http://dx.doi.org/10.35630/2199-885X/2021/11/2/5

INFLUENCE OF COPPER COMPLEXES $[Cu(PTA)_4[BF_4]$ AND $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ ON THE ORGANISM OF RATS IRRADIATED WITH RADIOISOTOPE TECHNETIUM

Received 8 May 2921;
Received in revised form 30 May 2021;
Accepted 2 June 2021

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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-organic complexes with high antioxidant activity are of particular interest.
In order to study the possible beneficial radioprotective effect of copper complex compounds $[Cu(PTA)_4[BF_4]$ and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ on an irradiated organism, we studied cytogenetic parameters in 4 groups of experimental animals: intact animals, animals exposed to the radioisotope technetium (Tc) — "pure irradiation", animals with "irradiation + compound $[Cu(PTA)_4[BF_4]$", and animals with "irradiation + compound $[Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$". The survival rate and cytogenetic parameters were studied: mitotic index (MI), chromosomal aberrations (CA) and polyploid cells (PC) in the bone marrow cells of the femur. The survival rate in the first and third groups was 100%, in the second group — 40%, and in the fourth — 80%. The dynamics of survival was described by regression curves and equations, which make it possible, using 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.
When analyzing the results in 4 groups of animals, we found a significant difference in cytogenetic parameters between these groups. Thus, for all 3 indicators, a significant difference is observed between intact and irradiated animals, i.e. these parameters can be considered as markers of Tc exposure. In terms of the mitotic index (proliferative activity), a significant difference was found in the irradiated compared with the groups: "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$", which indicates the radioprotective property of both compounds. By the number of polyploid cells, a significant difference was found between the groups: "pure irradiation" and "irradiation + $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$", which also proves the beneficial effect of this compound.
Multiregression analysis of cytogenetic parameters along with standard statistical methods confirmed the highest efficiency of $[Cu(PTA)_4[BF4]$ relative to $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$.

KEYWORDS — radiation, chromosomal aberrations, polyploid cells, mitotic index, survival.

INTRODUCTION

It is known that the main initiating event after irradiation of an organism is DNA damage, on the basis of which chromosome destabilization is considered one of the first and direct signs of the effect of ionizing radiation (IR) on a cell [1–4]. Radiation-induced damage to the karyotype is an important indicator both for biological indication of the severity of radiation injuries and for predicting 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-organic 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 were noted both in the scientific works of other authors [5, 6] and in our early works [7–10].

Aim: to study the possible beneficial radioprotective effect of copper complex compounds $[Cu(PTA)_4[BF_4]$ and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ on an irradiated organism using cytogenetic parameters.

MATERIALS AND METHODS

In order to study the possible beneficial radioprotective effect of copper complex compounds $[Cu(PTA)_4[BF_4]$ and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ on an irradiated organism, we studied cytogenetic parameters in 4 groups of experimental animals (white, outbred, sexually mature male rats with an average weight of 180 g,; 10 rats in each group).

The animals used in our research were maintained in compliance with European Union Legislation (Directive 2010/63/EU, amended by Regulation (EU) 2019/1010).

The first group included intact animals. The second group consisted of animals exposed to the radioi-

sotope technetium (Tc), which were injected intraperitoneally with an isotope with an activity of 4.8 mCi in a volume of 2 ml — "pure irradiation". The third group consisted of animals that were injected intraperitoneally with a copper 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 + copper compound $[Cu (PTA)_4[BF_4]$)»). The fourth group involved animals that, before irradiation, received the compound $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$ at a dose of 50 mg/ kg in a volume of 1 ml.

We studied the survival rate and cytogenetic parameters at the metaphase stage of the mitotic cycle (according to the Ford-Wallam method [11], the following were determined: the mitotic index (MI), chromosomal aberrations (CA) and polyploid cells (PC) in the bone marrow cells of the femur (counting in 1000 cells in each). The cytogenetic examination included the analysis of chromosomes using a J.B. Carnoy fixative and with Giemsa stain. The obtained cytogenetic preparations were analyzed according to the generally accepted method of cytogenetic analysis of bone marrow cells (BMC) of white rats according to G. McGregor [12] under a microscope at 900–1400 times magnification.

Data analysis was carried out using a number of specialized statistical packages: Statsoft and SPSS-10.0. We used regression, multi-regression and correlation methods of analysis [13, 14].

RESULTS

The experiment lasted 30 days. The survival rate of the second group was 40%. In the third group, with the injection of the compound [Cu(PTA)4[BF4], the survival rate was 100%, and in the fourth — 80%. The dynamics of survival was described by regression curves and equations (where y_1 is survival under pure irradiation, y_2 is when «irradiated + injection of $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$» and y_3 is when «irradiated + injection $[Cu(PTA)_4[BF_4]$)» shown in Fig. 1, which make it possible, using 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 obtained the cytogenetic indicators of these groups, the results of which are shown in the Table 1. Only reliable values of changes in cytogenetic parameters are given.

The types of karyotype disorders (polyploid cell and double fragment) detected after irradiation of animals with technetium are shown in Fig. 2.

When analyzing the results of the animal groups "pure irradiation", "irradiation + $[Cu(PTA)_4[BF_4]$" and

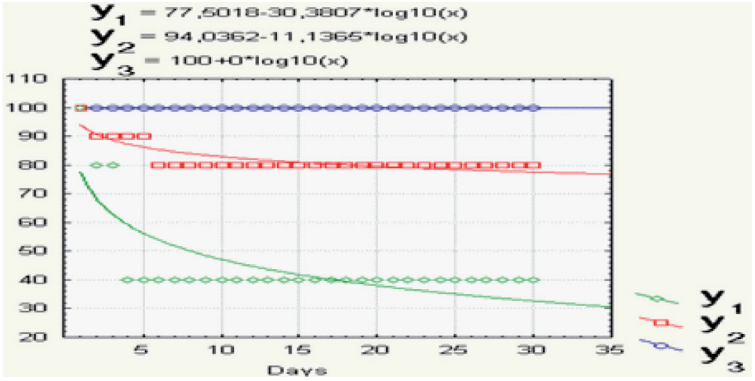

Fig. 1. Survival of the groups "pure irradiation", "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$"

"irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$", we found a significant difference in cytogenetic parameters between these groups. Thus, for all 3 indicators, a significant difference is observed between intact and irradiated animals, i.e. these parameters can be considered as markers of Tc exposure. In terms of the mitotic index (proliferative activity), a significant difference was found in the irradiated compared with the groups: "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$", which indicates the radioprotective property of both compounds. According to the number of polyploid cells, a significant difference was found between the groups: "pure irradiation" and "irradiation + $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$", which also proves the beneficial effect of this compound.

Fig. 3 shows the results of multi-regression dependences of the mutual influence of cytogenetic parameters upon injection of $[Cu(PTA)_4[BF_4]$ (a) and $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$ (b). In the formulas shown in the upper part of the figure (a) and (b) x is the coefficient characterizing the change in cytogenetic parameters under normal conditions, y is under the influence of Technetium, z1 is under the influence of Tc + $[Cu(PTA)_4[BF_4]$ (a) , and z_2 — under the influence of $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$ (b). Multiregression analysis of cytogenetic parameters along with standard statistical methods confirmed the highest efficiency of $[Cu(PTA)_4[BF_4]$ relative to $Cu(II)_2(3,5\text{-DIPS})_4(H_2O)_3$.

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

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Table 1. *Cytogenetic indices in 4 groups: "normal", "pure irradiation", "irradiation + $[Cu(PTA)_4[BF_4]$" and "irradiation + $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$" on the 30th day of the experiment*

groups / indicators	Norm (group 1)	Tc (group 2)	Tc+ $[Cu(PTA)_4[BF_4]$ (group 3)	Tc+ $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ (group 4)
Mitotic index (MI) ‰	20,1±2,8	10,9±0,35 $0,01<p_{n2}<0,02$	14,2±0,96 $0,01<p_{23}<0,02$	17,2±1,9 $0,002<p_{24}<0,01$
Chromosomal aberrations (ChA) %	3,0±0,22	6,2±0,5 $p_{n2}<0,001$	4,8±0,42 $0,002<p_{n3}<0,01$	4,8±0,5 $0,002<p_{n4}<0,01$
Polyploid cells (PC) %	0.001±0.0001	3,5±0,44 $p_{n2}<0,05$	3,4±0,47 $p_{n3}<0,05$	2,0±0,2 $p_{n4}<0,05$ $0.01<p_{24}<0.02$

p_{n2} — when comparing the indicators of the second group and the group of intact animals
p_{n3} — when comparing the indicators of the third group and the group of intact animals
p_{n4} — when comparing the indicators of the fourth group and the group of intact animals
p_{23} — when comparing the indicators of the second and third groups
p_{24} — when comparing the indicators of the second and fourth groups

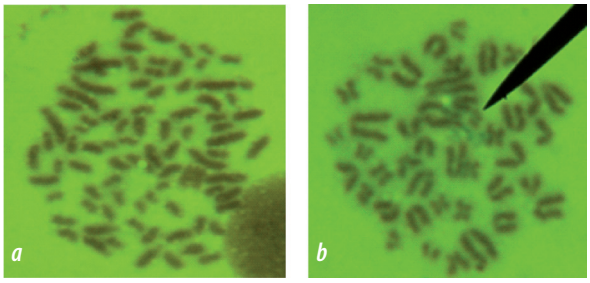

Fig. 2. *Polyploid cell (a) and double fragment (b) 45 days after exposure to technetium isotope*

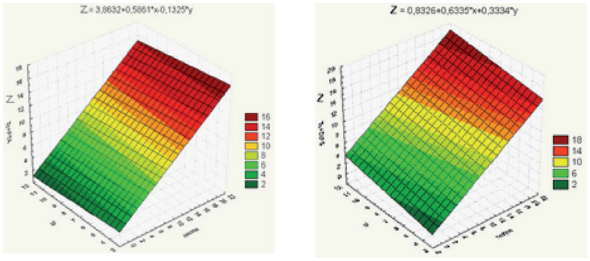

Fig. 3. *Interdependence of cytogenetic parameters upon injection of $[Cu(PTA)_4[BF_4]$ (a) and $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ (b)*

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