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CYTOGENETIC CHANGES İN IRRADIATED RATS UNDER INFLUENCE OF GUANINIUM-ZINC CHLORIDE COMPLEX

Aim. The main goal of this study was to study the anti-radiation effect of guaninium-zinc chloride complex on cytogenetic changes in irradiated rats. ***Methods.*** White rats of the Wistar line with an average weight of 110-125 grams served as the material for the detection of the anti-radiation effect of guaninium-zinc chloride complex. Animals were injected intraperitoneally with solutions of guaninium-zinc chloride complex at a concentration of 40 mg/kg 2 hours before irradiation and were subjected to general uniform gamma irradiation from a 60Co source in the Rukhund-20000 irradiation unit at a dose of 3 and 5 Gy. ***Results.*** Cytogenetic analysis of animals showed that with a single injection of guaninium-zinc chloride complex solutions before irradiation, the proportion of chromosomal abnormalities decreased markedly, and reached 2.93 %, and the amount of abnormal spermatozoa markedly decreased. Guaninium-zinc chloride complex softened the damaging effects of ionizing radiation. There was no suppression of cell division. ***Conclusions.*** It was found for the first time that, with the introduction of a solution of guaninium-zinc chloride complex in white Wistar rats before irradiation, it reduces the damaging effect of gamma irradiation. The obtained data gives grounds to assume that the guaninium-zinc chloride complex tested by us can be recommended as a promising anti-radiation agent.

Keywords: gamma irradiation, guanine, guaninium-zinc chloride complex, chromosomal aberrations, abnormal spermatozoa.

Currently, radiation is present in almost many areas of human activity. A potential threat to the life and health of people is created by the arsenal of nuclear weapons accumulated in different parts of the globe and the danger of using it for military purposes. The problem is actualized in connection with changes in the environment, including man-made origin. As, for example, cases of radiation contamination of the environment during the accident at the Chernobyl nuclear power plant in Ukraine and the Fukushima-1 nuclear power plant in Japan. And for the entire Caucasus, the Metsamor Nuclear Power Plant, built in a seismically hazardous zone, poses a potential threat. Nothing guarantees the safety of other installations operating on nuclear reactors, submarines, etc.

It is known that, as a result of interaction with ionizing radiation, changes occur in any molecular structures that make up a living cell: nucleic acids, proteins, fats, carbohydrates.

Analyzing the accumulated literature data on the potential danger of radiation for the whole of mankind, it is easy to imagine how important it is to search for and create new drugs that will protect organisms from the results of such man-made disasters.

To date, the radioprotective properties of extracts from various medicinal plants [1, 2], thousands of chemical compounds and only a few combs of them that are effective in the prevention of radiation sickness and have become pharmacopoeial preparations have been identified [3].

Research shows that nitrogen-containing heterocyclic compounds increase the body's internal defense mechanisms and its detoxification capacity. The presence in its composition of a combination of oxygen and nitrogen atoms, as well as unsaturated bonds increases the physiological activity of these polyfunctional compounds. It has been shown that derivatives of purine bases have pronounced anti-oxidant and radioprotective properties. Moreover, they showed the most effective radioprotective properties when they were introduced into the body shortly after irradiation [4].

One of these compounds is guanine $C_5H_5N_5O$, a nitrogenous base, an amino derivative of purine (2-amino-6-oxopurine), which is an integral part of nucleic acids. In the body, guanine plays a protective function, which is very important under the action of various stress factors, including radiation.

Therefore, the study of the radioprotective properties of guanine complexes with biogenic metals is very promising.

The main goal of this study was to study the anti-radiation effect of the guaninium-zinc chloride complex on cytogenetic changes in irradiated rats.

Material and methods

The synthesis of the guaninium-zinc chloride complex was carried out according to the procedure described in the literature [5].

White rats of the Wistar line with an average weight of 110-125 grams served as the material for the detection of the anti-radiation effect of guaninium-zinc chloride complex. The experiments involved 12 animals (1♀, 11♂), which were intraperitoneally injected with solutions of guaninium-zinc chloride complex at a concentration of 40 mg/kg 2 hours before irradiation. Animals were subjected to general uniform gamma irradiation from a source of 60Co in the irradiation unit "Rukhund-20000" at a dose of 3 and 5 Gy at an average dose rate of 0.260 rad/sec. (Table 1).

Animals were slaughtered one day after irradiation in accordance with Directive 210/63/EC of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes (ETSN 123, Strasbourg, March 18, 1986). The frequency of chromosomal abnormalities was registered on the mitotic cells of the bone marrow and on the germ cells of the testes. Meiotic chromosome preparations were prepared according to the method of Meredith [6] and Williams et al. [7]. Bone marrow preparations obtained by the method of Ford and Hamerton [8]. Preparations were stained according to Rajably, Kryukov [9], Sumner [10], Howell, Black [11]. The plates were selected according to the criteria proposed by Bochkov et al. [12] and Zakharov [13].

For the analysis of chromosome sets, an Amplival microscope with an automatic MF photo attachment (x100 objective, x4.1 Prohectiv eyepiece) was used. At least 200 metaphase plates of the bone marrow and 100 germ cells at different stages of division were examined for each animal.

Results and discussion

Cytogenetic analysis of bone marrow cells and germ cells in control white rats did not reveal structural damage to the chromosomes. The frequency of aberrations was 0.59 % and 6.3 %, respectively. These were mostly quantitative lesions such as polyploid and aneuploid plates (Fig. 1).

Administration of the guaninium-zinc chloride complex to animals did not affect the rate of spontaneous mutations (Table 2).

In the control variant, no structural disturbances were observed. Acute gamma irradiation at a dose of 3 Gy led to inhibition of cell division. The number of chromosomal aberrations increased to 4.37 % due to structural disorders (deletions, fragments, inversions). Animals that received a dose of 5 Gy with a single exposure, the frequency of chromosomal disorders increased to 9.50 %.

Table 1. Form of influence and description of the test material

Form of influence	Quantity of investigated rats	Quantity of scanned mitotic cells	Quantity of scanned sexual cells and spermatozoids
Control	1 ♂+ 1 ♀	357	108
3 Gy irradiation	2 ♂	572	261
5 Gy irradiation	2 ♂	591	363
Guaninium-zinc chloride complex	2 ♂	563	259
Guaninium-zinc chloride complex + 3Gy	2 ♂	613	298
Guaninium-zinc chloride complex + 5Gy	2 ♂	621	354

a)

b)

Fig. 1. a) polyploid metaphase plate 2n = 48; b) aneuploid metaphase plate 2n = 43.

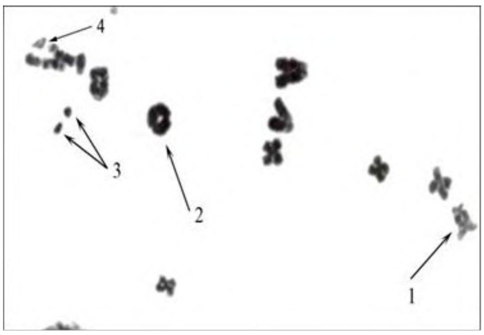
Table 2. Influence of guaninium-zinc chloride complex on frequency of chromosomal aberrations in bone marrow cells of white rats

Variants	Quantity of metaphses (all)	Number of metaphase – 2n = 42	Poliploidic metaphase	Aneuploidic metaphase	Structural damages	% damages
Control	363	361	1	1 (n>42)	-	0,55
3 Gy irradiation	572	547	6	7 (n>42) 3 (n<42)	9	4,37
5 Gy irradiation	589	533	18	11 (n>42) 4 (n<42)	23	9,50
Guaninium-zinc chloride complex	543	539	2	2 (n<42)	-	0,73
Guaninium-zinc chloride complex + 3 Gy	511	496	3	4 (n>42) 2 (n<42)	6	2,93
Guaninium-zinc chloride complex + 5 Gy	613	564	13	6 (n>42) 12 (n<42)	18	7,99

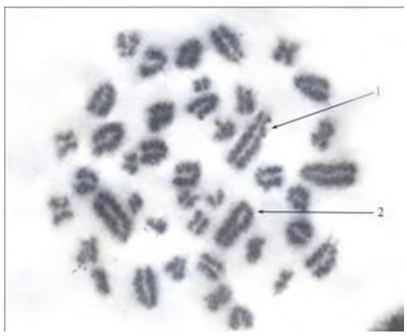
Note. *Statistically significant difference from control level ($p < 0.05$).

There was also a sharp increase in ring chromosomes, single fragments, dicentrics in the mitotic cells of the bone marrow of rats. A sharp increase in polyploid and aneuploid meiotic plates was registered at the stages of diakinesis-metaphase I and metaphase II (Fig. 2).

Inhibition of cell division is the result of exposure to ionizing radiation. As the radiation dose increases, more and more cells lose their ability to divide, or at least stop dividing, resulting in polyploid cells. Thus, in animals irradiated with a dose of 5 Gy, the number of tetraploid and octoploid cells sharply increased.



a)



b)

Fig. 2. a) 1. Dicentric; 2. Ring chromosome; 3. Chromosome fragments b) 1. Normal chromosome; 2. Inverted chromosome.

Under the influence of radiation, the number of abnormal spermatozoa increases, they differ both in shape and size, and with an increase in the dose of radiation, the number of such spermatozoa increases dramatically (Fig. 3).

Cytogenetic analysis of animals showed that with a single injection of guaninium-zinc chloride complex solutions before irradiation, the proportion of chromosomal disorders significantly decreased and reached 2.93 %. The introduction of this substance softened the damaging effects of ionizing radiation. There was no suppression of cell division (Table 2). The percentage of structural chromosome disorders was higher than in the control experiments and lower than in the experiments with acute irradiation (Table 3).

As can be seen from Table 4, the effect of guanine and guaninium-zinc chloride complex was insignificant, the highest percentage of violations was observed when animals were irradiated with a dose of 5 Gy – 23.22 %. However, germ cells turned out to be much more sensitive than bone marrow cells by almost 2.5 times. The number of spermatocytes with signs of degeneration at the stage in pachytene increased markedly, and the frequency of conjugation disorders between -X and -Y chromosomes increased. There was an increase in polyploid and aneuploid meiotic plates at the stages of diakinesis-metaphase I, metaphase II (Fig. 4). Analysis of germ cells at different stages of spermatogenesis showed their significant sensitivity to the action of acute gamma irradiation (Table 5).

The value of abnormally shaped and hyperhaploid spermatozoa reached 54.5 % of all registered aberrations. Nondisjunction of chromosomes in metaphase I may be the cause of such disorders.

Our experiments showed that guaninium-zinc chloride complex reduced the mutagenic effect of gamma irradiation. It not only contributed to the restoration of cell division, but also reduced the proportion of structural disorders.

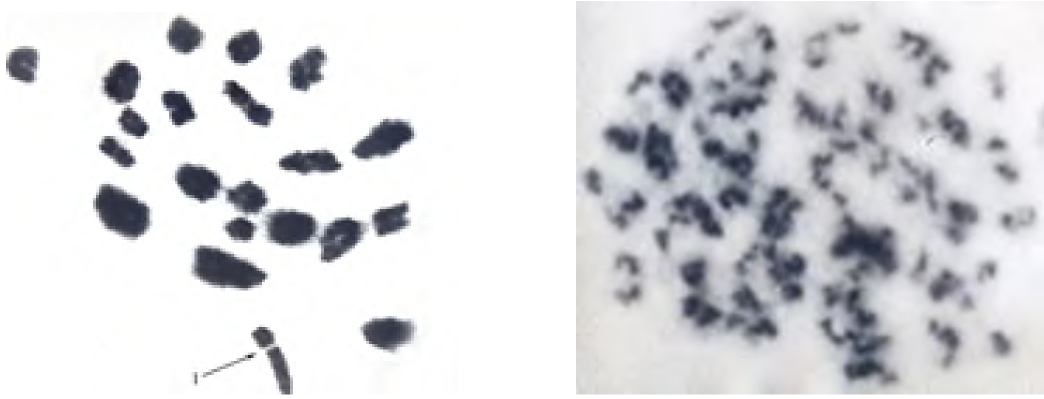
a) b) c)

Fig. 3. a) normal spermatozoid; b) abnormal spermatozoids; c) 1 – spermatozoid with two heads, 2 – normal spermatozoid.

Table 3. Influence of guaninium-zinc chloride complex on frequency of chromosomal aberrations in bone marrow cells of white rats

Variants	Number of metaphase (total)	Number of metaphases – 2n = 42	Polyploidic metaphases	Aneuploidic metaphases	Structural infrigments	% of infrigments
Guanine	395	393	1	1 (n>42) -	-	0,50
Guanine + 3 Gy	735	712	6	3 (n>42) 6 (n<42)	8	3,12
Guanine + 5 Gy	633	583	14	7 (n>42) 8 (n<42)	21	7,89
Guaninium-zinc chloride complex	555	539	2	- 4 (n<42)	-	1,08
Guaninium-zinc chloride complex + 3 Gy	478	464	4	- 4 (n<42)	6	2,92
Guaninium-zinc chloride complex + 5 Gy	596	553	12	2 (n>42) 6 (n<42)	23	7,21

Note. *Statistically significant difference from control level ($p < 0.05$).



a) b)

Fig. 4. a) Conjugation infringements between X and Y chromosomes b) Polyploid metaphase II.

Table 5. Influence of guanine and guaninium-zinc chloride complex on frequency of chromosomal aberrations in sexual cells

Variants	Number of cells	Aneuploids	Poliploids	Conjugation disorder between X and Y chromosomes	Abnormal spermatozoids	Total infrigments (%)
Control	112	1	2	-	2	4,46
3 Gy irradiation	261	7	8	6	16	14,17
5 Gy irradiation	366	18	16	14	37	23,22
Guanine	272	3	1	2	10	5,88
Guanine + 3 Gy	344	5	6	5	12	8,13
Guanine + 5 Gy	477	14	23	12	29	16,35
Guaninium-zinc chloride complex	317	3	5	3	4	4,73
Guaninium-zinc chloride complex + 3 Gy	385	5	6	5	17	8,57
Guaninium-zinc chloride complex + 5 Gy	435	9	14	4	33	13,79

Note. *Statistically significant difference from control level ($p < 0.05$).

In general, in terms of their cytogenetic results, the consequences of radiation turned out to be very similar to the data available in the literature on these consequences in different rodent species [14, 15, 16, 17]. This enhances the diagnostic and prognostic value of various outcomes.

Conclusions

For the first time, it was found that, with the introduction of a solution of the guaninium-zinc chloride complex synthesized by us in white Wistar rats before irradiation, the damaging effect of gamma irradiation decreases. At the same time, the proportion of chromosomal disorders and the magnitude of abnormal spermatozoa decreased markedly.

The data obtained as a result of our experiments suggests that the guaninium-zinc chloride complex tested by us can be recommended as a promising anti-radiation agent.

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ЦИТОГЕНЕТИЧНІ ЗМІНИ В ОПРОМІНЕНИХ ЩУРІВ ПІД ВПЛИВОМ ГУАНІН-ЦИНК ХЛОРИДНОГО КОМПЛЕКСУ

Мета. Основною метою цього дослідження було вивчення протирадіаційної дії гуанін-цинк хлоридного комплексу на цитогенетичні зміни в опромінених щурів. ***Методи.*** Матеріалом для виявлення протирадіаційної дії гуанін-цинк хлоридного комплексу послужили білі щури лінії Вістар із середньою масою 110-125 г. від джерела 60Co в установці опромінення Рухунд-20000 у дозах 3 та 5 Гр. ***Результати.*** Цитогенетичний аналіз тварин показав, що при одноразовому введенні розчинів гуанін-цинк хлоридного комплексу перед опроміненням частка хромосомних аномалій помітно зменшилася і досягла 2,93 %, а кількість аномальних сперматозоїдів помітно зменшилася. Гуанін-цинк хлоридний комплекс пом'якшував шкідливу дію іонізуючого випромінювання. Не було пригнічення поділу клітин. ***Висновки.*** Вперше встановлено, що при введенні розчину гуанін-цинк хлоридного комплексу білим щурам Вістар перед опроміненням він знижує ушкоджувальну дію гамма-опромінення. Отримані дані дають підстави припускати, що апробований нами гуанін-цинк хлоридний комплекс можна рекомендувати як перспективний протирадіаційний засіб.

Ключові слова: гамма-опромінення, гуанін, гуанін-цинк хлоридний комплекс, хромосомні аберації, аномальні сперматозоїди.