

## DNA PROTECTION FROM RADIATION DAMAGE

N. H. KARAPETYAN \*

*Chair of Molecular Physics YSU, Armenia*

The use of radioprotectors is important as one way to decrease radiation-related deleterious effects on normal tissues. The aim of this research was to study the radioprotective abilities of the newly synthesized compounds such as Cu(II) complexes with tryptophan, histidine and phenylalanine. The rats were exposed to X-ray at 5 Gy dose (LD<sub>50/30</sub>) after preliminary administration of 10 mg/kg compounds. At 3, 7, 14 and 28 days following irradiation DNA was isolated from rats' livers. In order to detect defects in the DNA structure on the specified days, the DNA melting parameters were determined, including the melting point, temperature interval of melting and hypochromic effect. The results showed that the Cu(II) complexes derived from tryptophan, histidine and phenylalanine exhibit radioprotective properties. The investigated compounds demonstrated positive effects on the melting parameters of liver DNA, testifying a preservation of the DNA secondary structure stability.

**Keywords:** radioprotectors, UV spectrophotometry, DNA melting.

**Introduction.** Ionizing radiation is a type of high-energy radiation capable of releasing electrons from atoms and molecules, thereby generating ions that can break covalent bonds. Ionizing radiation can cause various types of damage to DNA, RNA, proteins and other biomolecules. DNA is a major target of radiation induced damage. Ionizing radiation directly affects the DNA structure, causing DNA breaks, particularly, double strand breaks. Secondary effects are the generation of reactive oxygen species that oxidize proteins and lipids, as well as cause some DNA damage such as the formation of abasic sites and single strand breaks. Together, all these changes cause cell death and mitotic failure [1].

In connection with the increased use of ionizing radiation in various aspects of human life, the need arises to develop an effective and non-toxic radioprotector. Research into the development of radioprotectors worldwide has focused on screening a variety of chemical and biological compounds. Various natural or synthetic compounds possessing either antioxidant, or cytoprotective, or immunomodulatory properties, or combinations thereof, have been extensively evaluated for their radioprotective potentials. Many studies have focused on identifying agents that could protect cellular DNA from the radiation-induced chemical alterations. The compounds with antioxidant capacity could provide important protection against DNA damage [2, 3].

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\* E-mail: [nelkarapetyan@gmail.com](mailto:nelkarapetyan@gmail.com)

The goal of our study was to assess whether there is an improvement in the characteristics of rat liver DNA in case of pretreatment of animals with novel Cu(II) Schiff Base complexes before exposure to ionizing radiation. The DNA samples used during this study were analyzed using ultraviolet (UV) absorption spectroscopy.

**Materials and Methods.** Male albino rats of Wistar strain with 180–200 g body weight were used for experimental studies. The rats were bred at the vivarium of the Scientific Centre of Radiation Medicine and Burns (Yerevan, Armenia) under conventional laboratory conditions.

To be undergone to X-ray total body irradiation 10 rats were placed in well-ventilated box, located 50 cm from the radiation source. For irradiation, RUM-17 (Russia) therapeutic X-ray was used [3]. The radiation conditions were as follows: voltage 180 kV, current 15 mA, without filter, dose rate 1.78 Gy/min. The radiation dose was measured in units of gray (Gy), a measure of the absorbed dose of radiation, defined as the absorption of one joule of radiation energy per kilogram of matter.

The following 6 groups of animals were taken into consideration:

- intact animals of I group served as a control;
- rats of II group received 10 mg/kg Cu(II)–3-pyridine tryptophanate, Cu(II)–4-pyridine histidinate or Cu–3-pyridine phenylalaninate (the pure effect);
- animals of III group were exposed to X-ray irradiation at 5 Gy dose level (Lethal Dose, 50%, LD<sub>50</sub>) (irradiated control group);
- rats of IV group were pretreated using subcutaneous injection of 10 mg/kg Cu(II)–3-pyridine tryptophanate 1 h before exposure to X-ray irradiation at 5 Gy dose level;
- rats of V group received 10 mg/kg Cu(II)–4-pyridine histidinate subcutaneously 1 h prior to irradiation at 5 Gy;
- rats of VI group received 10 mg/kg Cu(II)–3-pyridine phenylalaninate subcutaneously 1 h prior to irradiation at 5 Gy.

Cu(II) complexes used in this study were administrated to the rats in the form of a suspension using deionizing water as a solvent. The animals were injected subcutaneously with 2 mg Cu(II) chelates in solution of 0.5 mL per 200 mg body weight 1 h before irradiation.

All used chemicals and reagents of Analytical Reagent grade, were obtained from “Sigma-Aldrich Stanford”, California, USA. Cu(II) complexes with Schiff Base ligands were used in studies. These new complexes were synthesized and kindly presented by Dr. V. Matosyan and Dr. V. Tonoyan from the Scientific Centre of Radiation Medicine and Burns (Yerevan, Armenia) [4, 5].

In order to perform analyses, rats were sacrificed under anesthesia on days 3, 7, 14, and 30 after irradiation (5 rats per each point). The DNA extraction material was kindly provided by Dr. M.H. Malakyan [5]. DNA was isolated from animals’ livers.

The toxicity of synthesized Schiff Bases was characterized based on calculation of the value of LD<sub>50/7</sub>, that is, the dose of a compound, at which the lethality of 50% animals was observed for 7 days after subcutaneous administration of the compound into the organism. In experiments on mice the toxicity of compounds was calculated using the Behrens integration method [6]. According to the test results Cu(II) complexes with Schiff Bases proved to be low toxic.

**Spectroscopy.** The absorption spectra and ultraviolet melting curves of DNA samples were recorded on a Lambda 800 UV/VIS spectrometer (Perkin-Elmer). DNA concentrations calculated in base pairs, were determined spectrophotometrically with  $\varepsilon = 1.31 \cdot 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at 260 nm [7]. All spectroscopic measurements were performed in buffer solution 0.1 BPSE (1 BPSE=6 mM Na<sub>2</sub>HPO<sub>4</sub> + 2 mM NaH<sub>2</sub>PO<sub>4</sub> + +185 mM NaCl + 0.1 mM EDTA), pH 7.2.

Melting experiments were carried out in the temperature range 35–95°C, using 10 mm thermostatic quartz cuvettes. The heating rate was 0.5°C/min, while absorbance at 260 nm was recorded. The degree of DNA denaturation  $1 - \theta$ , versus temperature was calculated using the following formula:

$$1 - \theta = \frac{A - A_{\min}}{A_{\max} - A_{\min}},$$

where  $\theta$  is the degree of helicity;  $A$ ,  $A_{\min}$  and  $A_{\max}$  are the absorbances of the experimental curve, the lower baseline (before melting) and the upper (after melting) baseline, respectively, at a given temperature  $T$  [8]. The melting temperature ( $T_m$ ) is defined as the temperature at which half of the total base pairs are “melted”, i.e.  $1 - \theta = 0.5$ , and  $\Delta T$  is the width of the melting interval equal to the difference of temperatures, at which the tangent in the bend point crosses the levels  $\theta = 0$  and  $\theta = 1$ , i.e.

$$\Delta T = \left( \frac{\partial \theta}{\partial T} \right)_{T=T_m}^{-1}.$$

The hypochromic effect ( $\Delta h$ ) was calculated by the following formula:

$$\Delta h = \left( \frac{A_{95^\circ\text{C}}}{A_{25^\circ\text{C}}} - 1 \right) \cdot 100\%,$$

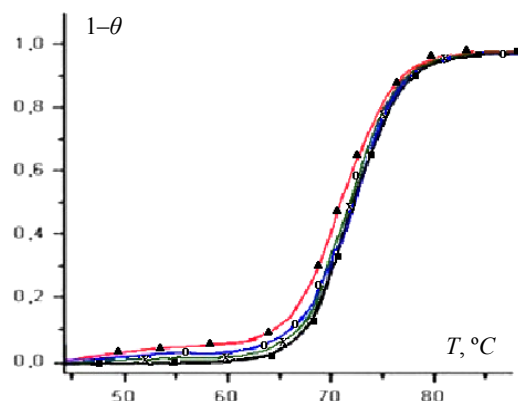
where  $A_{25^\circ\text{C}}$  and  $A_{95^\circ\text{C}}$  are the absorbances corresponding to the entire helical and entire coiled conditions of DNA [8].

**Results and Discussion.** The net effect of Cu(II) complexes with tryptophan, histidine and phenylalanine on the stability of rat liver DNA was investigated on the 1 day after administration of the preparations. The compounds were administered subcutaneously at a concentration of 10 mg/kg. The DNA was isolated from rats' livers using standard chloroform technique, and then investigated using thermal denaturation method. The melting temperature, the width of the melting interval and hypochromicity provided information on DNA stability and secondary structure defects. Figure shows the normalized melting curves of DNA isolated from I and II groups of rats. The melting curves of DNA extracted from the livers of rats of group II do not differ from melting curves of healthy rats' DNA.

So, the results showed that the Cu(II) complexes of tryptophan, histidine, and phenylalanine do not change the DNA melting parameters (minor changes within the experimental error). This indicates the stability of the DNA molecule in the presence of the Cu(II) complexes.

Significant changes in melting parameters were observed for DNA rats irradiated with X-rays (III group) compared with DNA of healthy rats from the

control group (I group). The melting temperature of DNA of irradiated rats decreased, the width of the melting interval increased, the hypochromicity decreased as compared with normal DNA. The same results related the melting parameters of irradiated DNA are described in the literature [9]. The DNA melting parameters calculated from obtained melting curves are summarized in Table.



The melting curves of DNA isolated from I norm control (■) and II group of rats on 1 day after administration of Cu(II)-3-pyridine tryptophanate (x), Cu(II)-4-pyridine histidinate (o) and Cu-3-pyridine phenylalaninate (▲).

*The melting parameters of DNA isolated from I (norm control), III (irradiated control), and IV, V, VI groups of rats on 3, 7, 14 and 28 days after irradiation*

Days after exposure	Study group	$T_m, ^\circ\text{C}$ Norm: $71.1 \pm 0.2$	$\Delta T, ^\circ\text{C}$ Norm: $7.5 \pm 0.15$	$\Delta h, \%$ Norm: $31.5 \pm 0.2$
3	5 Gy	$68.5 \pm 0.15$	$17.0 \pm 0.2$	$22.0 \pm 0.25$
	Cu-3-pyr. tryp	$71.0 \pm 0.2$	$9.15 \pm 0.1$	$27.0 \pm 0.2$
	Cu-4-pyr. hist	$70.1 \pm 0.1$	$7.9 \pm 0.2$	$29.5 \pm 0.2$
	Cu-3-pyr. pha	$70.0 \pm 0.15$	$9.5 \pm 0.15$	$29.5 \pm 0.15$
7	5 Gy	$67.5 \pm 0.2$	$23 \pm 0.15$	$16.0 \pm 0.2$
	Cu-3-pyr. tryp	$71.3 \pm 0.15$	$12.0 \pm 0.1$	$25.0 \pm 0.25$
	Cu-4-pyr. hist	$70.0 \pm 0.2$	$7.6 \pm 0.2$	$27.0 \pm 0.2$
	Cu-3-pyr. pha	$68.9 \pm 0.15$	$12 \pm 0.25$	$19.4 \pm 0.1$
14	5 Gy	$64.3 \pm 0.15$	$25.0 \pm 0.1$	$15.0 \pm 0.25$
	Cu-3-pyr. tryp	$69.0 \pm 0.2$	$10.5 \pm 0.2$	$20.7 \pm 0.15$
	Cu-4-pyr. hist	$70.2 \pm 0.1$	$8.2 \pm 0.15$	$26.5 \pm 0.1$
	Cu-3-pyr. pha	$68.5 \pm 0.25$	$14.9 \pm 0.2$	$19.2 \pm 0.2$
28	5 Gy	$62.2 \pm 0.15$	$27.0 \pm 0.25$	$14.0 \pm 0.25$
	Cu-3-pyr. tryp	$70.3 \pm 0.2$	$14.0 \pm 0.15$	$21.0 \pm 0.2$
	Cu-4-pyr. hist	$70.0 \pm 0.15$	$8.4 \pm 0.2$	$28.8 \pm 0.15$
	Cu-3-pyr. pha	$68.0 \pm 0.25$	$18.7 \pm 0.2$	$19.5 \pm 0.25$

The melting temperature  $T_m$  of irradiated rats' DNA decreased to 68.5, 67.5, 64.3 and 62.2°C on 3, 7, 14 and 28 days, respectively, compared with 71.1°C for normal DNA. The decrease in  $T_m$  indicates the destabilization of DNA molecules. This can probably be explained by the DNA strand breaks and breaks of hydrogen bonds between the DNA strands during irradiation.

The width of the melting interval  $\Delta T$  of irradiated DNA increased to 17, 23, 25 and 27°C on 3, 7, 14 and 28 days, respectively, in comparison with 7.5°C for normal DNA. The hypochromicity of irradiated DNA decreased to 22, 16, 15 and 14% on 3, 7, 14 and 28 days, respectively, while the norm is equal to 31.5%. This indicates the presence of partially molten various fragments of DNA molecules.

The results suggested that pretreatment of the irradiated rats with novel Cu(II) complexes (IV, V, VI groups) improves the liver DNA characteristics. It is noteworthy that the DNA melting parameters shift back to norm. Thus, for pretreated rats' DNA, after 3, 7, 14 and 28 days, there were observed an increase in  $T_m$  and  $\Delta h$ , a decrease in  $\Delta T$  compared with irradiated rats' DNA.

**Conclusion.** The newly prepared Cu(II) Schiff Base compounds were screened for their radioprotective activity. Examination of the DNA melting curves revealed an improvement in the DNA characteristics of rat liver, if animals were pretreated with novel Cu(II) Schiff Base complexes prior to exposure to ionizing radiation. Cu(II) complexes with Schiff bases derived from isomeric tryptophan, histidine and phenylalanine may be useful for the development of radioprotective agents.

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