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STUDY OF COPPER COMPLEXES $[CU(L^{CF3})_2]$ AND $[CU(L^{CUR})_2]H_2O$ AS RADIOPROTECTIVE COMPOUNDS

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ABSTRACT

One of the first and direct signs of the impact of ionizing radiation (IR) on a cell is the destabilization of chromosomes. 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. The search for new, effective radioprotective compounds is a priority task of modern radiobiology. In this area, metal-based compounds with high antioxidant activity are of particular interest.

The purpose of this work is to determine the possible radioprotective properties of Cu(II) complexes, $[Cu(L^{CF3})_2]$ and $[Cu(L^{cur})_2]H_2O$, supported by the anions of the β-diketone ligands 1,1,1,5,5,5-hexafluoro-2,4-pentanedione (HL^{CF3}) and 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione or curcumin (HL^{cur}).

In this research work we determined survival, life expectancy and cytogenetic parameters: mitotic index, chromosome aberrations and % of polyploid cells in the bone marrow cells of the femur (count in 1000 cells in each preparation).

Group I included intact animals; Group II consisted of animals exposed to the radioisotope technetium; Group III consisted of animals that were intraperitoneally injected with copper complex $[Cu(L^{CF3})_2]$ 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 $[Cu(L^{CF3})_2]$); Group IV included animals that received the compound $[Cu(L^{cur})_2]H_2O$ before irradiation.

The groups with the injection of $[Cu(L^{CF3})_2]$ complex had the highest survival.

In terms of chromosomal aberrations and number of polyploid cells, as indicators, a significant difference was found in those irradiated compared with the "irradiation + $[Cu(L^{CF3})_2]$" group (both after 15 and after 30 days), which indicates the radioprotective property of the compound. In terms of the mitotic index, a tendency towards normalization was noted after 15 days and a significant difference between Groups 2 and

3 by the end of the study (after 30 days), which also proves the beneficial effect of this compound. Analysis of survival and changes in cytogenetic parameters multiregression analysis using both complexes confirms the greatest efficiency of the $[Cu(L^{CF3})_2]$ complex with respect to $[Cu(L^{cur})_2]H_2O$, because when using $[Cu(L^{cur})_2]H_2O$ in Group IV and comparing it with "pure irradiation" only a tendency toward normalization of cytogenetic parameters was observed.

Multiregression analysis of cytogenetic parameters also confirmed the highest efficiency of the $[Cu(L^{CF3})_2]$ compound in comparison with $[Cu(L^{cur})_2]H_2O$.

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: radiation, copper complexes, technetium, proliferative activity, chromosome aberrations, cell ploidy

INTRODUCTION

It is known that the main initiating event after irradiation of the body 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 [1,2]. Radiation-induced karyotype damage 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 IS.

Over the years, research has been conducted to find sensitive biological markers specific to radiation exposure [3,4].

Currently, one of the few biological marker indicators (along with EPR spectroscopy of tooth enamel) are chromosomal aberrations in peripheral blood lymphocytes [5,6].

One of the priority tasks of modern radiobiology is the search for new, effective radioprotective compounds. In this area, metal-based complexes with high antioxidant activity are of particular interest. The ability to protect the body from the damaging effects of ionizing radiations of such complexes was observed both in scientific papers [7-9] and in the works of employees of the L. A. Orbeli Institute of Physiology NAS RA [10, 11].

The work carried out cytogenetic screening of the new synthesized copper(II) complexes.

MATERIALS AND METHODS

The copper(II) complex $[Cu(L^{CF3})_2]$ (Figure 1) was synthesized by a one-step synthetic protocol involving the reaction of the β-diketone ligand 1,1,1,5,5,5-hexafluoro-2,4-pentanedione (HL^{CF3}) with copper(II) acetate monohydrate, in 2:1 stoichiometric ratio (ligand:copper salt), in ethanol/water solution (1:1) at room temperature, according to general procedures detailed in previous literature for analogous homoleptic copper(II) complexes [12-14]. The reaction mixture was stirred at room temperature for 24 hours and, after filtration, the precipitate was dried in vacuo and recrystallized from methanol to give complex $[Cu(L^{CF3})_2]$ as analytically pure complex. A similar procedure was used for the synthesis of the complex $[Cu(L^{cur})_2]H_2O$ (Figure 1) by dissolving the ligand 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (or curcumin) (HL^{cur}) in a water/ethanol solution (1:1) containing the copper(II) acetate monohydrate, in 2:1 stoichiometric ratio (ligand:copper salt). The reaction mixture was stirred at room temperature for 24 hours and, after filtration, the precipitate was dried in vacuo and recrystallized from methanol to give the complex $[Cu(L^{cur})_2]H_2O$ as an analytically pure compound that matched all the spectroscopic parameters of the literature compound [15].

In order to study the possible beneficial radioprotective effect of the copper compounds $[Cu(L^{CF3})_2]$ (complex 1) and $[Cu(L^{cur})_2]H_2O$ (complex 2) on an irradiated organism, we studied cytogenetic parameters in 4 groups of experimental animals (white, outbred, mature male rats average weight 180 g, 10 rats in each group). The maintenance of the animals was conducted in accordance with the guidelines for the adequate care of animals (Directive 86/609/EEC). Cytogenetic examination included chromosome analysis with Giemsa staining. Group I included intact animals. Group II consisted of animals exposed to the radioisotope technetium (Tc), 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 complex $[Cu(L^{CF3})_2]$ at a dose of 50 mg/kg in a volume of 2 mL, one hour before the administration of the Tc isotope ("irradiation + $[Cu(L^{CF3})_2]$, complex 1). Group IV included animals that

received $[Cu(L^{cur})_2]H_2O$ (complex 2) before irradiation.

We studied survival and cytogenetic parameters (by the Ford-Wollam 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).

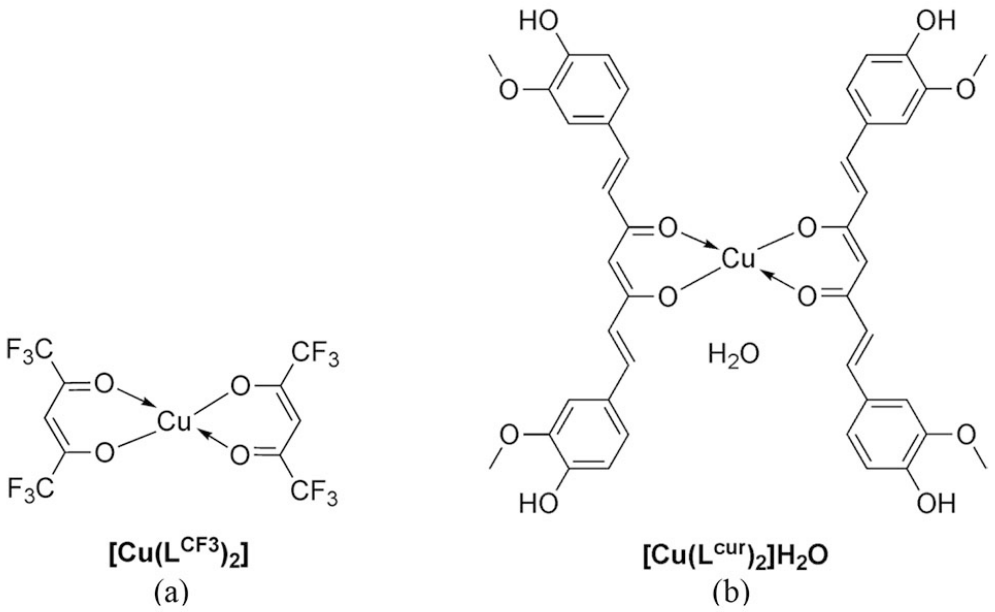

Figure 1. Chemical structure of $[Cu(L^{CF3})_2]$ (a) and $[Cu(L^{cur})_2]H_2O$ (b).

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

RESULTS

The survival rate of animals in the 4 groups was calculated. In the Group I of intact animals, the survival rate was 100%; the survival rate of Group II was 40%, in Group III was 90%, and in Group IV was 80%. The dynamics of survival were described by logarithmic regression equations:

$y_1 = 100 + 0 \lg(x)$

$y_2 = 77{,}5018 - 30{,}38 \lg(x)$

$y_3 = 104{,}38 - 10{,}53 \lg(x)$

$y_4 = 101{,}9 - 15{,}05 \lg(x)$

where x is the day of the experiment, y_1 is the survival rate of intact animals, y_2 is the survival rate with "pure irradiation", y_3 – with "irradiation + injection of $[Cu(L^{CF3})_2]$", and y_4 – with "irradiation + injection of $[Cu(L^{cur})_2]H_2O$". The given regression equations 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.

By analyzing the karyotype and proliferative activity of the above cells, we obtained cytogenetic indicators of these groups, the results of which are shown in the table. Only reliable values of changes in cytogenetic parameters are given.

Table 1. Cytogenetic parameters in 4 groups: "norm", "pure irradiation", "irradiation + $[Cu(L^{CF3})_2]$" and "irradiation + $[Cu(L^{cur})_2]H_2O$" on the 15th and 30th days of the experiment

Indicators	Norm (I group)	Tc (II group)	Tc + $[Cu(L^{CF3})_2]$ (III group) 15 days	Tc + $[Cu(L^{CF3})_2]$ (III group) 30 days	Tc + $[Cu(L^{cur})_2]H_2O$ (IV group) 15 days	Tc + $[Cu(L^{cur})_2]H_2O$ (IV group) 30 days
MI, %	20,35±2,8	9,8±0,96	11,4±0,16	15,8±0,17*	10,6±1,4	12,4±1,6
CA, %	2,6±0,26	6,8±0,74	5,0±0,51*	4,4±0,48*	6,2±0,66	5,8±0,6
PPC, %	0,5±0,08	4,6±0,53	2,2±0,24*	2,1±0,26*	4,0±0,48	3,6±0,4

** Significant difference when comparing the indicators of Groups II and III;*

When analyzing the results of a study of animal groups "pure irradiation", "irradiation + complex 1," and "irradiation + complex 2," we found a significant difference in cytogenetic parameters between these groups. Thus, for all 3 indicators there is a significant difference between intact and irradiated animals ($p<0.05$), i.e. these indicators can be considered as markers of Tc exposure. In terms of chromosomal aberrations and number of polyploid cells, a significant difference was found in those irradiated compared to the group "irradiation + $[Cu(L^{CF3})_2]$ (both after 15 and after 30 days), which indicates the radioprotective property of the compound. In terms of the mitotic index (proliferative activity), a tendency towards normalization was noted after 15 days and a significant difference between Groups II and III at the end of the study (after 30 days). Survival and changes in cytogenetic parameters when using $[Cu(L^{CF3})_2]$ confirms the greatest effectiveness of this compound with respect to $[Cu(L^{cur})_2]H_2O$, because when using $[Cu(L^{cur})_2]H_2O$ in Group IV and comparison, we did not get a significant difference from the group with "pure irradiation" (although a tendency towards normalization is observed).

Figure 2 shows the results of multi-regression relationships between the mutual influence of cytogenetic parameters upon injection of $[Cu(L^{CF3})_2]$ (a) and $[Cu(L^{cur})_2]H_2O$ (b). The equations for the multi-regression relationship between MI, CA and PPC in normal conditions (x), with pure irradiation (y) and with the use of copper(II) complexes (z) are also given.

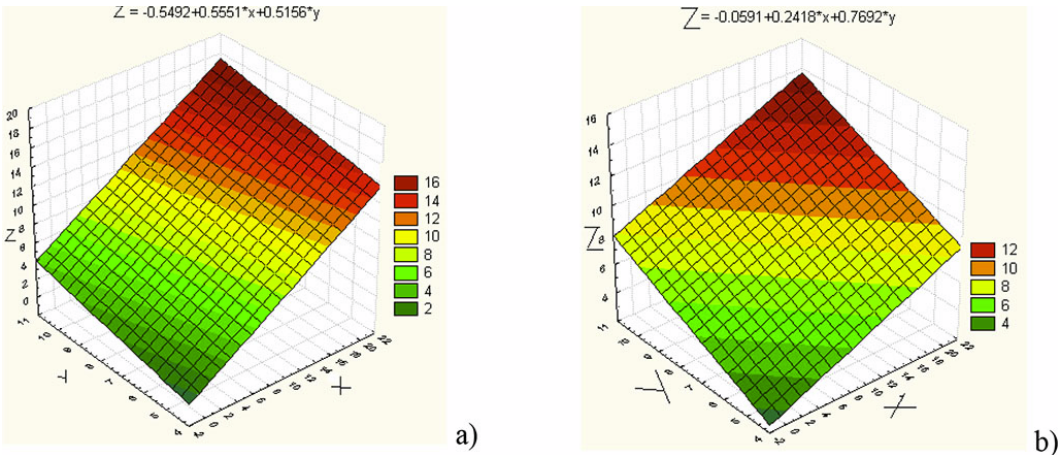

Figure 2. Results of multi-regression analysis of the mutual influence of cytogenetic parameters upon injection of $[Cu(L^{CF3})_2]$ (a) and $[Cu(L^{cur})_2]H_2O$ (b).

Multi-regression analysis of cytogenetic parameters, along with standard statistical methods, confirmed the greatest effectiveness of complex $[Cu(L^{CF3})_2]$ in comparison to $[Cu(L^{cur})_2]H_2O$.

When irradiated with Tc, the following disorders were discovered: double fragment, deletion and polyploid (Figure 3). Their quantity was sharply reduced after the introduction of complexes $[Cu(L^{CF3})_2]$ and $[Cu(L^{cur})_2]H_2O$, which also confirms the effectiveness of their beneficial effects.

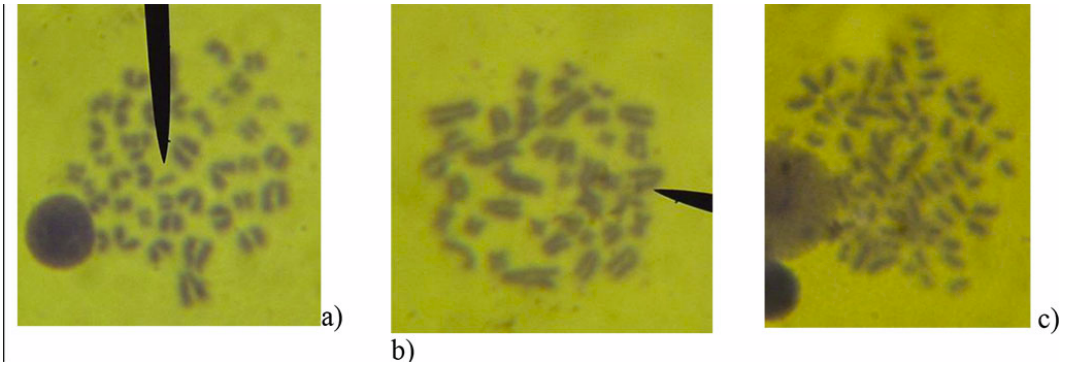

Figure 3. Cytogenetic disorders discovered under the influence of technetium: a) double fragment, b) deletion, c) polyploid.

CONCLUSION

Examining the cytogenetic indicators, a significant difference was found between intact and irradiated animals for all 3 indicators, that can be considered as markers of exposure to the technetium isotope. Having determined survival and cytogenetic indicators (mitotic index, chromosomal aberrations and the percentage of polyploid cells in the bone marrow cells of the femur) it was revealed that the group with injection of complex $[Cu(L^{CF3})_2]$ had the highest survival rate.

In terms of chromosomal aberrations and the number of polyploid cells, a significant difference was found in animals irradiated compared to the "irradiation + $[Cu(L^{CF3})_2]$" group (both after 15 and 30 days), which indicates the radioprotective property of the compound. In terms of the mitotic index (proliferative activity), a tendency towards normalization was noted after 15 days and a significant difference between Groups 2 and 3 was observed by the end of the study (after 30 days), which also proves the beneficial effect of this compound. Analysis of survival, changes in cytogenetic parameters, multi-regression analysis using the two complexes confirms the greatest efficiency of $[Cu(L^{CF3})_2]$ in comparison with $[Cu(L^{cur})_2]H_2O$, because using $[Cu(L^{cur})_2]H_2O$ in Group IV and comparing the results with "pure irradiation" only a tendency toward normalization of cytogenetic parameters was observed.

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

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