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ROLE OF RADIOPROTECTORS IN MINIMIZATION OF STOCHASTIC EFFECTS OF RADIATION INCIDENTS

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The real threat of emergency situations in Ukraine dictates the need to take into account the experience of previous radiation accidents, during which a significant part of the population was exposed to low-dose radiation. In such a case clinical manifestations of irradiation were mostly absent, while the danger of stochastic (carcinogenic) effects remained. Therefore, at present, the strategy of radiation protection of the population should be aimed at revising and choosing effective and low-toxic anti-radiation means. The main criterion for the development of stochastic consequences of exposure is radiation-induced genome instability, which is a promoter of carcinogenesis. The use of radiomitigators, which are able to weaken the harmful effect of ionizing radiation on critical highly radiosensitive systems of the human body, is promising. Our research showed the radiomitigative effect of inosine in cultured human T-lymphocytes on the genetic level with the significant decrease in the frequency of gamma-induced chromosome aberrations. The results experimentally justified an expediency of use of radiomitigators in the conditions of an emergency situation to minimize the occurrence and development of stochastic effects in population.

***Key Words*: radiation emergency, stochastic effects, genome instability, chromosome aberrations, radiomitigators.**

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Protection of the genome, tissues, organs and organism as a whole of persons exposed to radiation due to various circumstances remains one of the urgent problems of clinical radiobiology and radiation medicine. The search for effective substances that would reduce the level of radiation-induced damage began over 70 years ago, when it was proposed to use cysteamine and its disulfide, cystamine, as radioprotective agents for acute irradiation of the human body. Back in the middle of the last century, the prominent radiobiologist N.V. Luchnik discovered that RNA preparations protect irradiated organisms from induced damage when administered both before and after irradiation. The involvement of significant contingents of the population exposed to prolonged and fractionated irradiation in elimination of the consequences of the Chernobyl accident contributed to the intensification of scientific and practical interest in the search for effective and low-toxic means to minimize radiation-induced disturbances [1].

There has been observed some progress in the development of the means to prevent long-term stochastic effects of radiation (leukemia, solid tumors). The most promising is the use of radiomitigators, which are able to weaken the damaging effect of irradiation on critical highly radiosensitive systems of the body, regardless of the timing of their use [2]. The nature of the effect of radiomitigators on the genetic structures of somatic cells in the body of exposed individuals remains insufficiently studied [3].

Today, taking into account the real threat of the emergency situation of exposure of the population of Ukraine, it is necessary to learn from the experience of previous radiation incidents [4, 5], in which a significant part of the population located far from the site of a nuclear incident was exposed to low doses of radiation that increased the risk of the stochastic effects in future. Therefore, at present, the strategy for radiation protection of the population should be aimed at revising and choosing effective radioprotective agents, primarily radiomitigators, whose action is effective under exposure to a wide range of doses.

MODERN VIEW ON STOCHASTIC EFFECTS OF IONIZING RADIATION

One of the main paradigms of radiobiology and radiation medicine is the reasoned division of the medical and biological effects of ionizing radiation (IR) into deterministic and stochastic effects. Deterministic effects are considered as a pathological condition caused by IR in high doses. Its severity depends on the radiation dose and has a dose threshold below which effects are not observed. Above the threshold dose, the probability of an effect occurring is 100%. These are skin reactions (erythema, epilation), radiation burns, fibrosis, impaired hematopoiesis, etc.

Stochastic, that is, probabilistic, effects are characterized by a linear non-threshold dependence of their occurrence on the IR dose. In this case, only the probability of the events under consideration, and not their severity, depends on the dose of IR. This means that even the smallest exposure increases the probability of the formation of a stochastic effect. The stochastic effects of irradiation include point mutations, rearrangements of chromosomes, and those radiobiological reactions that do not have a dose threshold and are of a probabilistic nature. It is important to focus researchers' attention on the fact that radiation carcinogenesis is also referred to as long-term stochastic results of low-dose irradiation [3].

As is known, the genome is one of the main targets of the damaging effect of IR. The main criterion for the development of stochastic consequences of irradia-

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Abbreviations used: DMF – dose modifying factor; IR – ionizing radiation.

tion is the accumulation of genetic changes, an early sign of which is genome instability, which is a promoter of radiation carcinogenesis [6–8]. It manifests itself even under exposure to doses, which are only slightly higher than the levels of natural background radiation [9]. Not only the nature, but also the period of development of radiation injury depends on the cell proliferation rate, the mechanisms of the reaction to irradiation, and the blood supply of irradiated tissues. Early radiation reactions are often caused by damage to stem cells and progenitor cells, resulting in a lack of functional mature cells. For example, a rapid decrease in the counts of highly radiosensitive mature lymphocytes is due to their apoptosis. Late radiation reactions in tissues are accompanied by a low rate of renewal of their cellular composition.

We also draw the attention of the professional community to the potential change of the radiosensitivity in COVID-19 convalescents. It is known that the effect of IR on the immune system manifests itself in early and long-term periods after irradiation. In this case, the death of individual subpopulations of lymphocytes is possible under irradiation in the low dose range. In the early terms after irradiation, a decrease in the content and proliferative activity of T-lymphocytes, as well as their migration activity, was revealed. Increased systemic inflammatory activity, which persists for a long time in COVID-19 convalescents, in combination with post-radiation inflammation can significantly modify cell radiosensitivity [10–11]. This justifies "a differentiated approach to predicting the occurrence of the IR stochastic effects…" [12].

ROLE OF RADIOPROTECTIVE AGENTS IN MINIMIZING STOCHASTIC EFFECTS

The main mechanisms for the formation of radioprotective effects are the suppression of free-radical oxidation processes and stimulation of post-radiation recovery. However, many traditional radioprotectors proved to be ineffective under long-term exposure due to high toxicity [13, 14]. The absence of universal radioprotective agents effective in a wide range of IR doses, according to some researchers, is due to the difference in the main molecular mechanisms of the detrimental effects of irradiation at low and high doses. In the case of protecting the body from acute irradiation in sublethal and lethal doses the main attention is paid to synthetic compounds and cytokines, while in a case of low-dose irradiation the preference is given to substances of natural origin [15, 16]. The contribution of antioxidants to the radioresistance of the body depends on the severity of radiation injury and linear energy transfer of radiation. Synthetic antioxidants are more effective as radioprotectors at doses that cause bone marrow syndrome as well as under the linear energy transfer increase. Since most radioprotectors of synthetic origin are highly toxic, it is more expedient to use a natural compounds or those synthesized on the basis of natural raw materials, which are less toxic to the human body [17, 18].

Radiobiologists focused their research interest on the radioprotective action of cytokines [19]. The protective effect of cytokines is determined by their hemo- and immunostimulatory activity, as well as the ability to increase the endogenous background of radioresistance and enhance the host antitumor response.

Organoselenium compounds (for example, selenium tetracysteine) possessing antioxidant activity are recognized as promising means for preventing radiation injuries due to their low toxicity [20]. In experimental studies, it was found that the radioprotective effectiveness of organoselenium compounds depends on the oxidation state of selenium. The optimal time for prophylactic administration of preparations with Se^{4+} is 24 h, and Se^{2+} is 1 h [21]. The results of epidemiological studies have shown that cancer rates are significantly higher in selenium-deficient regions.

The action of radioprotective agents is competitive with respect to the formation of a radiobiological effect. The radioprotective efficacy of different radioprotectors is expressed in terms of dose modifying factor (DMF). The DMF or reduction factor is defined as the ratio of radiation dosage producing similar effects in the presence or absence of the compound. Some increase in DMF value is achieved by using a mixture of two or more radioprotectors with different mechanisms of protective action. When using the most effective radioprotectors, DMF can reach values of 1.8–2.0.

Currently, purine compounds (xanthosine, caffeine, inosine) are considered as promising prophylactic agents to reduce radiation risks. It has been shown that the efficiency of reparation in a case of using purine compounds is associated with the activation of poly(ADP-ribose) polymerase, one of the key DNA repair enzymes [22]. Purine compounds can influence DNA repair systems indirectly through signaling pathways; also they affect the human brain under hypoxia via mitogen-activated protein kinase signaling pathways [23, 24]. It was concluded that the basis of the radioprotective properties of purine compounds is, on the one hand, their ability to neutralize reactive oxygen species and long-lived protein radicals, and, on the other hand, their ability to activate cellular mechanisms of post-radiation restoration and DNA repair processes.

Depending on the intended purpose of radioprotective agents, certain requirements are imposed on them, taking into account the effectiveness, duration of the protective effect, toxicity and tolerability in single and repeated use, the possibility of using under different irradiation in a wide dose range, and storage stability [1]. On this basis, a modern classification of radioprotectors was developed and proposed along with "modernization of the relevant terminology and differentiation of key definitions of the implementation's mechanism of radioprotective agents" [25]:

- Radioprotectors (chemical protection) are the agents that exert a protective effect at the physicochemical and biochemical levels, preventing the "oxygen effect" as a radiobiological phenomenon in the process of absorption of IR energy during DNA radiolysis. The action of radioprotectors begins from the moment the substance enters the tissues and is limited to 1–3 h, which is determined by the high rate of their metabolism in the body. The advantage of radioprotectors is their ability to increase the body's radioresistance within a few minutes, including the situation of exposure

to superlethal IR doses (10–15 Gy). This advantage is typical only for radioprotective agents of this classification group.

- Radiomodulators (biological protection) are the agents and nutritional supplements that increase the body's resistance to adverse environmental factors including IR [26]. This is a large group of natural compounds with antioxidant, antimutagenic, anti-inflammatory properties. The mechanism of their action is characterized by an increase in the general (nonspecific) resistance of the body along with a decrease in carcinogenic risk [27]. Radiomodulators include natural antioxidants and the most important components of the antioxidant system of cells (vitamins C, E, A, bioflavonoids, trace elements, etc.), natural stimulators of protein and nucleic acid synthesis (nucleosides), antihypoxants (melatonin, mexidol, etc.). Radiomodulators are low toxic and have no side effects at recommended doses. The radioprotective effect of these agents is directly related to adaptive reactions at the cellular and organism levels by modulating gene expression [25].
- Radiomitigators are radioprotective agents that realize their effect at the system level "by accelerating the post-radiation restoration of radiosensitive tissues through the activation of a number of anti-inflammatory signaling pathways and increased secretion of hematopoietic growth factors, used in the early stages after irradiation before the development of clinical manifestations of acute radiation injury" [25]. These agents differ from other radioprotectors in terms of the mechanism of realization of their antiradiation properties, since they are not directly related to the primary radiation-induced chemical and biochemical processes in cells. The optimal antiradiation effect is noted when they are used in the period from several hours to 4 days before irradiation at doses that cause bone marrow syndrome of acute radiation sickness. Radiomitigators include hormonal preparations of steroid structure and their non-steroid analogues; adjuvants of immunological reactions (vaccines, endotoxins, polysaccharides, polynucleotides, etc.); cytokines (tumor necrosis factor, growth factors, interferons, etc.); immunoregulatory peptides (for example, thymalin, thymogen, taktivin, thymoptin, etc.). The mechanism of radiomitigators action is associated with their ability to accelerate post-radiation regeneration of cells of a highly radiosensitive hematopoietic system [28, 29].

For a number of years, the team of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the National Academy of Sciences of Ukraine, has been studying the bioindication and modification of radiation effects at the genetic level in the culture of human peripheral blood lymphocytes. The radiomodifying effect of caffeine, verapamil, thymalin, etc. has been investigated and proven theoretically and experimentally [3]. Particular attention has been focused on the antioxidant inosine, exerting multivariant mechanisms of action, causing a wide range of biological activities, increasing the total radioresistance, and mobilizing the body's protective resources. We have shown for the first time that inosine decreased significantly the frequency of spontaneous chromosomal aberrations in non-irradiated peripheral blood T-lymphocytes of healthy individuals (Table 1) [30].

In the presence of inosine, the frequency of total chromosome aberrations decreased by 3.5 times, the frequency of chromatid-type aberrations decreased by 3.8 times, and more complex chromosomal rearrangements (translocations) that cause reproductive cell death, "disappeared".

The radiomitigating effect of inosine on human immunocompetent cells (T-lymphocytes) at the genetic level was also established in the studied range of gamma radiation doses (0.1–1.0 Gy) (Table 2) [30].

Inosine as radiomitigator decreased the frequency of gamma-induced chromosome aberrations in the studied dose range by 1.2–3.8 times. The most significant radiomitigating effect was observed in a case of irradiation in the low-dose range (0.1–0.2–0.3 Gy), reaching the average values of the spontaneous genetic rearrangements in T-lymphocytes of general population. By increasing the activity of reparation enzymes, inosine ensures the resistance of the human genome in a wide range of IR doses. This gives grounds to recommend inosine

Table 1. Effect of inosine on the frequency of spontaneous chromosomal aberrations in peripheral blood lymphocytes of healthy donors (mean group values)

Cytogenetic parameter	Frequency of aberrations per 100 metaphases	
Total chromosomal aberrations	2.8 ± 0.3	
Total chromosomal aberrations in presence of inosine	0.8 ± 0.1	$p \leqslant 0.05$
Chromosome-type aberrations	0.9 ± 0.2	
Chromosome-type aberrations in presence of inosine	0	$p \leqslant 0.05$
Chromatid-type aberrations	1.9 ± 0.1	
Chromatid-type aberrations in presence of inosine	0.5 ± 0.2	$p \leqslant 0.05$

Table 2. The frequency of radiation-induced aberrations of chromosomes in peripheral blood lymphocytes of healthy donors treated with inosine (mean group values)

Dose, Gy	Cytogenetic parameters per 100 metaphases			
	Aberrant cells,%	Total chromosomal aberration rate	Chromosome-type aberrations	Chromatid-type aberrations
0.1	6.1 ± 0.5	6.1 ± 0.5	2.99	3.07
In + 0.1	1.3 ± 0.1	1.6 ± 0.1	1.1	0.6
0.2	7.0 ± 1.6	7.1 ± 1.6	3.3	3.8
In + 0.2	2.6 ± 0.4	2.6 ± 0.4	1.6	1.1
0.3	7.5 ± 1.0	7.8 ± 1.0	4.2	3.6
In + 0.3	2.2 ± 0.6	2.2 ± 0.6	1.2	1.0
0.5	10.9 ± 1.4	11.3 ± 1.4	5.2	5.9
In + 0.5	3.5 ± 1.0	4.5 ± 1.0	3.5	1.0
1.0	17.4 ± 1.5	18.8 ± 1.8	11.4	7.2
In +1.0	14.8 ± 1.1	15.5 ± 1.0	8.6	6.7

Note: In – inosine 30 min before irradiation of lymphocyte culture.

as the radiomitigator in order to reduce the carcinogenic risk in an emergency situation of public exposure to minimize the probable stochastic effects.

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РОЛЬ РАДІОПРОТЕКТОРІВ У МІНІМІЗАЦІЇ ВИНИКНЕННЯ СТОХАСТИЧНИХ ЕФЕКТІВ ПРИ РАДІАЦІЙНИХ ІНЦИДЕНТАХ

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Сьогодні з урахуванням реальної загрози надзвичайних ситуацій в Україні необхідно враховувати досвід попередніх радіаційних аварій, за яких значна частина населення зазнала опромінення в низьких дозах. При цьому клінічні прояви переважно були відсутні, а небезпека виникнення стохастичних (канцерогенних) ефектів зберігалася. Тому на цей час стратегія радіаційного захисту населення має бути спрямована на перегляд та вибір ефективних та малотоксичних протипроменевих засобів. Головним критерієм розвитку стохастичних наслідків опромінення вважається радіаційно-індукована нестабільність геному, що є промотором канцерогенезу. Перспективними є використання радіомітигаторів, які здатні послаблювати шкідливу дію іонізуючих випромінювань на критичні високорадіочутливі системи організму людини. У наших дослідженнях встановлено радіомітигувальний ефект інозину на генетичному рівні в культурі Т-лімфоцитів людини. Під впливом препарату знижується частота гамма-індукованих аберацій хромосом. Одержані результати слід розглядати як експериментальне обґрунтування цільового призначення радіомітигаторів в умовах надзвичайної ситуації опромінення населення для мінімізації виникнення та розвитку стохастичних ефектів.

Ключові слова: надзвичайна ситуація, опромінення, стохастичні ефекти, нестабільність геному, хромосомні аберації, радіомітигатори.