



Biolog. Journal of Armenia, 1 (68), 2016

STUDY OF L-TYROSINE AND PYRIDINECARBOXALDEHYDES DERIVED SCHIFF BASE COPPER COMPLEXES AS POTENTIAL RADIOPROTECTIVE AGENTS

M.H. MALAKYAN, S.A. BAJINYAN, D.E. YEGHIAZARYAN

Scientific Centre of Radiation Medicine and Burns
ritamalakyan@email.com

Radioprotective properties of copper complexes with Schiff Bases derived from L-Tyrosine aromatic amino acid and 2-, 3-, or 4-pyridinecarboxaldehydes (Cu.2-Tyr, Cu.3-Tyr, and Cu.4-Tyr) were investigated in rats subcutaneously or orally treated with metallocomplexes at 10 or 40 mg/kg doses 1 or 24 hours before X-ray irradiation at 650 R that composes LD50/30 for untreated control irradiated animals. According to the study results, at all the applied schemes of animals preventive treatment Cu.2-Tyr failed to manifest radioprotection. However, Cu.3-Tyr and Cu.4-Tyr demonstrated expressed radioprotective activity that might be mainly related to their respective support of endogenous non-enzymatic antioxidant defence system of the organism.

It is concluded that the position of carboxaldehyde group with regard to nitrogen of pyridine ring, viz. 3 or 4, is of great importance for manifestation of radioprotective activity of Schiff Base copper complexes derived from pyridinecarboxaldehydes and L-Tyrosine.

Ionizing radiation – copper – complex – L-Tyrosine – pyridinecarboxaldehyde – radioprotection – antioxidant

Անոնների վրա կատարված փորձերում հետազոտվել են L-թիրոզին ամինաթթվի և 2-, 3- և 4-պիրիդինկարբոքսալդեհիդների ածանցյալ Շիֆֆի հիմքերի հետ Cu(II) կոմպլեքսների (Cu.2-Tyr, Cu.3-Tyr, և Cu.4-Tyr) ճառագայթապաշտպանիչ հատկությունները: Մետաղական կոմպլեքսները ներմուծվել են օրգանիզմ ենթամաշկային կամ օրալ 10 կամ 40 մգ/կգ դոզաչափով 1 կամ 24 ժ 650Ռ դոզայով ճառագայթահարումից առաջ, ինչը կազմում է ՄԴ50/30, այսինքն՝ այն դոզան, որը 30 օրվա ընթացքում բերում է ստուգիչ խմբի կենդանիների 50%-ոց մահացությանը: Համաձայն ստացված արդյունքների՝ նախնական ներմուծման բոլոր կիրառված սխեմաների դեպքում Cu.2-Tyr-ը չի դրսևորել ճառագայթապաշտպանիչ ազդեցություն: Սակայն Cu.3-Tyr-ը և Cu.4-Tyr-ը ցուցաբերել են արտահայտված ճառագայթապաշտպանիչ ակտիվություն, որը կարող է կապված լինել օրգանիզմի բնածին՝ ոչ ֆերմենտային հակաօքսիդանտային պաշտպանության համակարգի համապատասխան մակարդակը պահպանելու նրանց ունակության հետ:

Ելնելով ստացված տվյալներից, կարելի է եզրակացնել, որ L-թիրոզինի և 2-, 3- և 4-պիրիդինկարբոքսալդեհիդների ածանցյալ Շիֆֆի հիմքերի հետ պղնձի կոմպլեքսների ճառագայթապաշտպանիչ ակտիվության դրսևորման համար կարևոր նշանակություն ունի կարբոքսալդեհիդային խմբի տեղակայումը, այն է՝ պիրիդինային օղակի ազոտի նկատմամբ 3 և 4 դիրքերը:

Իոնացնող ճառագայթում – պղնձ, կոմպլեքս – L-թիրոզին – պիրիդինկարբոքսալդեհիդ – ճառագայթապաշտպանիչ – հակաօքսիդանտ

В опытах на крысах были исследованы радиопротекторные свойства комплексов меди с Шиффовыми основаниями, производными ароматической аминокислоты L-тирозина и 2-, 3-, или 4-пиридинкарбоксальдегидов (Cu.2-Tyr, Cu.3-Tyr и Cu.4-Tyr). Металлокомплексы вводили в организм подкожно или перорально в дозах 10 и 40 мг/кг за 1 ч или 24 ч перед рентгеновским облучением в дозе 650 Р, что составляет LD50/30 для контрольно облу-

ченных нелеченых животных. Согласно результатам исследования, при всех примененных схемах превентивного введения Cu.2-Тур не оказывал радиозащитного действия. Однако Cu.3-Тур и Cu.4-Тур проявляли выраженную радиопротекторную активность, что может быть связано с их способностью поддерживать соответствующий уровень эндогенной системы неферментативной антиоксидантной защиты организма.

Исходя из полученных данных, можно заключить, что важное значение в проявлении радиозащитной активности комплексов меди с Шиффовыми основаниями, производными L-тирозина и 2-, 3-, или 4-пиридинкарбоксальдегидов, имеет расположение карбоксальдегидной группы, а именно 3 и 4, относительно азота пиридинового кольца.

Ионизирующая радиация – медь, комплекс – L-тирозин – пиридинкарбоксальдегид – радиозащита – антиоксидант

The increased use of ionizing radiation in various aspects of human life has necessitated development of effective and non-toxic radioprotectors against harmful effects of radiation exposures. Despite the substantial amount of research aimed at development of radioprotectors, there is still a need in reliable and efficient radioprotectors. Experimental findings demonstrate that prophylactic treatment with essential metalloelement chelate complexes provides radioprotection and facilitates tissue repair processes required for recovery from radiation injury [7, 8].

Many small molecular mass Cu organic complexes have been reported as SOD-mimetics enabling them to prevent accumulation of toxic metabolic products, promote biochemical, cellular and tissue repair processes required for recovery from radiation injury [1, 2].

Apart from the metal ion, the biologically relevant ligands coordinated to metal also predetermine the biological activity of metallocomplex [4, 5]. Schiff bases, the condensation products of primary amines and aldehydes, have high chelating ability and therefore are an important class of ligands. Because aromatic aldehydes and aromatic amino acids play an significant role in chemical and biological recognition [3, 10], their Schiff base derivatives are interesting for use as ligands. Pyridinecarboxaldehyde isomers bearing the carboxaldehyde group at 2-, 3- or 4-position with regard to nitrogen of pyridine ring are useful precursors for complex forming Schiff bases, because they might have similar physiological effect with pyridoxal-amino acid systems, which are significant in many metabolic reactions intermediated with amino acid and pyridoxal.

In this study the radioprotective properties of copper complexes with Schiff Bases derived from L-Tyrosine aromatic amino acid and isomeric 2-, 3-, or 4-pyridinecarboxaldehydes (Cu.2-Tyr, Cu.3-Tyr, and Cu.4-Tyr) were investigated, and the role of aldehyde group location in pyridine ring in the aspect of manifestation of radioprotection was ascertained.

Materials and methods. 1. Experimental design. Albino Wistar rats weighing 180-200 g were used in the study. RUM-17 roentgen facility was applied for animal radiation exposure in 2 experimental sets. In the 1st set of experiments, animals were subcutaneously or orally treated with tested copper complexes at 10 or 40 mg/kg doses 1 or 24 hours before X-ray irradiation at 650 R that composed LD_{50/30} for untreated control irradiated rats, i.e. the specified dose level was lethal for 50% control animals by Day 30 post irradiation. The radiomodifying effects of the substances were evaluated on the base of animal survival and their average life-span (ALS) in 30 days after irradiation.

The 2nd set of experiments was designed to reveal some biological effects of the titled copper organic complexes that might be responsible for their radioprotective properties. With this aim animals were subcutaneously administered 10 mg/kg of the copper complexes 1 hour before irradiation at 500R dose. On days 3, 7, 14 and 28 after exposure the intensity of lipid peroxidation (LPO) processes in blood plasma and the integral antioxidant activity (AOA) of endogenous non-enzymatic water-soluble low molecular antioxidants (NEWSLMAO) were analyzed.

2. Blood plasma LPO activity was measured based on determination of malone dialdehyde (MDA) content [11]. Briefly, the mixture of 0.1 ml blood plasma with 2.5 ml of 20% trichloroacetic acid solution and 1 ml of 0.68% solution of sodium thiobarbiturate was placed in a boiling water bath for 30 min. After cooling and adding 4 ml of butanol the mixture was shaken for 30 min and then centrifuged at 3000 rpm. MDA content was determined spectrophotometrically on UNICO 2100 UV-VIS spectrophotometer using its molar extinction coefficient: $\epsilon = 1.56 \times 10^{-5} \text{ moles}^{-1} \text{ cm}^{-1}$. The results were expressed as nanomoles MDA per 1 ml of blood plasma.

3. The integral AOA of NEWSLMAO was analyzed using photochemiluminescent analyzer PHOTOCHEM and the ACW kit (Analytik Jena, Germany). The method is based on determination of the degree of inhibition of photochemiluminescence detector signal produced by 10 microliters of blood serum in comparison with the activity of ascorbic acid as Standard. AOA of the sample studied was expressed in conventional units (c.u.) equal to quantity of Standard in nanomoles with the equivalent activity [6].

4. Statistical analysis. The results of blood biochemical analyses were expressed as mean \pm S.E. The Student's t-test was used to make a statistical comparison between the groups. The significance level was set at $p < 0.05$.

Results and Discussion. According to the results of radioprotective studies, at all the applied schemes of animals preventive treatment Cu.2-Tyr failed to manifest radioprotection. Moreover, at 40 mg/kg dose level both at subcutaneous and oral administration Cu.2-Tyr revealed radiosensibilizing effects and induced enhanced rats mortality rate in comparison with control irradiated group: in the treated groups survival made 30 %, while in untreated irradiated group this index achieved 50 % (tabl. 1).

In opposite to Cu.2-Tyr, the copper complexes containing 3- or 4-pyridinecarboxaldehydes, namely, Cu.3-Tyr and Cu.4-Tyr demonstrated expressed radioprotective activity. Thus, by Day 30 post irradiation in groups of animals either orally or subcutaneously given Cu.3-Tyr at both considered doses 1 hour before irradiation 100% survival was observed. The same results were registered at subcutaneous treatment with 10 mg/kg Cu.3-Tyr 24 hours prior to exposure (tabl.1).

In case of rats prophylactic treatment with Cu.4-Tyr pronounced radioprotective effects were obtained at 40 mg/kg dose level both at oral and subcutaneous mode of administration 1 hour before irradiation that facilitated animal survival increase up to 90%-100% and extended rats average life-span (tabl. 1). It should be noted that at lower dose level Cu.4-Tyr was non-effective as radioprotector.

According to the results of biochemical analyses, in animals of the Control group exposed to irradiation at 500 R a high level of LPO intensity along with a significantly decreased activity of NEWSLMAO were registered in all study days post exposure (tabl. 2).

In rats treated with 10 mg/kg copper complexes the index of LPO activity tended to be maintained within normal values during almost the whole observation period being at significantly lower level compared to Control. As to NEWSLMAO, the preliminary administration of copper complexes to rats before irradiation did not prevent a radiationinduced decrease in the level of this parameter during the early post-exposure period. In case of pre-treatment with Cu.2-Tyr and Cu.3-Tyr the significantly raised NEWSLMAO activity was registered on days 14 and 28. In the late post-exposure period, namely, on day 28, in rats treated with Cu.2-Tyr, Cu.3-Tyr, and Cu.4-Tyr the significantly high level of NEWSLMAO activity was recorded in comparison with Norm and the Control irradiation group as well.

Copper Schiff base complexes, Cu.3-Tyr and Cu.4-Tyr, can be considered as potent radioprotectors equally effective both at oral and subcutaneous treatment prior to radiation exposure. Apparently, the position of carboxaldehyde group with regard to nitrogen of pyridine ring, viz. 3 or 4, is of great importance for manifestation of radioprotective activity of the specified copper complexes derived from pyridine-carboxaldehydes and L-Tyrosine.

Table 1. Survival rate and ALS of animals in 30 post-irradiation days after total body irradiation at 650 R on the background of preliminary subcutaneous or oral administration of 10 and 40/mg/kg copper complexes 1 h or 24 h before exposure (Irradiated Control: 50% survival and 18.7 days ALS)

Copper complex	Time and mode of the metallocomplex administration to rats before irradiation		Survival, %		ALS, days	
			10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg
Cu.2-Tyr	1 h	subcutaneous	60	30	23.4	17.8
		oral	60	30	23.0	16.7
	24 h	subcutaneous	50	30	24.7	16.8
		oral	50	30	20.9	16.5
Cu.3-Tyr	1 h	subcutaneous	100	100	30	30
		oral	90	100	27.4	30
	24 h	subcutaneous	100	80	30	25.5
		oral	70	70	26	24.7
Cu.4-Tyr	1 h	subcutaneous	70	100	24	30
		oral	80	90	26.7	28
	24 h	subcutaneous	60	90	24.8	27.6
		oral	50	60	21.8	22.2

Table 2. Blood plasma LPO processes intensity and integral AOA of endogenous NEWSLMAO on days 3, 7, 14 and 28 after rats irradiation at 500 R performed on the background of 10 mg/kg copper complexes at preliminary subcutaneous administration

Indices	Days	Control: 500 R	Cu.2-Tyr + 500 R	Cu.3-Tyr + 500 R	Cu.4-Tyr + 500 R
LPO Norm: 1.10±0.11 nM MDA/ml pl.	3	*1.70±0.04	1.11±0.04**	1.12±0.08**	*1.48±0.03**
	7	*1.71±0.05	*2.39±0.18**	0.87±0.06**	0.85±0.02**
	14	*1.95±0.05	1.41±0.07**	1.01±0.07**	*1.46±0.04**
	28	*1.64±0.09	0.97±0.07**	1.32±0.06**	1.24±0.05**
AOA of NEWSLMAO Norm: 6.84±0.11 c.u.	3	*4.56±0.25	*5.91±0.06	*5.44±0.19**	*5.13±0.04**
	7	*2.66±0.034	*3.12±0.02**	*4.88±0.10**	*3.47±0.196**
	14	*5.43±0.12	*7.61±0.10**	*8.4±0.0.15**	*4.89±0.15
	28	*6.09±0.17	*9.82±0.11**	*9.6±0.0.16**	*8.23±0.18**

Note: * - statistically significant changes compared to Norm; ** - statistically significant changes compared to indices in animals of Control group in the same time intervals after irradiation

Efficient radioprotective properties exhibited by copper organic complexes might be mainly related to their respective support of endogenous non-enzymatic antioxidant defence system and their known SOD- and Catalase-mimetic activities [1, 2, 9].

Acknowledgement. The study was supported by ISTC A-1321 Project and 13-1F342 grant of the State Committee of Science of Armenia.

REFERENCES

1. Autzen S., Korth H., Boese R., Groot H., Sustmann R. Studies of Pyridinyl-Containing 14-Membered Macrocyclic Copper(II) Complexes, Eur. J. Inorg. Chem., **7**, p. 1401-1410, 2003.

2. *Durackova Z., Labuda J.* Superoxide-dismutase mimetic activity of macrocyclic CU(II)-tetraanhydroaminobenzaldehyde (TAAB) complex, *J. Inorg. Biochem*, *58*, p. 297-303, 1995.
3. *Meyer E.A., Castellano R.K., Diederich F.* Interactions with aromatic rings in chemical and biological recognition, *Angew. Chem. Int. Ed. Engl.*, *42*, 11, p. 1210-1250, 2003.
4. *Mishra N., Poonia K., Kumar D.* An overview of biological aspects of Schiff base metal complexes, *International Journal of Advancements in Research & Technology*, *2*, 8, p. 52-66, 2013.
5. *Kostova I., Saso L.* Advances in research of Schiff-base metal complexes as potent antioxidants, *Curr. Med. Chem.*, *20*, 36, p. 4609-4632, 2013.
6. *Popov I.N., Lewin G.* Photochemiluminescent detection of antiradical activity: II. Testing of nonenzymic water-soluble antioxidants, *Free Radic. Biol. Med.*, *17*, 3, p. 267-271, 1994.
7. *Sorenson J.R.J.* Cu, Fe, Mn, and Zn chelates offer a medicinal chemistry approach to overcoming radiation injury, *Current Med. Chem.*, *9*, p. 639-662, 2002.
8. *Sorenson J.R.J., Soderberg L.S., Chang L.W.* Radiation protection and radiation recovery with essential metalloelement chelates, *Proc. Soc. Exp. Biol. Med.*, *210*, 3, p. 191-204, 1995.
9. *Thummaruk S., Supaluk P., Theeraphon P., Chartchalerm I., Chanin N., Virapong P.* Copper Complexes of Nicotinic-Aromatic Carboxylic Acids as Superoxide Dismutase Mimetics, *Molecules*, *13*, 12, p. 3040-3056, 2008.
10. *Waters M.L.* The Role of Aromatic Interactions in Biomolecular Recognition: Contributions to Affinity and Specificity, *Proceedings of the Beilstein-Bozen Symposium "Molecular Interactions-Bringing Chemistry to Life"*, p.111, Bozen, Italy May 15th – 19th, 2006.
11. *Yoshioka T., Kawada K., Shimada T., Mori M.* Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood, *Amer. J. Obstet. & Gynecol.*, *135*, 3, p. 372-376, 1979.

Received on 18.01.2016