MORPHOLOGY, PATHOLOGY, PHYSIOLOGY

Cite as: Archiv EuroMedica. 2022. 12; 3: e1. DOI 10.35630/2199-885X/2022/12/3.5

Received 1 May 2022;
Received in revised form 14 May 2022;
Accepted 14 May 2022

CHANGES IN BLOOD INDICATORS IN CASE OF USE OF BIS[BIS(3,5-DIMETHYLPYRAZOL-1-YL)ACETATO]COPPER(II) COMPLEX AFTER BURN INJURIES

Anahit Karapetyan[1] ✉, ***Carlo Santini[2]***, ***Maura Pellei[2]***, ***Vahan Grigoryan[3,4]***, ***Ashot Dallakyan[1]***, ***Jasmina Petrosyan[1]***

[1]*National Burn Center, Yerevan, Armenia*
[2]*School of Science and Technology, Chemistry Division, University of Camerino, Camerino Italy*
[3]*University of Traditional Medicine of Armenia, Yerevan, Armenia*
[4]*L. A. Orbeli Institute of Physiology NAS RA, Yerevan, Armenia*

✉ ***ncrmio@web.am***

ABSTRACT

Burn injuries cause profound changes in hematological parameters. The degree of severity of such changes and the speed of development of disturbances depend on the quantitative parameters of the injuries (area and degree of damage).

One of the most urgent modern healthcare problems is the search and development of new effective approaches and medications for healing burn injuries. Radioprotectors differ by their effectiveness and duration of their protective action. For the purpose of promotion of tissue regeneration after burn injuries, medications with anti-inflammatory, analgesic and pro-regenerative properties are used. In this regard, copper-based complexes are of particular interest.

The biogenic role of copper includes its participation in the processes of hematopoiesis. Copper also plays an important role in neutralizing the toxins of microorganisms, in prolongation of the action of antibacterial medications, as well as in reducing inflammatory reactions. The use of copper sulfate promotes the acceleration of skin regeneration in burn wounds, and Cu complexes show low toxicity.

The aim of the study is to identify a possible positive effect of the bis[bis(3,5-dimethylpyrazol-1-yl)acetato]copper(II) complex, $Cu[HC(COO)(pz^{Me2})_2]_2$, in case of burns. As a result of hematological and cytogenetic studies, it was found that $Cu[HC(COO)(pz^{Me2})_2]_2$ has the ability to prevent or reduce the pathological processes in case of deep burns. The findings were also confirmed by survival assessments (100%).

Keywords: burn, copper(II) complexes, number of erythrocytes, leukocytes, hemoglobin level, hematocrit.

INTRODUCTION

It is known that the hematopoietic system, as an actively proliferating tissue, is extremely sensitive to the influence of various external factors, including injuries such as burns. Therefore, the issue of the influence of pathogenic factors on the blood system and hemostasis presents a considerable interest.

Extensive deep burns and radiation in relatively high doses cause a complex of pathological functional and morphological changes in the internal organs and systems of the body. Burn injuries also cause profound changes in hematological parameters.

One of the most important problems in modern combustiology is the search for novel effective methods for accelerating the regeneration of burn wounds. Such medications should have high efficiency and duration of action. According to Saxonov et al. [1], the requirements for such medications are: the drug must be sufficiently effective and not cause any severe adverse reactions; it must act quickly and for a relatively long time; it must be non-toxic; it should not have even a short-term negative effect on the body; it must have a dosage form convenient for oral administration or injection; it should be stable during storage, retain its pharmacological properties for a long time.

In burn injuries, when blood flows through tissues, thermal damage and destruction of red blood cells (RBC) takes place, with subsequent release of free hemoglobin into the plasma [2-4]. Burn disease also causes a pronounced leukocyte reaction which is described by many researchers in clinical and experimental studies [5,6].

For tissue regeneration in burns, medications with anti-inflammatory, analgesic and pro-regenerative properties are used [3]. In this regard, copper-based metal-organic complexes with high antioxidant activity are of particular interest.

The main biogenic role of copper is its participation in hematopoiesis [7]. This microelement is involved in the synthesis of hemoglobin, which transports oxygen throughout the body, as well as in increasing the rate of blood circulation. Copper also plays an important role for nervous tissue: it is a constituent part of the myelin sheaths that insulate nerve fibres. Copper is actively involved in carbohydrate metabolism: it activates glucose oxidation, slows down the cleavage of glycogen in the liver. Copper is also important for the immune system. It is involved in the neutralization of the toxins of microorganisms, as well as in prolongation of the action of antibacterial drugs [8,9] and reduction of inflammatory reactions.

Copper sulphate promotes the acceleration of skin regeneration in burn wounds and its use has been proposed in regenerative medicine [10,11]. According to our early studies [12,13], copper-based complexes have low toxicity.

The aim of this study was to identify a possible positive effect of the copper complex $Cu[HC(COO)(pz^{Me2})_2]_2$ in case of burn injuries. This complex has been chosen because such compounds have a number of advantages: they are inexpensive, stable, easy to use, have low toxicity and prolonged action. $Cu[HC(COO)(pz^{Me2})_2]_2$ is a homoleptic compound and we dissolved it in DMSO in the experiment. We made an attempt to compare its therapeutic effect in case of thermal burns with its chemical-physical properties.

MATERIALS AND METHODS

The bis(3,5-dimethylpyrazol-1-yl)acetic acid, $[HC(COOH)(pz^{Me2})_2]$, was prepared by literature method [14]. The copper(II) bis(3,5-dimethylpyrazol-1-yl)acetato complex, $Cu[HC(COO)(pz^{Me2})_2]_2$ (Fig. 1), was synthesized by a modified literature method [15,16], by a one-step synthetic protocol involving the reaction of the sodium salt of bis(3,5-dimethylpyrazol-1-yl)acetic acid, $Na[HC(COO)(pz^{Me2})_2]$, with $CuCl_2{\cdot}2H_2O$ in water solution. Pale-blue crystals of $Cu[HC(COO)(pz^{Me2})_2]_2$ started to precipitate at room temperature in a few hours. They were filtered off after 12 hours, washed with water and dried in vacuo. The result of the synthesis was an analytically pure compound that matched all the spectroscopic parameters of the literature compound [15].

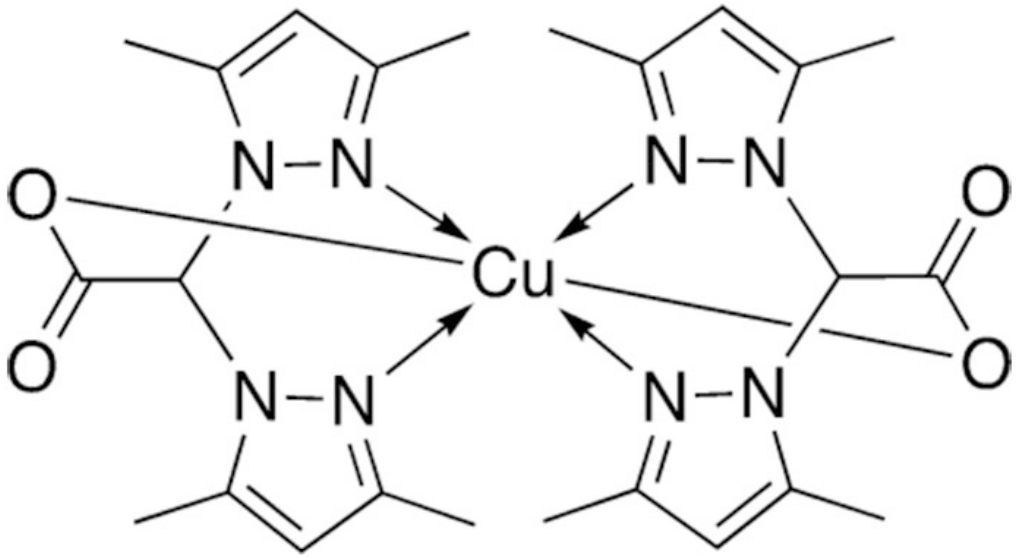

Fig. 1. Chemical structure of Cu[HC(COO)(pz^{Me2})$_2$]$_2$

In order to reveal the therapeutic effect of Cu[HC(COO)(pz^{Me2})$_2$]$_2$ in thermal burns, a series of *in vivo* experiments was carried out on white outbred mature rats with an average weight of 180 g. The maintenance and use of animals in the study was in compliance with Directive 2010/63/EU.

Animals were divided into 3 groups: I: intact animals (without burns), II: with thermal burn without treatment, III: thermal burn + injection of the Cu[HC(COO)(pz^{Me2})$_2$]$_2$ complex.

Animals were subjected to a thermal burn lesion of III AB degree on the epilated skin in the back area, affecting 30% of the body surface. After this procedure, the animals were intraperitoneally injected with the complex dissolved in DMSO, at a dose of 40 mg/kg. The compound was administered one hour after the burn lesion (1st injection). Afterwards, the compound was administered intraperitoneally every other day at a dose of 20 mg/kg until the wound scab was rejected. Hematological parameters of peripheral blood (from the tail vein) were assessed on the days 3,7,14,21, and 30 of the experiment. The following indicators were determined: blood clotting time, leukocyte count (according to the classical method using a Goryaev camera); the level of hemoglobin, RBC count, platelet count, hematocrit. Visual monitoring of the burn wound was also carried out until the wound was completely healed.

The activity of this compound was also evaluated in terms of survival and mean life expectancy. Survival and mean life expectancy of animals were monitored, describing the dynamics of the lethal outcomes in experimental rats during the 30-day period of the experiment (after the burn).

Statistical analysis of the obtained data was carried out using a number of computer programs designed for statistical processing of digital data arrays. Along with the programs developed by us, specialized statistical packages Statsoft-7, SPSS-10.0, MedCalc and StatGraphics Plus were used. The analysis was carried out using correlation and regression methods [16-18].

RESULTS

Visual inspection and monitoring of the wound healing process showed that regeneration of the epithelium and hair growth was more active in the group with intraperitoneal injection of the complex than in the group with a clean burn.

The results (shown in Table 1) in respect to the survival and average life expectancy of rats in three groups showed that in the group III (rats with the injected complex), the indicators were significantly better (100%) than in animals with burns without treatment. Thus, the aforementioned parameters were identical between group III and group I (intact rats).

Table 1. Survival and average life expectancy of rats

Group	Survival (in	Average life expectancy (in

	%)	days)
Intact animals (I)	100	30
Burn without treatment (II)	57	20,57
Burn + injection (III)	100	30

The survival dynamics curve in the case of Group II (burn lesion without treatment) is described by a logarithmic regression equation: y = 109.93-39.72lg(x), where y represents survival and x represents the days of experiment. The resulting equation makes it possible to predict the outcome in longer periods using the approximation method. Survival data indicate a significant beneficial effect of the compound on burn injury.

Hematological parameters were analyzed in dynamics during the entire period of the experiment. As can be seen from the results shown in Table 2, the burn lesion without treatment (Group II) causes a significant increase in the leukocyte count in the blood of animals until the end of the study, meanwhile in the group with the injection of the compound (Group III), moderate leukocytosis is observed, which resolves in normalization of this indicator by the end of the study.

Table 2. Blood parameters in rats with burns without treatment (Group II) and Cu[HC(COO)(pz^{Me2})$_2$]$_2$ injections (Group III)

Indicators	day	Burn without treatment - Group II (control)	Burn + Injection - Group III
Blood clotting time (norm: 363,0±23,03 sec)	3	(*) 264,0±12,88	372,2±16,96 *
	7	(*) 215,0±14,32	314,4±23,49 *
	14	(*) 225,6±13,09	(*) 296,0±13,56 *
	21	(*) 277,8±15,77	(*) 301,4±14,99
	30	330,0±18,44	357,0±23,75
Leukocytes (norm: 8,6±0,68 x10^9/L)	3	(*) 14,72±0,81	(*) 11,64±1,05 *
	7	(*) 16,92±0,618	(*) 13,24±1,22 *
	14	(*) 11,320±0,89	(*) 12,4±0,75
	21	(*) 13,64±0,67	(*) 11,28±0,73 *
	30	(*) 15,96±0,46	8,12±1,31 *
Platelets (norm: 522000,0± 10560,0 N/μL)	3	(*) 627000,0±10793,52	492000,0±17930,4 *
	7	(*) 681400,0±62572,38	(*) 471000±19455,08 *
	14	609000,0±52115,26	(*) 431000±24969,98 *
	21	590000,0±37524,98	522000±20832,67
	30	(*) 571000,0±22934,69	589000,0±48228,62

Red Blood Cells (norm: 6,2±0,35 x10^{12}/L)	3	5,92±0,13	(*) 5,08±0,33 *
	7	(*) 3,13±0,1	5,89±0,82 *
	14	6,56±0,18	5,38±0,27 *
	21	6,47±0,10	(*) 4,68±0,2 *6
	30	6,38±1,9	(*) 5,35±0,19
Hemoglobin, (norm: 158,0±14,6 g/L)	3	134,6±6,06	131,0±5,76
	7	136,5±5,5	131,5±2,77
	14	163,3±10,13	170,0±16,15
	21	162,3±5,95	147,9±4,02 *
	30	161,3±1,76	158,76±4,14
Hematocrit (norm: 37,2±1,75 %)	3	(*) 47,6±1,4	43,5±3,53
	7	(*) 49,1±0,91	35,3±1,95 *
	14	(*) 47,3±1,11	34,68±1,23 *
	21	(*) 48,3±1,18	39,54±3,92 *
	30	(*) 44,5±1,71	(*) 43,1±1,96

(*) Significant differences between the groups intact and burn without treatment; burn + complex

** Significant differences when comparing groups: burn without treatment and burn + complex*

On the 30th day, a significant difference was revealed between the leukocyte counts in the Groups II (burn without treatment) and Group III (burn + injection of the complex), which indicates a beneficial effect of $Cu[HC(COO)(pz^{Me2})_2]_2$ on burn injury. According to literature, burns cause an increase in blood clotting and viscosity, which is confirmed by the experimental data obtained by us. A burn (without treatment) affecting 30% of the surface led to a significant reduction in clotting time (normalization of this indicator was noted only by the 30th day), and in the case of treatment with $Cu[HC(COO)(pz^{Me2})_2]_2$, the blood clotting time of this group was comparable to normal values(in intact animals - Group I), which also indicates a mitigation of the influence of the factors of the burn lesion, from the very beginning of the experiment.

Burn injury leading to inhibition of hematopoiesis causes severe erythropenia and anemia. According to Table 2, on the day 7 of the experiment, a significant decrease in the RBC count was observed in Group II (burn lesion without treatment), in contrast to the group with the administered complex (Group III), which is also indicative of a beneficial effect of the complex. There was also a significant increase in hematocrit in Group II, in contrast to the hematocrit levels in Group III (burn + $Cu[HC(COO)(pz^{Me2})_2]_2$), which only slightly differed from normal values, which also indicates a mitigation effect.

CONCLUSION

As a result of hematological studies, it was found that the copper(II) compound $Cu[HC(COO)(pz^{Me2})_2]_2$ has the ability to prevent or reduce the effect of deep burns on the animal body, which makes it possible to continue research on the properties and biological activity of this complex as a promising and effective compound.

FUNDING

The work was supported by the Science Committee of RA, in the frame of the research project No 21T-1F126

REFERENCES

1. Saxonov P.P., Shashkov V.S., Sergeev P.V. Radiation pharmacology. Moscow, Medicine, 1976
2. Loginov L.P., Smirnov S.V., Khvatov V.B. et al. Blood transfusions in burnt patients in the process of skin restoration // Sat. scientific tr. II Congress of Combustiologists of Russia, Moscow, 2008, p. 108-109
3. Murazyan R.I., Ilyukhin A.V. On the destruction of erythrocytes in extensive burns. Clinical Medicine, 1971, No. 6, p. 44-49
4. Slesarenko S.V. Anemia in burn disease and the possibility of its correction. Vestnik khirurgii by I.I. Grekov, 1997, vol. 156, No. 4, p. 37-41
5. Gubler E.V., Khrebtovich V.N., Subbota A.G. Thermal burns and burn disease. In book. Disease Modeling / Ed. S.V. Andreeeva. Moscow, Medicine, 1972, p. 46-59
6. Zinoviev E.V. etc. New St. Petersburg medical records, 2006, No. 3, p. 73-74
7. Rodimin E.M. Treatment with copper and precious metals, NEU STC "Universitetsky", Moscow, 2009, 42pp., ISBN 978-5-9900135-7-5
8. Karomatov I.D. Copper and its importance in medicine. Electronic scientific journal "Biology and Integrative Medicine", 2017, No. 11
9. Shut V.N., Mozzharov S.E., Yanchenko V.V. Physical and antibacterial properties of ultrafine copper powders. Bulletin of Vitebsk State Technological University, 2(31), 2016, p. 97-105
10. Barralet J., Gbureck U., Habibovic P., Vorndran E, Gererd C. and Doillon J., Tissue Eng, Part A, 2009, No. 15, p. 1601-1609
11. Narayanan G., Bharathidevi S.R., Vuyyuru H., Muthuvel B. and Natrajan S.K., PLoS One, 2013, No. 8, p. e71982
12. 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
13. Poghosyan 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 21st Century. Proceedings, Yerevan, 2012, p. 85-86
14. Beck A., Weibert B.; Burzlaff N. Monoanionic N,N,O-Scorpionate Ligands and their Iron(II) and Zinc(II) Complexes: Models for Mononuclear Active Sites of Non-Heme Iron Oxidases and Zinc Enzymes. Eur. J. Inorg. Chem. 2001, No. 2, p. 521–527
15. Kozlevčar B., Gamez P., de Gelder R., Driessen W. L., Reedijk J. Unprecedented Change of the Jahn-Teller Axis in a Centrosymmetric Cu^{II} Complex Induced by Lattice Water Molecules - Crystal and Molecular Structures of Bis[bis(3,5-dimethylpyrazol-1-yl)acetato]copper(II) and Its Dihydrate. Eur. J. Inorg. Chem. 2003, No. 1, p. 47-50
16. Pellei M., Gandin V., Marchiò L., Marzano C., Bagnarelli L., Santini C. Syntheses and Biological Studies of Cu(II) Complexes Bearing Bis(pyrazol-1-yl)- and Bis(triazol-1-yl)-acetato Heteroscorpionate Ligands. Molecules 2019, Vol. 24, No. 9, p. 1761
17. Borovikov V.P. A popular introduction to modern data analysis in the STATISTICA system. Moscow, Telecom, 2013, 288 p., ISBN 978-5-9912-0326-5
18. 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
19. Vukolov E.A. Fundamentals of statistical analysis. Workshop on statistical methods and operations research using Statistica and Excel packages, M., Forum-Infra-M, 2004

back