that received the 0.9% glucose solution. As expected, the radioprotective effect of the Cr aqueous solution, given its relatively worse absorption, turned out to be somewhat lower, 20.5%, compared with the corresponding control group. Thus, this series of experiments unambiguously supports the radioprotective efficacy of Cr, which increased the resistance and adaptability of the body to ionizing radiation.

Data on the CK activity and Cr content in the brains of rats that survived in the control group of animals 30 days after irradiation at a dose of 6.3 Gy point to significant native adaptive properties of CK and the participation of the Cr–CK system in the survival process. The data on the control irradiated rats (Fig. 3, group 3) that received water instead of a Cr solution in which 3 rats survived 30 days after irradiation from 12 animals are of particular interest. In these animals, a significantly elevated level of activity of cerebral CK was determined (by more than 2 times compared to intact rats) with a normal level of Cr in it, which clearly indicates that the increased natural resistance of these animals to the IR effects involved the brain Cr–CK system. It may be assumed that the mechanisms of long-term adaptation associated with the additional biosynthesis of the enzyme protein become activated during this post-radiation period. Hence, the data on a significant two-fold increase in the Cr content in the blood serum of these animals, which correlates (r_3 = 0.865, p_3 = 0.05) with an equivalent drop in the activity of serum CK, are of considerable interest. As an explanation of this phenomenon, the following assumption may be made: a compensatory increase in the level of CK activity during the adaptation of cell energy metabolism to the effects of IR in the brain, as well as in other organs, requires additional synthesis of endogenous Cr, which is performed in the liver, kidneys, and spleen, and is transported to other organs and tissues by blood [13], which apparently also leads to an increase in the Cr content in serum and to substrate inhibition of CK activity by high Cr content [19]. The data we obtained relative to the control group of intact animals that received an aqueous Cr solution show that in the absence of sugars in the brain Cr accumulation (almost 1.5 times higher than the control level) occurs, which causes a significant, while smaller compared to the Cr glucose solution, radioprotective effect of an aqueous Cr solution.

CONCLUSIONS

(1) The dynamics of post-radiation changes in the levels of activity of cerebral CK, which is induced by total X-ray irradiation of rats at a sublethal dose of 4.5 Gy, in the presence and absence of Cr was oscillatory and indicates the high level of radiosensitivity and significant adaptability of the enzyme; however, the Cr level in the brain of these animals remains within the control level.

(2) The indicated dynamics of post-radiation changes points to the radioprotective efficacy of the Cr in relation to the Cr–CK system of the brain.

(3) The radioprotective effects of the Cr solution in 0.9% glucose and water, which was calculated according to the Kaplan–Meier statistical survival model,

after irradiation of rats at an $LD_{70/30}$ = 6.3 Gy dose are 30.3 and 20.5%, respectively.

(4) The Cr–CK system of the rat brain has certain native adaptive properties to the action of IR, which significantly increases in the presence of Cr as a dietary supplement.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical approval. All applicable international and institutional guidelines for the care and use of animals were followed.

REFERENCES

1. Wallimann, T., Tokarska-Schlattner, M., and Schlattner, U., *Amino Acids,* 2011, vol. 40, pp. 1271–1296.
2. Schlattner, U., Tokarska-Schlattner, M., and Wallimann, T., *Biochim. Biophys. Acta,* 2006, vol. 1762, pp. 164–180.
3. Nersesova, L.S., *Zhurn. Evolyuts. Biokhim. Fiziol.*, 2011, vol. 47, no. 2, pp. 120–127.
4. Ames, A., *Brain Res. Rev.,* 2000, vol. 34, pp. 42–68.
5. Kutsenko S.A., *Voennaya toksikologiya, radiobiologiya i meditsinskaya zashchita* (Military Toxicology, Radiobiology, and Medical Protection), Moscow: Foliant, 2004.
6. Catravas, G.N. and McHale, C.G., *J. Neurochem.,* 1975, vol. 24, no. 4, pp. 673–667.
7. Burlakova, E.B., Konradov, A.A., and Mal'tseva, E.L., *Khim. Fizika,* 2003, vol. 22, no. 2, pp. 21–40.
8. Somayaji, Y., Vidya, V., Vishakh, R., Shetty, J., Peter, A.J., and Kumari, S., *Cogent Biology*, 2016, vol. 2, no. 1, pp. 1–14.
9. Nersesova, L.C., Gazaryants, M.G., Mkrtchyan, Z.C., Meliksetyan, G.O., Pogosyan, L.G., Pogosyan, S.A., Pogosyan, L.L., Karalova, E.M., Avetisyan, A.S., Abroyan, L.O., Karalyan, Z.A., and Akopyan, Zh.I., *Radiats. Biologiya. Radioekologiya,* 2013, vol. 53, no. 1, pp. 55–62.
10. Pastula, D.M., Moore, D.H., and Bedlack, R.S., *Cochrane Database Syst. Rev.,* 2012, pp. 1–26.
11. Brewer, G.J. and Wallimann, T., *J. Neurochem.,* 2000, vol. 74, pp. 1968–1978.
12. Adcock, K.H., Nedelcu, J., Loenneker, T., Wallimann, T., and Wagner, B.P., *Dev. Neurosci.,* 2002, vol. 24, pp. 382–388.
13. Salomons, G.S. and Wyss, M., *Subcell. Biochem.,* 2007, vol. 46, no. 17, p. 351.
14. Goel, M.K., Khanna, P., and Kishore, J., *Send to Int. J. Ayurveda Res.,* 2010, vol. 1, no. 4, pp. 274–278.
15. Petrova, T.A. and Lyzlova, S.N., *Vestn. LGU,* 1985, no. 24, pp. 88–90.
16. Ennor, A.H. and Rosenberg, H., *Biochem. J.,* 1954, vol. 57, p. 295.
17. Dobgenski, V. Santos, M.G., D., and Kreider, R., *J. Nutri. Health May,* 2016, vol. 2, no. 1, pp. 1–5.
18. Costallat, B.L., Miglioli, L., Silva, P., Novo, N.F., and Joao, L.G., *Rev. Bras. Med. Esporte,* 2007, vol. 13, no. 1, pp. 18–21.
19. Lyslova, S.N. and Stefanov, V.E., *Phosphagenkinases,* 1991, Boston.
20. Malone, J. and Ullrich, R., *Radiat. Res.,* 2007, vol. 167, no. 2, pp. 176–184.
21. Aksenov, M., Aksenova, M., Butterfield, D.A., and Markesbery, W.R., *J. Neurochem.,* 2000, vol. 74, pp. 2520–2527.
22. Lenz, H., Schmidt, M., Welge, V., Schlattner, U., Wallimann, T., Elsasser, H.-P., Wittern, K.-P., Wenck, H., Stab, F., and Blatt, T., *J. Invest. Dermatol.,* 2005, vol. 124, pp. 443–452.
23. Kay, L., Nicolay, K., Wieringa, B., Saks, V., and Wallimann, T., *J. Biol. Chem.,* 2000, vol. 275, pp. 6937–6944.
24. Meyer, L.E., Machado, L.B., Santiago, A.P., Silva, W.S., De Felice, F.G., Holub, O., Oliveira, M.F., and Galina, A., *J. Biol. Chem.,* 2006, vol. 281, pp. 37361–37371.
25. Nersesova, L.S., Petrosyan, M.S., Gazaryants, M.G., Mkrtchyan, Z.S., Meliksetyan, G.O., Pogosyan, L.G., and Akopyan, Zh.I., *Radiats. Biologiya. Radioekologiya,* 2014, vol. 54, no. 5, pp. 522–530.
26. Koufen, P. and Stark, G., *Biochim. Biophys. Acta,* 2000, vol. 1501, no. 1, pp. 44–50.
27. Hochachka, P.W. and Somero, G.N. *Biochemical adaptation*, 1984, New Jersey: Princeton University Press.