ORIGINAL ARTICLES/
MORPHOLOGY, PATHOLOGY, PHYSIOLOGY

Cite as: Archiv EuroMedica. 2023. 13; 5: e1. DOI 10.35630/2023/13/5.501

Received 15 September 2023;
Accepted 18 October 2023;
Published 21 October 2023

INFLUENCE OF COPPER COMPLEXES $[CU(PTA)_4]BF_4$ AND $[CU(ACAC)_2]$ ON RATS IRRADIATED WITH RADIOISOTOPE TECHNETIUM

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ABSTRACT

One of the priority tasks of modern radiobiology is the search for new, effective radioprotective compounds. In this area, metal complexes with high antioxidant activity are of particular interest.

In order to study the possible favorable radioprotective effect of two copper complexes in different oxidation states, $[Cu(PTA)_4]BF_4$ and $[Cu(acac)_2]$, on the irradiated organism, we studied cytogenetic parameters in 4 groups of experimental animals. Group I included intact animals; Group II consisted of animals exposed to technetium (Tc) radioisotope, which were intraperitoneally injected with an isotope with an activity of 4.8 mCi in a volume of 2 mL - "pure irradiation"; Group III consisted of animals that were intraperitoneally injected with the copper(I) complex $[Cu(PTA)_4]BF_4$ at a dose of 50 mg/kg in a volume of 2 mL one hour before the administration of the Tc isotope ("irradiation + $[Cu(PTA)_4]BF_4$"). Group IV included animals that received the copper(II) complex $[Cu(acac)_2]$ before irradiation.

We studied survival and cytogenetic parameters, determined the mitotic index, chromosomal aberrations and the percentage of polyploid cells. The survival rate of group II was 40%. In the group of intact animals, as well as in groups III and IV the survival rate was 100%. The dynamics of survival was described by regression equations, which make it possible, by means of extrapolation, to determine the change in the percentage of survival in the long term of the experiment and to predict the further outcome of the experiment. Investigating cytogenetic indicators, for all 3 indicators, a significant difference was found between intact and irradiated animals, i.e. these indicators can be considered as markers of technetium isotope exposure.

In terms of proliferative activity, a significant difference was found in irradiated animals compared with groups III and IV, which indicates the radioprotective property of both the copper compounds. As for the $[Cu(acac)_2]$, when it was used in group IV and compared with others, we obtained a pronounced significant difference from the group with "pure irradiation" in all studied cytogenetic parameters.

It was found that the copper complexes $[Cu(PTA)_4]BF_4$ and $[Cu(acac)_2]$ have the ability to prevent or mitigate the effect of ionizing radiation on an animal's body. Based on cytogenetic parameters, it can be

concluded that these compounds promote reparative processes in the bone marrow cells of irradiated animals.

Multiregression analysis of cytogenetic parameters confirmed the highest efficiency of the copper (II) complex [Cu(acac)$_2$]. The results of the research indicate the need to continue work in the direction of searching for agents that have a therapeutic effect in radiation injuries.

Keywords: ionizing radiation, copper complexes, mitotic index, chromosomal aberration, percentage of polyploid cells

INTRODUCTION

Over many years, studies have been carried out regarding the search for sensitive biological markers specific to radiation exposure, that would be informative both in the early and late periods after exposure [1,2]. As a result, the principles of biological indication and the requirements for biological markers were formulated, the main of which include: specificity (i.e. the ability to respond to exposure to radiation is much stronger than to any other impact of a non-radiation nature), and a quantitative dependence on the dose of radiation exposure.

Currently, one of the few biological indicators (along with Electron paramagnetic resonance spectroscopy of tooth enamel) that fully meet these requirements are chromosomal aberrations in peripheral blood lymphocytes [3,4].

It is known that the main initiating event after irradiation of the body is DNA damage, on the basis of which one of the first and direct signs of the impact of ionizing radiation (IR) on the cell is considered chromosome destabilization [5,6]. Radiation-induced damage to the karyotype is an important indicator both for biological indication of the severity of radiation injuries and for prediction of the development of long-term adverse effects of IR.

One of the priority tasks of modern radiobiology is the search for new, effective radioprotective compounds. In this area, metal complexes with high antioxidant activity are of particular interest. The ability to protect the body from the damaging effects of ionizing radiation in such complexes has been previously noted and reported in scientific works [7,8,9], including the works of specialists from L.A. Orbeli Institute of Physiology NAS RA [10, 11].

MATERIALS AND METHODS

The analytically pure copper(I) complex [Cu(PTA)$_4$]BF$_4$ (Figure 1) was synthesized by a one-step reaction of [Cu(CH$_3$CN)$_4$]BF$_4$ with an excess of 1,3,5-triaza-7-phosphaadamantane (PTA) in an acetonitrile solution at room temperature. The reaction mixture was stirred overnight, filtered and the white residue was washed with chloroform and diethyl ether and then dried under vacuum [12]. The bis(acetylacetonato)copper(II) complex [Cu(acac)$_2$] (Figure 1) was synthesized by a one-step synthetic protocol involving the reaction of the acetylacetone with copper(II) acetate monohydrate, in 2:1 stoichiometric ratio, in ethanol/water solution at room temperature [13]. After filtration, the precipitate was dried in vacuo and recrystallized from methanol to give the analytically pure complex [Cu(acac)$_2$] [14].

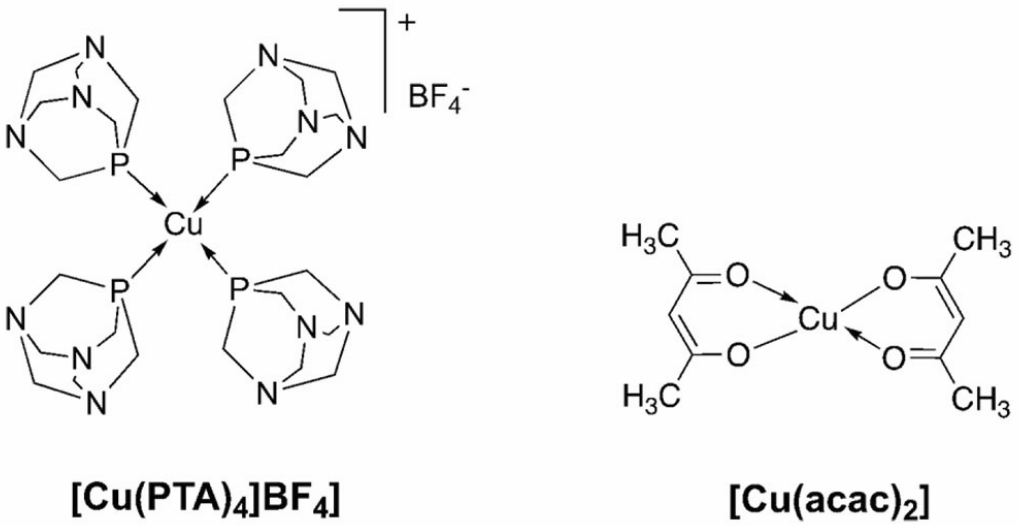

Figure 1. Chemical structure of the copper complexes [Cu(PTA)$_4$]BF$_4$ and [Cu(acac)$_2$].

In order to study the possible favorable radioprotective effect of the copper(I) complex $[Cu(PTA)_4]BF_4$ and the copper(II) complex $[Cu(acac)_2]$ on the irradiated organism, we studied cytogenetic parameters in 4 groups of experimental animals (white, outbred, mature male rats with an average weight of 180 g, 10 rats in each group). The experiments were performed in compliance with current best practices and standards of care in laboratory animals. Cytogenetic examination included analysis of chromosomes with Giemsa stain.

Group I included intact animals; Group II consisted of animals exposed to technetium (Tc) radioisotope, which were intraperitoneally injected with an isotope with an activity of 4.8 mCi in a volume of 2 mL - "pure irradiation"; Group III consisted of animals that were intraperitoneally injected with $[Cu(PTA)_4]BF_4$ at a dose of 50 mg/kg in a volume of 2 mL one hour before the administration of the Tc isotope ("irradiation + $[Cu(PTA)_4]BF_4$"). Group IV included animals that received $[Cu(acac)_2]$ before irradiation.

We studied survival and cytogenetic parameters (by the Ford-Woollam method), determined the mitotic index (MI), chromosomal aberrations (CA) and the percentage of polyploid cells (PPC) in the bone marrow cells of the femur (counting in 1000 cells in each preparation).

Data analysis was carried out using a number of specialized statistical packages: Statsoft and SPSS-10.0. We used regression and correlation methods of analysis.

RESULTS

Survival and average life expectancy (ALS) of animals were calculated. The survival rate of group II was 40% (SLE = 13.8). In the group of intact animals, as well as in groups III and IV, with the injection of compounds: $[Cu(PTA)_4]BF_4$ and $[Cu(acac)_2]$, the survival rate was 100% (ALS = 30). The dynamics of survival was described by regression equations:

$$y_1=y_3= y_4=100+0lg(x)$$

$$y_2=77.5018-30.38lg(x)$$

where y_1 is the survival rate of intact animals, y_2 is the survival rate under "pure irradiation", y_3 is under "irradiation + injection of $[Cu(PTA)_4]BF_4$" and y_4 - under "irradiation + injection of $[Cu(acac)_2]$". The above regression equations make it possible, by means of extrapolation, to determine the change in the percentage of survival in the long term of the experiment and to predict the further outcome of the experiment.

Analyzing the karyotype and proliferative activity of the above cells, we assessed the cytogenetic indicators of these groups, the results of which are shown in the table. Only significant values of changes in cytogenetic parameters are given.

Table 1. Cytogenetic parameters in 4 groups: "Norm", "Pure irradiation", "Irradiation + $[Cu(PTA)_4]BF_4$" and "Irradiation + $[Cu(acac)_2]$" on the 30th day of the experiment.

Indicators	**Norm (I group)**	**Tc (group II)**	**Tc+$[Cu(PTA)_4]BF_4$ (group III)**	**Tc+ $[Cu(acac)_2]$ (group IV)**
Mitotic index, %	20,1±2,8	10,9±0,35 $0,01<p_{n2}<0,02$	14,2±0,96 $0,01<p_{23}<0,02$	19,1±0,8 $p_{24}<0,001$
Chromosomal aberrations, %	3,0±0,22	6,2±0,5 $p_{n2}<0,001$	4,8±0,42 $0,002<p_{n3}<0,01$	3,7±0,4 $p_{24}<0,001$
Polyploid cells, %	0,001 ±0,0001	3,5±0,44 $p_{n2}<0,05$	3,4±0,47 $p_{n3}<0,05$	1,2±0,3 $p_{24}<0,001$

p_{n2} - when comparing the indicators of groups I and II of animals; p_{n3} - groups I and III; p_{n4} - groups I and IV; p_{23} - groups II and III; p_{24} - groups II and IV.

When analyzing the results in animal groups, "pure irradiation" – "irradiation + $[Cu(PTA)_4]BF_4$" and "irradiation + $[Cu(acac)_2]$" we found a significant difference in cytogenetic parameters between these groups. Thus, for 2 indicators, there is a significant difference between intact and irradiated animals, i.e.

these indicators can be considered as markers of Tc exposure. In terms of the mitotic index (proliferative activity), a significant difference was found in irradiated animals compared to the groups: "irradiation + $[Cu(PTA)_4]BF_4$" and "irradiation + $[Cu(acac)_2]$", which indicates the radioprotective properties of both the copper complexes. As for the $[Cu(acac)_2]$ compound, in case of its use in group IV and comparison with others, we obtained a pronounced significant difference from the group with "pure irradiation" in all studied cytogenetic parameters ($p<0.001$).

Figure 1 shows the results of multi-regression dependences of the mutual influence of cytogenetic parameters upon injection of $[Cu(PTA)_4]BF_4$ (a) and $[Cu(acac)_2]$ (b). The equations of the multiregression dependence between MI, CA and PC in the norm (x), with pure irradiation (y) and the use of copper-organic complexes (Z) are also given: $[Cu(PTA)_4]BF_4$ (a) and $[Cu(acac)_2]$ (b).

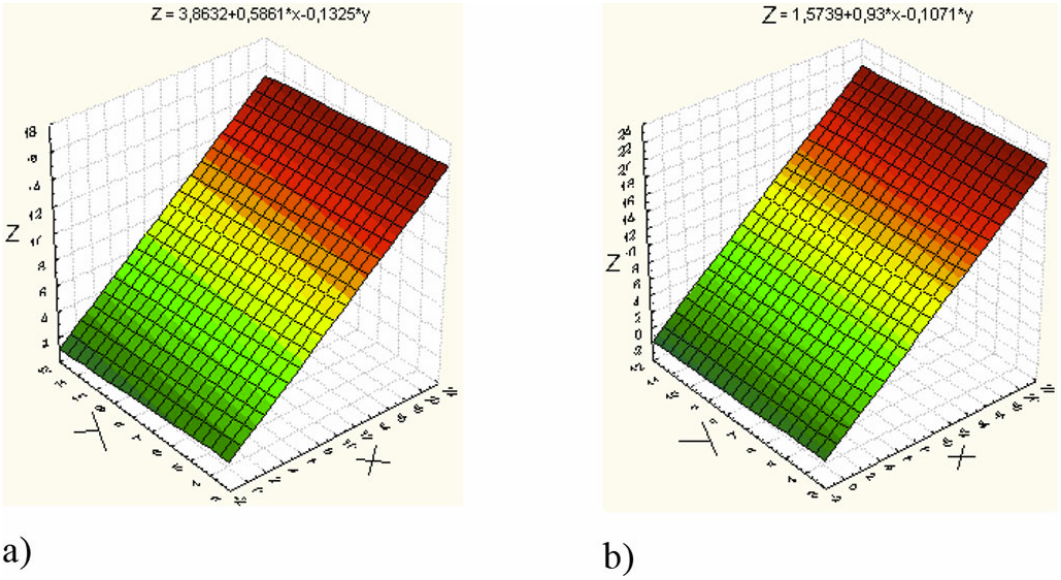

Figure 1. Results of multi-regression analysis of the mutual influence of cytogenetic parameters upon injection of $[Cu(PTA)_4]BF_4$ (a) and $[Cu(acac)_2]$ (b).

Multi-regression analysis of cytogenetic parameters, along with standard statistical methods, confirmed the higher efficiency of $[Cu(acac)_2]$ in comparison to $[Cu(PTA)_4]BF_4$.

CONCLUSION

After determining the survival rate, average life expectancy and cytogenetic parameters (mitotic index, chromosome aberrations and the percentage of polyploid cells in the bone marrow cells of the femur), we found the highest survival rates and average life expectancy were observed in groups with injection of $[Cu(PTA)_4]BF_4$ and $[Cu(acac)_2]$.

Investigating cytogenetic indicators, for 2 indicators, a significant difference was found between intact and irradiated animals, i.e. these indicators can be considered as markers of technetium isotope exposure. In terms of proliferative activity, a significant difference was found in irradiated animals compared with groups: III and IV ("irradiation + complexes"), which indicates the radioprotective property of both the copper complexes. As for $[Cu(acac)_2]$, when it was used in group IV and compared with others, we obtained a pronounced significant difference from the group with "pure irradiation" in all studied cytogenetic parameters ($p<0.001$). With "pure irradiation", the following genetic disorders were observed (Figure 2).

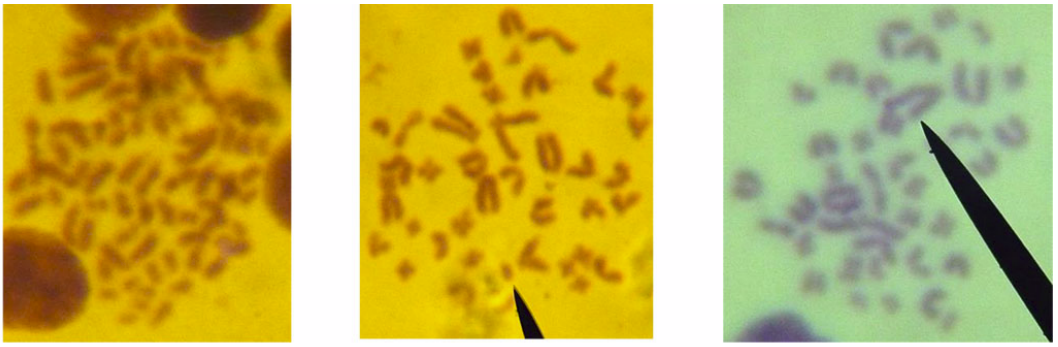

Figure 2. Cytogenetic disturbances detected on day 30 after incorporation of Tc with activity of 15.78 mCi: a) polyploid cell as a result of cytokinesis block in bone marrow tissue; b) a double fragment formed from a break in a chromosome arm; c) deletion of chromosome arm, pair I.

When comparing the cytogenetic parameters of groups II and III, a significant difference in the proliferation of bone marrow cells (BMC) was revealed. It was also noted that chromosomal aberrations in the form of fragments and the number of polyploid cells tend to decrease in group III.

As a result of studies of cytogenetic indicators, survival and average life expectancy, it was found that the complexes $[Cu(PTA)_4]BF_4$ and $[Cu(acac)_2]$ have the ability to prevent or mitigate the effect of ionizing radiation on an animal's body. Based on cytogenetic parameters, it can be concluded that these copper complexes promote reparative processes in the bone marrow cells of irradiated animals.

Multi-regression analysis of cytogenetic parameters also confirmed the highest efficiency of the the Cu(II) complex $[Cu(acac)_2]$ with respect to the Cu(I) complex to $[Cu(PTA)_4]BF_4$.

The results of the research indicate the need to continue work in the direction of searching for agents that have a therapeutic effect in radiation injuries.

FUNDING

The work was supported by the Science Committee of RA, in the frame of the research project No 21T-1F126

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