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PATHOPHYSIOLOGICAL MECHANISMS OF NITROGEN METABOLISM DYSREGULATION UNDER THE INFLUENCE OF IONIZING RADIATION

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The purpose of the study was to investigate the pathophysiological mechanisms of dysregulation of nitrogen metabolism in animals after exposure to different doses of ionizing radiation. Male Wistar rats were exposed to ionizing radiation. Alanine- and aspartate aminotransferase activities were determined in the animals' liver, blood, skeletal and cardiac muscles. The total nitrogen and its components content was determined in the urine and blood of rats. Data obrained demonstrated that ionizing radiation induces a pronounced, dose-dependent suppression of alanine- and aspartate aminotransferase activities in mitochondrial and cytoplasmic fractions of liver and cardiac muscle tissues, with the most severe impairments observed at 5.82 Gy, indicating significant depletion of cellular functional capacity and energy failure. In skeletal muscle, the decrease was mainly limited to mitochondrial fractions, while cytoplasmic enzyme activity remained relatively stable, suggesting higher tissue resistance to radiation injury. Blood analysis revealed a significant increase in transaminase activity, indicating systemic cytolysis, which may serve as a biomarker of radiation injury severity. In addition, radiation exposure led to a marked reduction in daily urine output, along with a significant increase in urinary nitrogen excretion and a dramatic rise in protein catabolism. These findings highlight the complex metabolic and functional impairments in vital organs and systems caused by ionizing radiation. The identified biochemical alterations may be utilized as informative biomarkers for early diagnosis, severity assessment, and monitoring of radiation injury, as well as for evaluating the effectiveness of radioprotective interventions.

Key words: total body gamma irradiation, ionizing radiation, muscle tissue, liver, alanine aminotransferase, aspartate aminotransferase, total urinary nitrogen, pathophysiological mechanisms.

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ПАТОФІЗІОЛОГІЧНІ МЕХАНІЗМИ ДИЗРЕГУЛЯЦІЇ АЗОТИСТОГО ОБМІНУ В УМОВАХ ВПЛИВУ ІОНІЗУЮЧОГО ОПРОМІНЕННЯ

Метою дослідження було вивчення патофізіологічних механізмів порушення регуляції азотного обміну у тварин після впливу різних доз іонізуючого випромінювання. Щурів-самців лінії Вістар піддавали впливу іонізуючого опромінення. В печінці, крові, скелетному та серцевому м'язах тварин визначали активність аланін- та аспартатамінотрансферази. У сечі та у крові щурів визначали вміст загального азоту та його компонентів. Отримані дані свідчать, що іонізуюче опромінення викликає виражене дозо-залежне пригнічення активності аланін- та аспартатамінотрансферази у мітохондріальних і цитоплазматичних фракціях печінки та серцевого м'яза. Найбільш помітні порушення спостерігались при дозі 5,82 Гр, що свідчить про значне виснаження функціонального потенціалу клітин і розвиток енергетичної недостатності. У скелетному м'язі зниження активності стосувалося переважно мітохондріальної фракції, тоді як цитоплазматичні показники залишалися відносно стабільними, що свідчить про більшу стійкість цієї тканини до радіаційного впливу. У крові відмічалося достовірне підвищення активності трансаміназ, що свідчить про розвиток системного цитолізу і може бути використане як маркер тяжкості радіаційного ураження. Окрім цього, опромінення супроводжувалося зниженням добового діурезу на фоні значного підвищення екскреції загального азоту з сечею та різким зростанням інтенсивності білкового розпаду. Отримані дані свідчать про комплексне ураження обміну речовин і функціонального стану життєво важливих органів під впливом іонізуючого випромінювання. Виявлені зміни можуть бути використані як інформативні біомаркери для ранньої діагностики, прогнозування і моніторингу тяжкості радіаційного ураження та ефективності радіопротекторних заходів.

Ключові слова: тотальне γ-опромінення, іонізуюча радіація, м'язова тканина, печінка, аланінамінотрансфераза, аспартатамінотрансфераза, загальний азот сечі, патофізіологічні механізми.

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Disturbances in nitrogen metabolism are one of the early and sensitive markers of metabolic disorders that occur under the influence of ionizing radiation (IR). The relevance of studying the pathophysiological mechanisms of these disorders is due to the increasing use of IR sources in medicine, energy engineering, industry and in conditions of military conflicts. A deeper understanding of changes in nitrogen metabolism is important for the development of effective diagnostic and therapeutic approaches to the prevention and treatment of radiation injuries [7, 9].

Enzymatic systems that ensure the maintenance of metabolic balance in the body are especially sensitive to the action of the radiation factor. Among them, transaminases, in particular alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which catalyze transamination reactions, which are key processes in the regulation of protein and energy metabolism, occupy an important place.

The study of the activity of transamination enzymes attracts great attention, since the process of transamination is a source of α-amino acids, which is especially important for the renewal of body proteins.

Furthermore, the amino acids formed as a result of transamination are sources of physiologically active amines and peptides, which is especially important for nervous tissue [5, 14]. It has been proven that the brain, liver and myocardium are characterized by high activity of aminotransferases, which explains the importance of transamination processes for these organs [4, 6, 11].

Transamination provides integration of amino acid and carbohydrate-energy metabolism, promotes formation of substrates for tricarboxylic acid cycle (Krebs cycle) and participates in synthesis of glucose through gluconeogenesis. Thus, ALT and AST play a critical role not only in maintaining amino acid balance, but also in providing cells with energy resources.

A number of studies are devoted to the problem of elucidating the activity of transamination enzymes under various influences [10, 15,], including irradiation [8, 12]. However, considering that transdeamination of amino acids is a universal mechanism of amino acid involvement in energy and gluconeogenic processes, and most amino acids are deaminated through glutamic acid, it was interesting to investigate the mechanisms of dysregulation of transamination processes under the influence of ionizing radiation.

The purpose of the study was to investigate the pathophysiological mechanisms of dysregulation of nitrogen metabolism in animals after exposure to different doses of ionizing radiation.

Materials and methods. The studies were conducted on 30 sexually mature male Wistar rats weighing 180–220 g, maintained on a standard vivarium diet.

The maintenance, handling and manipulation of animals were carried out in accordance with the "General Ethical Principles of Animal Experiments" approved by the Fifth National Congress on Bioethics (Kyiv, 2013), while being guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985), the methodological recommendations of the State Clinical Research Center of the Ministry of Health of Ukraine "Preclinical Studies of Drugs" (2001) and the rules for the humane treatment of experimental animals and conditions approved by the Bioethics Commission of Odessa National Medical University (record No. 32D dated 17.03.2016).

For the experiment, sexually mature animals were subjected to total single-time gamma irradiation with ^{60}Co in special chambers made of organic glass, in the morning on an empty stomach on the "Agat" telegammatherapy device, the distance to the absorption source was 75 cm, the dose rate was 0.54 Gy/min, the absorbed dose was 3.0 Gy (group I) and 5.82 Gy (group II), (10 experimental animals were irradiated in each group). The control group consisted of 10 intact animals.

For biochemical studies, the animals were removed from the experiment by euthanasia under propofol (iv, 60 mg/kg) anesthesia. After the animals were dissected, blood was collected, previously centrifuged at 3000 rpm for 10 min to obtain blood serum. To determine biochemical parameters in tissues, the liver, heart and anterior thigh muscle group were removed, washed with chilled saline, ground and homogenized in a 9-fold volume of 0.32 mol sucrose in 0.05 mol Tris buffer, pH=7.36 in a homogenizer with Teflon surfaces and subjected to differential centrifugation.

The nuclei were pelleted at 1000 rpm for 10 min, then the mitochondria – at 12000 rpm for 20 min, resuspended in a homogenizer in the separation medium containing 0.1 % Triton X-100 solution at the rate of 1 ml of 0.1 % Triton solution per 500 mg of tissue, and left on ice for 30–35 min.

For biochemical studies, mitochondria, mitochondrial supernatant, blood, and urine of the experimental animals were used.

Determination of total urine nitrogen was performed by the Kjeldahl method [1]. The total amount of protein for calculating the specific activity of enzymes in liver fractions and blood was estimated spectrophotometrically by the biuret method. Electrophoresis of serum proteins was performed on acetate cellulose with subsequent densitometry [3].

Determination of AST and ALT activity in tissues and blood serum was performed spectrophotometrically [2]. The activity of aminotransferases in tissues was expressed in μmol pyruvate/g per hour, in blood – μmol pyruvate/mL per hour of incubation at a temperature of 37 °C.

The obtained data were subjected to statistical processing using the method of estimating the mean, as described in "T tables," and the $\chi 2$ criterion, along with computer programs. The minimum statistical probability was determined at $p < 0.05$.

Results of the study and their discussion. Both the ALT and AST activitiers were determined in isolated subcellular fractions (mitochondria and cytoplasm) of liver, heart and skeletal muscle, as well as in blood serum. The distribution of AST and ALT activity in subcellular fractions of liver, heart and skeletal muscle of sexually mature rats was different, but in individual organs the ratio of mitochondrial and cytoplasmic forms of isoenzymes is similar for each of the enzymes (Table 1).

Table 1

ALT, AST activity in tissues of intact and irradiated animals at different doses (M±m)

Tissues		Indicator	Groups of animals (n=30)		
			Intact animals	Irradiated at a dose of 3.0 Gy	Irradiated at a dose of 5.82 Gy
Liver	mitochondria	ALT	8.73±0.76	5.22±0.56*	4.36±0.32*
		AST	7.02±0.78	6.58±0.42	4.14±0.46*
	Cytoplasm	ALT	5.14±0.42	3.67±0.36*	2.83±0.26*
		AST	2.98±0.34	2.25±0.26	2.08±0.26
Cardiac muscle	mitochondria	ALT	4.47±0.37	3.98±0.32	1.74±0.22*
		AST	4.32±0.36	4.92±0.34	2.56±0.31*
	Cytoplasm	ALT	7.60±0.59	5.84±0.36*	2.79±0.28*
		AST	10.34±0.92	9.86±0.88	7.32±0.64*
Skeletal muscle	mitochondria	ALT	2.54±0.26	2.36±0.24	2.18±0.22
		AST	4.06±0.28	3.84±0.38	2.04±0.21*
	Cytoplasm	ALT	7.66±0.58	7.22±0.56	7.08±0.52
		AST	9.72±0.86	9.26±0.78	8.89±0.72
Blood		ALT	2.77±0.26	3.89±0.34*	6.64±0.48*
		AST	2.85±0.28	3.24±0.32	5.36±0.38*

Notes: 1. ALT and AST activity in tissues is expressed in μmol pyruvate (PK)/g •hr, in blood – in μmol pyruvate (PK)/l •hr; * – $p<0.05$ – statistical differences of the investigated indexes compared with the corresponding indicators in intact animals.

Thus, the activity of AST in the liver of sexually mature rats prevails in the mitochondrial fraction in comparison with the supernatant by approximately 2.4 times, and in skeletal muscle, on the contrary, AST prevails in the supernatant in comparison with the mitochondrial one by 2.4 times.

The activity of ALT and AST in the liver of sexually mature rats prevails in the mitochondrial fraction by 1.7 times, and in the heart there is an inverse relationship between the mitochondrial and cytoplasmic fractions of ALT isoenzymes. Similar results, which highlighted the same type of distribution of ALT, were registered in skeletal muscle with a significant predominance of the cytoplasmic isoenzyme in the supernatant (by 3 times).

In the liver, the greatest activity of ALT is registered in mitochondria. AST in the liver, although it exhibits significant activity, is localized both in mitochondria and in the cytoplasm.

In the heart muscle, AST dominates in the cytoplasm with a relatively high activity of ALT. In the blood, the activity of both enzymes is the lowest, which indicates the physiological integrity of cells and the limitation of the release of enzymes into the extracellular environment in healthy animals.

Total γ-irradiation of rats at a dose of 3.0 and 5.82 Gy leads to regular changes in the activity of aminotransferases, both in the supernatant and in the mitochondrial fractions of the liver, cardiac and skeletal muscles.

In the liver, inhibition of the function of both aminotransferases was registered, and ALT to a greater extent, especially in mitochondria. The decrease is especially pronounced at a dose of 5.82 Gy, where ALT activity in mitochondria is halved ($p<0.05$). AST activity remains more stable, although it also shows a decrease.

The predominant direction of changes in ALT activity in the heart muscle is the suppression of enzyme activity at a dose of 3.0 Gy by 1.3 times against the background of a slight increase in AST activity in mitochondria, which may indicate compensatory-adaptive responses of cells to radiation stress. However, a further increase in the dose to 5.82 Gy is accompanied by a sharp decrease in mitochondrial and cytoplasmic ALT activity by 2.57 and 2.72 times, respectively, and AST by 1.69 and 1.4 times, respectively, which indicates the depletion of compensatory mechanisms and the development of structural and functional damage to cardiomyocytes.

The predominant direction of ALT shifts in skeletal muscle is a moderate inhibition of activity in mitochondria at a dose of 3.0 Gy by 7 %. With a further increase in the dose to 5.82 Gy, ALT activity in mitochondria decreases by 1.17 times compared to control values. At the same time, cytoplasmic ALT activity remains relatively stable and decreases by only 8 % at 5.82 Gy, which indicates the preservation of basic amino acid metabolic processes in the cytosol even at high doses of radiation.

We observed similar changes in radiation-induced enzymatic activity for AST.

ALT activity in the blood at a dose of 3.0 Gy increases by 1.4 times, and at 5.82 Gy – by 2.4 times compared to control ($p<0.05$).

The activity of AST in the blood at 3.0 Gy increases by 1.14 times, and at 5.82 Gy – by 1.88 times compared to the control ($p<0.05$).

Along with changes in the activity of aminotransferases under the conditions of exposure to ionizing radiation, the concentration of total urine nitrogen increases by 16.9 % at a dose of 3.0 Gy, and at a dose of 5.82 Gy it increases by more than 1.3 times against the background of a decrease in daily diuresis, the indicator

of which in animals irradiated with a dose of 5.82 Gy is almost 1.6 times significantly lower compared to intact animals (P<0.05; Table 2).

Table 2

Daily diuresis and nitrogen metabolism in intact and irradiated animals at different doses (M±m)

Indicator	Intact animals (n=10)	Radiation doses (n=20)	
		3.0 Gy	5.84 Gy
Daily diuresis (ml/day)	8.76±0.63	7.68±0.56	5.54±0.32*
Total Urine Nitrogen (mg/mL)	7.16±0.50	8.28±0.76	9.76±0.74*
Total urine nitrogen (mg/day)	76.52± 3.74	89.10±6.46	100.80±6.73*
Protein breakdown (mg/day)	478.2±23.4	556.90±40.4	630.00±42.1*
Serum protein content (g/l)	58.97±1.09	55.73±1.24	52.36±1.26*

Note: * – p<0.05 – statistical differences of the investigated indexes compared with the corresponding indicators in intact animals.

There is an increase in the concentration of total nitrogen in 1 ml of urine at a dose of 3.0 Gy by 15.6 %, and at a dose of 5.82 Gy it significantly increases by almost 1.4 times. The protein-synthesizing function of the liver also undergoes significant changes, since there is a shift in the electrophoretic fractions of blood serum proteins towards hypoalbuminemia, this is especially pronounced in animals irradiated at a dose of 5.82 Gy. Along with this, there is an increase in protein breakdown, the indicator of which in those irradiated at a dose of 3.0 Gy exceeds that of the control group by 16.5 %, and in those irradiated with a dose of 5.82 Gy – by almost 32 % (p<0.05).

Therefore, the conducted studies allow to explain the reaction of tissues to the action of ionizing radiation through the prism of the disruption of the functional state of cellular and subcellular structures, primarily mitochondria and cytosol pathogenetically.

The results obtained indicate that ionizing radiation causes pronounced tissue-specific, dose-dependent disturbances in the activity of ALT and AST in the liver, cardiac and skeletal muscles and blood. The liver and cardiac muscle were found to be the most sensitive to radiation damage, in which already at a dose of 3.0 Gy there is a significant inhibition of transaminase activity in mitochondria and cytoplasm, which increases at a dose of 5.82 Gy.

We observed earlier the analogous enzymatic activity in the descendents of irradiated rats [13].

In skeletal muscle, the decrease in the enzyme activity is less pronounced and mainly affects the mitochondrial fraction, while cytoplasmic activity remains relatively stable, which indicates the relative radioresistance of skeletal muscle.

The increase in the level of ALT and AST in the blood is clearly expressed and dose-dependent, which indicates the systemic release of enzymes from damaged tissues and the formation of cytolytic syndrome, which can be used as a diagnostic criterion for the degree of damage to the body. This situation is also comparable with data [11] who indicated tissue damage with plasma membrane disruption or apoptosis and at least plasma membrane bleb formation as the result of AST serum activity increrased.

Violation of amino acid metabolism is accompanied by a significant increase in the concentration of total nitrogen in the urine and a decrease in diuresis, which indicates the activation of protein catabolism and increased protein breakdown. The analogous idea was stidied and explained detaily – i.e. increased α-amino nitrogen atoms remove from amino acids during their oxidative degradation with theis successive urine excrteation to prevent high ammonium potential toxicity – as the result of epigenetic disturbances of amino acids metabolism [14].

Hypoalbuminemia and shifts in the electrophoretic fractions of blood proteins indicate the suppression of the protein-synthesizing function of the liver. Increased protein breakdown against the background of a decrease in their synthesis is a typical sign of the catabolic syndrome that develops under the influence of ionizing radiation. Similar effects were also found in workers involved in the radioactive fuel leak cleanup at the Fukushima nuclear power plant and were continued for 9-18 months after the incident [8].

In terms of the probable pathophysiological mechanism of radiation-induced liver dysfunction, amino acid metabolism disorders, metabolic disorders of the blood, muscles, etc., one can refer to laboratory studies of 326 mRNA in which altered expression of the transcription factor P53 was detected after cellular radiation damage [9]. The authors proved the leading role of p53 in metabolism regulation in conditions of nitrogen, glutathione and arachidonic acid metabolism disruption together with glycolysis/gluconeogenesis impairment due to radiation exposure of cells.

Thus, the established changes in nitrogen metabolism against the background of irradiation reflect profound disorders of amino acid and energy metabolism, which may underlie the pathogenesis of radiation sickness. The obtained data are of promising importance for the further development of biochemical criteria for early diagnosis and monitoring of the severity of radiation damage, as well as for assessing the effectiveness of protective and therapeutic measures when exposed to ionizing radiation.

Conclusions

1. Ionizing radiation causes a dose-dependent decrease in the activity of ALT and AST in the tissues of the liver, heart and skeletal muscles, which reflects damage to energy metabolism and structural integrity of cells. In particular, at a dose of 5.82 Gy, the activity of ALT in mitochondria decreases by half, which indicates the suppression of the functional activity of hepatocytes, disruption of membrane integrity and degradation of mitochondrial structures, which are critically important for ensuring energy-dependent processes.

2. The predominant direction of changes in cardiac ALT is the suppression of the enzyme activity in the supernatant at a dose of 3.0 Gy by 1.3 times against the background of a slight increase in AST activity in mitochondria, which may indicate compensatory-adaptive reactions of cells to radiation stress. However, a further increase in the dose to 5.82 Gy is accompanied by a sharp decrease in mitochondrial and cytoplasmic activity of both ALT by 2.57 and 2.72 times, respectively, and AST by 1.69 and 1.4 times, respectively, which indicates the depletion of compensatory mechanisms and the development of structural and functional damage to cardiomyocytes.

3. A systemic increase in the activity of transaminases in the blood is an indicator of cytolysis. Thus, the activity of ALT in the blood increased by 2.4 times at a dose of 5.82 Gy, and of AST – by 1.88 times. This indicates a massive release of enzymes from damaged cells of the liver, heart and skeletal muscle, which can be used as an early diagnostic marker of radiation damage.

4. Violations of nitrogen metabolism under the influence of ionizing radiation are manifested by a decrease in diuresis. At the same time, there was an increase in the excretion of total nitrogen in the urine, an increase in the intensity of protein breakdown, and a decrease in the protein content in the blood serum, which indicates the development of hypoproteinemia.

Prospects for furthers researches include further studies to establish the prognostic efficacy of protein and nitrogen metabolism dysregulation markers in terms of ищер intraorganuc and systemic dysfunctions determining in conditions of ionizing radiation exposure.

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