http://dx.doi.org/10.35630/2199-885X/2020/10/12

EVALUATION OF PERIPHERAL BLOOD INDICATORS AND CYTOGENETIC INDICATORS USING COPPER(I) COMPLEXES FOR BURNS

Nikoghos Hovhannisyan[1], Anahit Karapetyan[1]✉, Marina Porchia[2], Carlo Santini[3], Ashot Dallakyan[1], Nektar Harutyunyan[1]

[1] *National Burn Center, Yerevan, Armenia*
[2] *ICMATE, National Research Council (CNR), Padua, Italy*
[3] *School of Science and Technology, University of Camerino, Italy*

✉ *ncrmio@web.am*

KEYWORDS — burn, copper complexes, proliferative activity, chromosome aberrations, ploidy of cells , number of red, white blood cells, hemoglobin level.

INTRODUCTION

Extensive deep burns cause a complex of peculiar pathological functional and morphological changes of internal organs and systems of the body. According to numerous literature data, a significant factor in further development of damage of tissues after burn injury is excessive activation of lipid peroxidation of cell membranes and the appearance of an excessive quantity of oxygen free radicals, generated by activated leukocytes, as well as those formed during tissue proteolysis [1, 2].

Burn injury also causes deep changes in hematological parameters. In case of blood flow through tissues during the burn period, we can view thermal damage and destruction of red blood cells with the release of free hemoglobin into the plasma [3, 4, 5].

Burn disease also causes a expressed leukocyte reaction, described by many researchers in clinic and during experiment [6, 7].

For tissue healing in burn cases, drugs that have anti-inflammatory, analgesic and regenerative properties are used [8]. In this field, metal-organic complexes based on copper and possessing high antioxidant activity are of special interest.

The copper biological role is diverse. Vital enzymes, performing complex functions in the body, comprise such a metal. Copper is the key component of the enzyme cytochrome oxidase, carrying out cellular respiration in all organs and tissues and is a constituent of vitamins, hormones and pigment substances. Copper has an impact on the synthesis of sex hormones, normalizes the work of the endocrine system, activates insulin [9].

The biogenous role of copper is participating in the processes of hematopoiesis. The trace element taking part in the synthesis of hemoglobin, carrying out the transfer of oxygen in the body, increases the speed of blood circulation. Copper takes part in the synthesis of collagen and elastin, supports skin turgor; without it connective tissue loses its resilience, bones and cartilage lose elasticity. Copper is also important for nerve tissue, it is a constituent part of the myelin sheaths of nerve cells isolating nerve fibers. Copper has an active participation in the metabolism of carbohydrates: activates the oxidation of glucose, slows down the destruction of glycogen in the liver. Copper is of great importance for the immune system. The metal neutralizes the toxins of microorganisms, prolongs the impact of antibacterial drugs [10, 11], reduces inflammatory reactions.

Copper derivatives are used for burns healing [12]. The use of copper sulphate determines a faster closure of the dermal wounds so the application of copper sulfate has been proposed in regenerative medicine [13].

According to literary sources and also our early studies [14, 15, 16, 17], several copper-based complexes are of low toxicity and expressed radioprotective properties.

In order to identify a possible positive effect on burns, we studied two copper(I) complexes, namely: [Cu (PTA)$_4$] BF$_4$ and [Cu (PCN) (HBP$_{z3}$)] PTA=1, 3, 5-triaza- 7-phosphaadamantane. PCN = tris (cyanoethyl) phosphine and HBP$_{z3}$ = trispyrazolylborate. Such compounds (hereinafter referred to as PTA and PCN) have been chosen because they have already demonstrated remarkable cytotoxic toward cancer cells both in vitro and in vivo tests [18]. Moreover as compound PTA is an homoleptic, water-soluble compound, whereas compound PCN is a mixed-ligand neutral complex insoluble in water (Fig. 1), we tried to correlate their therapeutic effect in case of thermal burns with their chemico-physical features.

MATERIALS AND METHODS

The synthesis of copper(I) complexes [[Cu(PTA)$_4$[BF$_4$] (PTA=PTA=1,3,5-Triaza-7-phosphadamantane) and [HB(pz)$_3$Cu(PCN)]

($HB(pz)_3$=tris(pyrazolyl)borate, PCN=tris (cyanoethyl)phospine was carried out according to published procedures [18, 19]

The in vivo experiments were performed on sexually mature white, outbred rats with an average weight of 180–200 g. All animal experiments were performed in according to the compliance with EC Directive 86/609/EEC.

Rats were divided into 4 groups: I — only with a thermal burn (clean burn); II — thermal burn with injection of the (I) complex PTA; III — thermal burn with injection of the Cu (I) complex PCN; IV — animals without burn (intact group)

On the epilated surface of the skin in the back of animals was applied thermal burn of III AB degree on 30% of the body surface. 30 minutes after the applied burn, Cu complexes of PTA and PCN were administered intraperitoneally to various groups of animals at a dose of 50 mg/kg in the form of an aqueous suspension. The control group consisted of animals with a clean burn. Injection was performed every 2 days during 14 days before the start of rejection of the wound scab.

The activity of these compounds was evaluated by survival, average life expectancy that showed the dynamics of death of experimental rats during a 30-day monitoring.

Visual monitoring of a burn wound was carried out. Observations were carried out during 60 days, when the wounds healed completely and were covered with coat (pelage).

For hematological analysis blood was taken from the tail vein at certain terms (on the 3, 7, 14 and 30th days). The following indicators were determined: blood coagulation time; the leukocyte number (according to the classical method with the help of the Goryaev camera); the hemoglobin level (hemoglobin was studied on the device *Mindray BA-88A*), red blood cells and platelets (was studied using an optical microscope at a magnification of x300).

For cytogenetic analysis (by the method of Ford-Wollam) the bone marrow was taken from the femur. Cytogenetic analysis was performed on an optical microscope. We studied the mitotic index (MI) with an increase of ×300, chromosomal aberrations (CHA) ×900, polyploid cells (PC) ×1440.

RESULTS

On the 60th day after complete wound healing, visual monitoring showed that epithelial regeneration, fouling were more actively viewed in the group with intraperitoneal injection of PTA than in the control groups and PCN injection.

On the fifth day after the burn, when the rats received 3 injections of metal complexes, the analysis of the karyotype showed that all cytogenetic indicators in three groups of experimental animals statistically significantly differ from the karyotype of intact individuals. When comparing the cytogenetic indices of both groups of rats with introduced copper complexes with control data, a slight variability of the parameters was observed without statistically significant shifts, as shown in the table 1.

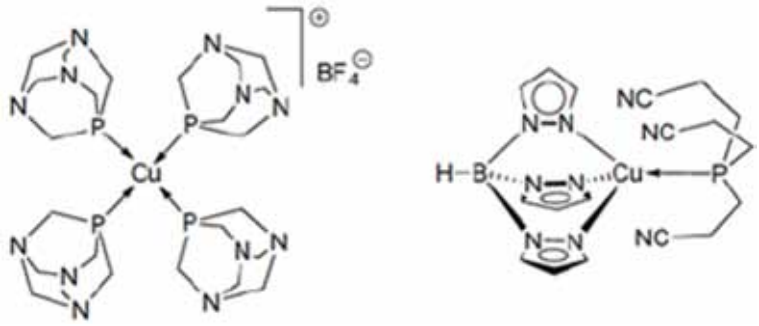

Fig. 1. *Chemical structure of $[Cu(PTA)_4]BF_4$ (PTA) left and $[Cu(PCN)(HBP_{z3})](PCN)$ right*

As can be seen from the table, when comparing the cytogenetic parameters of the last term of the experiment of three experimental groups with the intact group, there was a tendency to increase the mitotic index (MI). In the control and PCN groups, this indicator is still significantly lower than normal, but in the PTA group it is closer to normal (the difference between the normal group and Burn + PTA is not significant). Aberrant chromosomes, mainly in the form of gaps, in the control and PCN groups are significantly higher than normal, and in the PTA group this indicator does not exceed a statistically significant level.

Comparison of the karyotypes of this period of study of the PCN and PTA groups with the control showed that the cytogenetic parameters of MI and PC of the PCN groups are significantly different. This means that, due to the combination of PTA, the indices MI and PC have a clear tendency to normalize.

Summing up the cytogenetic indices and visual monitoring criteria as well as the absence of animal death in the group with injection of the metal-organic complex PTA (vedi infra) we can conclude that this compound activates reparative processes, improving the cytogenetic status of experimental rats. In particular, it increases the proliferation of bone marrow cells, as a result of which the hematopoiesis of burnt animals improves.

Hematological parameters were analyzed in dynamics over the entire duration of the experiment and the results are shown in table 2.

As can be seen from table 2, the burn causes a significant change in the number of white blood cells

Table 1. *Change of cytogenetic parameters in 4 groups after burn*

terms after the study	5th day after the burn				30th day after the burn		
groups / indicators	intact group	control	Burn+PCN	Burn+PTA	control	Burn+PCN	Burn+PTA
MI%	20,1±2,8	9,8±1,2 P_1<0.05	10,1±1,5 P_1<0.05 P_2^1>0.05	11,2±1,7 P_1<0.05 P_2>0.05	12,8±1,8 P_1<0.05	13,2±1,9 P_1<0.05 P_2>0.05	17,0±1,2 P_1>0.05 P_2<0.05
CHA%	3,0±0,22	4,4±0,42 P_1<0.05	4,6±0,44 P_1<0.05 P_2>0.05	4,1±0,39 P_1<0.05 P_2>0.05	4,2±0,48 P_1<0.05	4,3±0,42 P_1<0.05 P_2>0.05	3,8±0,4 P_1>0.05 P_2>0.05
PC%	0	4,0±0,38	3,9±0,4 P_2>0.05	3,2±0,36 P_2>0.05	3,3±0,36	2,9±0,31 P_2>0.05	2,0±0,24 P_2<0.05

P_1 *— The significance of differences in the performance of groups with PCN and PTA with the norm group*
P_2 *— The significance of differences in the performance of groups with PCN and PTA with the control group*

Table 2. *Rat blood counts for a clean burn, PCN and PTA injection*

indicators	days	Clean burns (control)	Burn+PCN	Burn+PTA
Blood coagulation time (seconds)	3	415,0±7,63	413,8±47,75	340,0±35,46*
	7	327,5±32,5	230±11,55*	217,5±42,25*
	14	360,0±34,64	150,0±20,31*	207,4±12,53*
	30	316.7±52.47	280.7±35,51	195,0±36.63*
Leukocyte ($x10^9$/l)	3	8,3±0,59	9,5±1,6	7,5±1,2
	7	10,2±2,2	14,8±4,3	17,6±1,1*
	14	8,8±0,7	9,3±2,2	11,7±0,7*
	30	5,93±0,6	10,7±1,4*	11,54±2.1*
Platelets (N/µl)	3	588333,3±44378,42	568750±113933	489000±57649,8
	7	637500±2500	668333,3±64957,25	606250±48104,7
	14	495000±66583,28	392500±37052,89	405000±54815,71
	30	705000±5000	576666,7±71316,98	817000±43433,86*
red blood cells ($x10^{12}$/l)	3	5,92±0,13	5,52±0,9	5,45±0,4
	7	3,13±0,1	3,2±0,15	3,88±0,35*
	14	6,56±0,18	4,62±0,17*	5,23±0,19*
	30	6,38±1,9	6,0±0,8	5,62±0,26*
Hemoglobin (g/l)	3	134,6±6,06	133,5±10,57	138,2±9,24
	7	136,5±5,5	107,3±11,39*	97,3±1,2*
	14	163,3±10,13	136,2±2,52*	141,4±2,25*
	30	161,3±1,76	140,3±4,97*	139,2±3,39*

** — The significance of differences in the performance of groups with PCN and PTA with the control group*

in the blood of animals. So, on the 7th day after the injury in all groups there is an increase in the number of leukocytes, which indicates the course of reparative processes. The highest rates were obtained on the 7th day of the study, since at this time an infection joins the leukocyte reaction of the body. And if leukopenia is observed in rats from the control group at the end of the study, a tendency toward normalization is observed in groups with injection of compounds. On the 30th day, there was a significant difference in the level of leukocytes in the control group from groups with injection of PTA and PCN complexes.

By the 30th day, the level of leukocytes in groups of animals with burns+PTA and burns+PCN injection approaches the level of leukocytes in the normal group ($8.6\pm0.68 \cdot 10^9$/l).

Burn injury leading to inhibition of hematopoiesis is the cause of severe erythropenia and anemia. These two indicators in the early stages (7th day) of the study were significantly lower than the corresponding norms (erythrocytes: $6.2\pm0.35 \cdot 10^{12}$/l; hemoglobin; 158.0±14.6 g/l). As can be seen from the table, at the last observation periods (14th and 30th days), an increase in the number of red blood cells and a hemoglobin level. There was a significant difference between these indicators in the control group and the group with the PTA compound, which indicates a beneficial effect of this complex.

This conclusion is also confirmed when calculating the survival and average life expectancy of rats. Survival experiments were performed on 21 rats, 7 animals per group. Groups: Clean burn; PTA + burn and PCN + burn. An experiment to determine survival and average life expectancy showed that in the group of rats with the PTA complex, the indicators were significantly better (100% survival) than in animals with only burns (control), as shown in Table 3.

Table 3. *Survival and average life expectancy of rats*

Groups	Survivalc(%)	Average life expectancy in days
Control	57	20,57
Burns+PTA	100	30
Burns+PCN	57	19,57

According to the equations and logarithmic regression curves describing the dynamics of survival and shown in Fig. 2, the survival of the group with the injection of the PCN complex and the control group is identical and there will be a further decline in this percentage in contrast to the group introduced with the PTA complex.

Based on the results of survival, life expectancy, cytogenetics and hematology, we can conclude that the PTA complex under study exhibits tangible healing properties. According to all observed criteria, the PTA complex showed beneficial effects on experimental animals.

CONCLUSIONS

Basing on the survival results, average life expectancy, cytogenetic and hematological indicators, it can be concluded that studied complex PTA demonstrate noticeable healing properties.

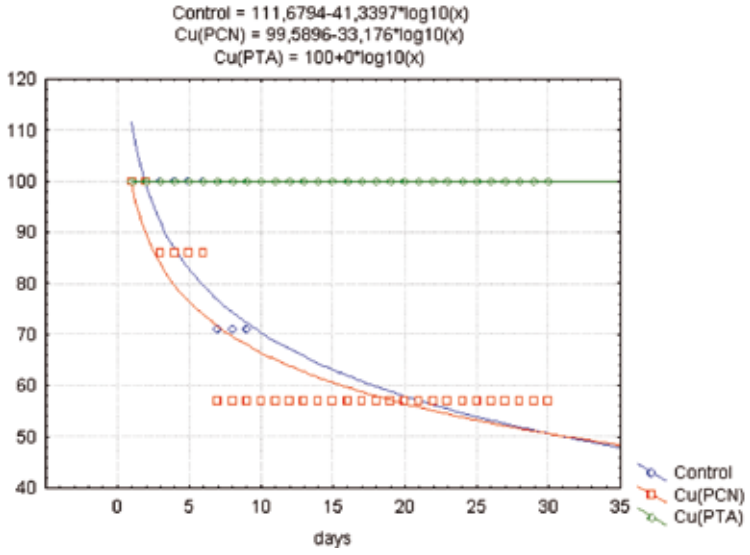

Fig. 2. *Equations and logarithmic regression curves describing the dynamics of survival*

In the early stages of analyzes (days 3 and 7), both compounds mitigate the damaging effects of burn injury, but in the last periods of observation (days 14 and 30), the group with PTA injection has many test values: (blood counts) approached normal values.

Based on the results obtained, it can be assumed that the studied Cu-1 complexes effectively promote reparative processes in bone marrow cells and has a therapeutic effect on thermal burns (especially PTA). The results of this yet preliminary research require continuation and search for new effective means for treating burn surfaces.

REFERENCES

1. **Smirnov S.V., Spiridonova T.G., Pakhomova G.V. et al.** Lipid peroxidation in patients with burn injury complicated by gastroduodenal bleeding // Combustiology, 2008, No. 1, pp. 10–13
2. **Ushakova T.A.** To the question of lipid peroxidation in patients with burn injury // Combustiology, 2008, No. 2, pp. 4–8
3. **Loginov L.P., Smirnov S.V., Khvatov V.B. et al** Blood transfusions in burned skin during the restoration of the skin // Proceedings of the II Congress of Combustiologists of Russia, Moscow, 2008, M., pp. 108–109
4. **Slesarenko S.V.** Anemia in burn disease and the possibility of its correction // Bulletin of Surgery. I.I. Grekova, 1997, v. 156, No. 4, p. 37–41
5. **Murazyan R.I., Ilyukhin A.V.** On the destruction of red blood cells with extensive burns. Clinic. Medicine, 1971, No. 6, p. 44–49

6. **Gubler E.V., Khrebtovich V.N., Subbota A.G.** Thermal burns and burn disease. In the book. Disease Modeling / Ed. S.V. Andreyeva. M, Medicine, 1972, p. 46–59
7. **Zinoviev E.V. et al.** New St. Petersburg medical statements. 2006, No.3, p. 73–74
8. **Buloyan S.A.** The effect of Dermafen ointment on the regenerative processes of the skin of white rats after an experimental thermal burn. International Journal of Applied and Fundamental Research, 2015, No. 10-2, p. 268–272
9. **Rodimin EM,** Treatment with copper and precious metals, M., 2009, -42c. ISBN 978-5-9900135-7-5
10. **Shut V.N., Mozhzharov S.E., Yanchenko V.V.** "Physical and antibacterial" properties of ultrafine copper powders ", Bulletin of Vitebsk State Technological University, 2 (31), 2016.
11. **Karomatov I.D.** Copper and its significance in medicine Electronic scientific journal "Biology and Integrative Medicine" 2017 №11
12. **Narauanan G., Bharathidevi S.R., Vuyyuru H., Muthuvel B. and Konerirajapuram Natrajan S.** PLoS One, 2013, 8, e71982
13. **Barralet J., Gbureck U., Habibovic P., Vorndran E,, Gererd C. and Doillon J.,** Tissue Eng, Part A, 2009, 15, 1601–1609
14. **Kornblatt A.P., Nicoletti V.G., A Travaglia** Jounal of Inorganic Biochemistry 161(2016), p1–8
15. **Das A., Sudhahar V., Chen G., Kim H.W.,Ushio-Fukai & T.** Fukai Scientific Reports/6:33783/DOI:10.1038/srep33783
16. **Bajinyan S.A., Malakyan M.G., Egiazaryan D.E., Dallakyan A.M., Vardevanyan L.A.,** Comparative study of the radioprotective activity of copper complexes with Schiff bases, derivatives of L-tyrosine and L-phenylalanine. Actual problems of radiation safety, Yerevan, 2014, p. 61–64
17. **Pogosyan A.S., Dallakyan A.M., Karapetyan A.G., Hovhannesyan A.N.** Role and probable mechanisms of polyploidy cells formation in irradiated organism. International Conference Radiation Safety challenges in the 21th Century. Proceedings, Yerevan, 2012, p. 85–86.
18. **Porchia M., Benetollo F., Refosco F., Tisato F., Marzano C., Gardin V.,** Synthesis and structural characterization of copper(I) complexes bearing N-methyl-1,3,5-triaza-7-phosphaadamantane (mPTA): Cytotoxic activity evaluation of a series of water soluble Cu(I) derivatives containing PTA, PTAH and mPTA ligands. J. Inorg. Biochem, 2009, 103(12), 1644–1651
19. **Gardin V., Tisato F., Dolmella A., Pellei M., Santini C., Giorgetti M., Marzano C., Porchia M.** In Vitro and in Vivo Anticancer Activity of Copper(I) Complexes with Homoscorpionate Tridentate Tris(pyrazolyl)borate and Auxiliary Monodentate Phospine Ligands. J.Med.Chem, 2014, 57(11), 4745–4760