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INFLUENCE OF A COPPER(II) COMPLEX SUPPORTED BY ACETYLACETONATES ON SURVIVAL, CYTOGENETIC PARAMETERS AND BLOOD PARAMETERS OF ANIMALS WITH III-AB DEGREE BURNS

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ABSTRACT

One of the priority tasks of modern medicine is the search for new effective means that contribute to the treatment of burn injuries. In this area, metal complexes based on copper are of particular interest.

The aim of the work is to evaluate the possible beneficial effect of a Cu(II) complex supported by acetylacetonates, $[Cu(acac)_2]$, on thermal burns by determining toxicity, analyzing survival and some cytogenetic and blood parameters.

The metal complex $[Cu(acac)_2]$ turned out to be a low-toxic compound: the $LD_{50/7}$ value was 1815 mg/kg. The study of survival, blood counts and cytogenetic parameters showed a beneficial effect of the $[Cu(acac)_2]$.

Studies have shown that in the early stages after the burn in animals injected with [Cu(acac)2], there is a significant decrease in blood clotting time (BCT) and hematocrit, an increase in the number of leukocytes. There is also a significant decrease in the levels of platelets, erythrocytes and hemoglobin. By the end of the experiment, there was a trend towards normalization of hematological parameters.

The dynamics of the survival of animals from the group "burn + injections of the metal complex" with the corresponding regression curve and an equation that allows using extrapolation to predict the change in the percentage of survival in the long term of the experiment is given.

The processes of wound healing, epithelialization, hair growth in the "burn + $[Cu(acac)_2]$" group are more intense than in the pure burn group, which indicates the beneficial effect of this metal complex on the burned organism.

Keywords: thermal burns, blood clotting time, leukocytes, platelets, hemoglobin, erythrocytes, copper(II) complexes

INTRODUCTION

It is known that in severe burn injuries, loss of functions of the skin, loss of plasma and metabolic disorders are observed. Changes in blood parameters are also noted. In particular, leukocytosis, hyperkalemia and hypoproteinemia, an increase in hematocrit and hemoglobin levels due to blood clotting are detected. The hematopoietic system, as an actively proliferating tissue, is extremely sensitive to the action of burns of various nature [1,2]. It was revealed that when blood flows through tissues during the burn period, thermal damage and destruction of erythrocytes occur with the release of free hemoglobin into the plasma [3,4]. Burn disease also causes a pronounced leukocyte reaction [5,6]. Therefore, to assess the state of the body of animals that have suffered a burn injury and the healing process, it is advisable to analyze blood parameters (the number of leukocytes, platelets, erythrocytes, hemoglobin levels, blood clotting time).

One of the priority tasks of modern medicine is the search for new effective means that contribute to the treatment of burn injuries. Requirements for such compounds should be commercially inexpensive, fairly stable, easy to use, low toxicity, and have a prolonged action. To heal tissues during burns, drugs that have anti-inflammatory, analgesic and regenerating properties are also used [7,8]. In this field, copper-based complexes with high antioxidant activity are of particular interest.

The aim of the work is to evaluate the possible beneficial effect of the $C_{10}H_{14}CuO_4$ metal complex on thermal burns by determining toxicity, analyzing survival and some cytogenetic and blood parameters.

MATERIALS AND METHODS

The bis(acetylacetonato)copper(II) complex $[Cu(acac)_2]$ (Fig. 1) was synthesized by a modified literature method [9] by a one-step synthetic protocol involving the reaction of the acetylacetone with copper(II) acetate monohydrate, in 2:1 stoichiometric ratio, in ethanol/water (1/1) solution. 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 $[Cu(acac)_2]$. The result of the synthesis was an analytically pure compound that matched all the spectroscopic parameters of the literature compound [10].

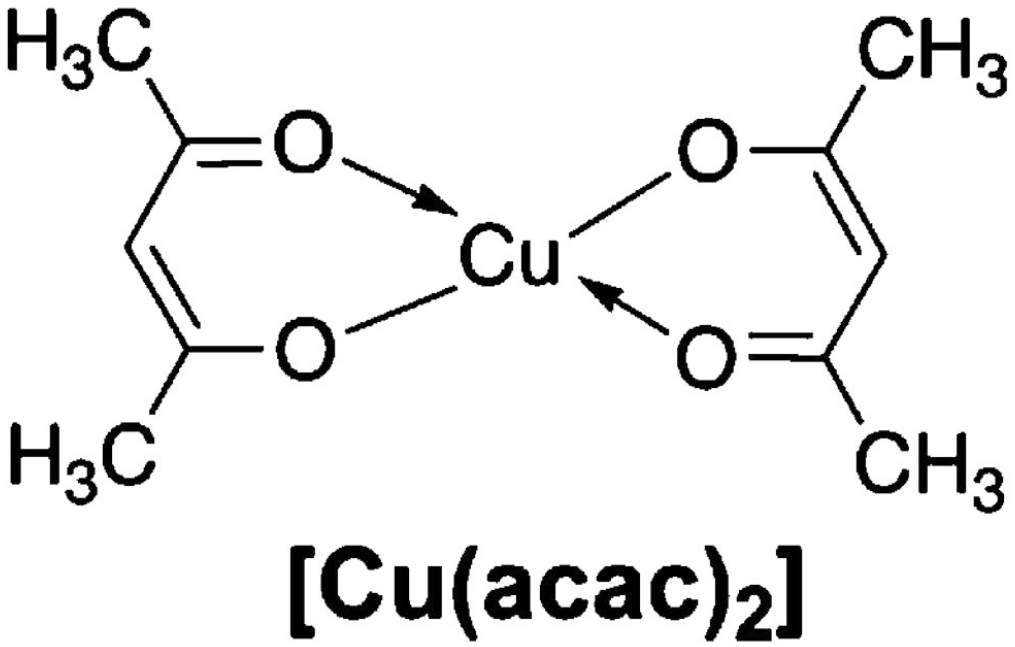

Figure 1. Chemical structure of the bis(acetylacetonato)copper(II) complex $[Cu(acac)_2]$.

To determine the possible therapeutic effect of the copper(II) complex $[Cu(acac)_2]$ in thermal burns, an experiment was carried out on white, outbred, mature rats of both sexes with an average weight of 180 g (group I - "pure burn", II - "burn + metal complex", III - norm). The experiments were performed in compliance with current best practices and standards of care in laboratory animals.

Animals on the epilated skin surface in the back area were subjected to a thermal burn (30% of the body surface) III-AB degree. 30 minutes after the burn, the test drug was injected intraperitoneally in the form of a suspension at a dose of 50 mg/kg. Further, blood sampling and analysis of the above blood parameters were carried out. The toxicity of the complex was also determined and the survival rate was analyzed (10 animals per group).

Determination of the toxicity of [$Cu(acac)_2$] was carried out in order to quantify the dose-effect relationship. The toxicity of the metal complex was characterized based on the calculation of the $LD_{50/7}$ index (1815 mg/kg), i.e. the dose of the compound at which the death of 50% of animals is observed within 7 days after subcutaneous administration of the substance into the body. For this purpose, copper complexes were administered to animals in gradually increasing doses, from the most ineffective to $LD_{100/7}$, i.e. up to that minimum dose, absolutely lethal for 100% of animals within 7 days (100 mg/kg - 3500 mg/kg). Using the integration method according to G. Behrens [11], in experiments on rats, the average lethal dose was calculated - $LD_{50/7}$.

Hematological parameters of experimental animals, namely the number of erythrocytes, leukocytes, platelets, hematocrit and hemoglobin content, as well as blood clotting time (BCT), were determined using standard laboratory equipment [12].

Using the method of integration according to G. Behrens, in experiments on rats, the average lethal dose $LD_{50/7}$ was determined, which is lethal for 50% of animals within 7 days after subcutaneous injection of [$Cu(acac)_2$] into the body.

Data analysis was carried out using a number of specialized statistical packages: StatSoft7, SPSS-10.0 and Stat Graphics Plus. We used regression and correlation methods of analysis.

RESULTS

DEFINITION OF TOXICITY

According to the test results, the copper(II) complex [Cu(acac)2] turned out to be a low-toxic compound: the LD50/7 value was 1815 mg/kg.

It should be noted that with subcutaneous administration in the dose range from 100 mg/kg to 700 mg/kg, no disturbances in behavioral reactions and death of animals were observed within 24 hours. In the case of high doses (3500 and > mg/kg), lethargy and lethargy of animal movements were often observed immediately after injection, with the onset of death after a few hours.

According to the data obtained, in the experimental burn group, the indicated conditions for introducing [Cu(acac)2] into the body did not cause the death of animals not only within 7 days, but also 30 days after the injection. Only at a dose of 750-800 mg/kg, transient external signs of the depressive effect of the complex on locomotor activity (hypodynamia) were noted in animals, which were no longer detected after 24 hours. By the end of the study, no significant differences in cytogenetic parameters were found between the group of intact animals and those injected with [Cu(acac)2] (800 mg/kg dose) (Table 1).

The condition of experimental animals (dose 270 mg/kg) was monitored, and hematological parameters were determined. At all periods of the experiment, no deviations in the behavior and appearance of rats were noted. No animal deaths were observed either.

Table 1. Cytogenetic indicators (when determining the toxicity of [Cu(acac)2] after 30 days).

Indicators	Intact	[$Cu(acac)_2$] (30 days)
Mitotic index (%)	20,35±2,8	21,2±3,4
Chromosomal aberrations (%)	2,6±0,26	2,72±0,3
Ppolyploid cells (%)	0,5±0,08	0,5±0,06

The results of the analysis of hematological parameters in the group of intact animals and the group with the injection of the metal complex are shown in Table. 2.

Table 2. Hematological indicators in the analysis of toxicity.

Days	Norm	3-rd	7-th	14-th	21-st	30-th
Blood clotting time (sec)	311,0 ±19,00	380,8 ±24,94 (*)	236,3 ±34,87	273,9 ±16,3	246,7 ±15,14 (*)	260,7 ±30,23

Leukocytes (N/μL)	11500,0 ±420,0	12180 ±700,76	17000 ±1568 (*)	15828,6 ±1853,92 (*)	12542,9 ±1026,06	12342,9 ±994,95
Platelets (N/μL)	520000 ±18230	409500 ±19442,94 (*)	307777,68 ±20751,69 (*)	417142,9 ±50105,33 (*)	477142,9 ±35031,57	393571,4 ±21484,06 (*)
Erythrocytes (N/μL)	5823000 ±278800	3761800 ±727104,24 (*)	5752222,2 ±275301,74	5429142,9 ±285458,31	4351428,6 ±256751,02 (*)	5477142,9 ±230854,67
Hemoglobin (g/L)	138,1 ±5,82	141,8 ±2,89	162,3 ±2,17 (*)	159,5 ±3,77 (*)	161,8 ±3,9 (*)	157,8 ±3,16 (*)
Hematocrit	43,8 ±2,1	46,2 ±2,2	40,0 ±2,11	48,7 ±1,31 (*)	52,8 ±2,76 (*)	51,2 ±1,37 (*)

(*) - p <0.05

According to this table, there is a significant increase in BCT on the 3rd day and a trend towards normalization at other times. On the 7th and 14th day there is a significant increase in the number of leukocytes (which occurs with any inflammatory processes) with further normalization. There is also a significant increase in hemoglobin and hematocrit, a decrease in platelet levels almost throughout the experiment, which indicates a significant effect of the compound on the animal organism. By the end of the experiment, there is a tendency towards normalization of most hematological parameters.

DETERMINATION OF SURVIVAL AFTER BURN + [CU(ACAC)$_2$]

The drug in the form of a suspension, at a dose of 50 mg/kg, was administered every 2 days until the scab of the burn wound was rejected. The survival rate was 90% (in contrast to the "pure burn" - 80%). When analyzing the dynamics of survival of animals from the group “burn + injections of [Cu(acac)2]”, a regression curve and an equation describing the dynamics were obtained: y=105.49-8.164lg(x), where y is the percentage of surviving animals, x is the day of the experiment.

Visual monitoring of the state of burn wounds of 2 groups of experimental rats (“burn” and “burn + [Cu(acac)2]”) was also carried out, which showed that the rejection of the scab was observed approximately at the same time, within 12-16 days after the burn was applied. As for wound healing, epithelialization, hair growth, in the “burn + [Cu(acac)2]” group, these processes are more intense than in the “burn” group, which indicates a positive effect of this metal complex on the burned organism.

ANALYSIS OF HEMATOLOGICAL PARAMETERS IN THE "BURN" AND "BURN + [CU(ACAC)$_2$]" GROUPS

At certain times (days 3, 7, 14, 21, 30), blood was taken from the tail vein for hematological analysis. The results of the analysis are given in Table. 3. Values of indicators in brackets correspond to the "burn" group.

Table 3. Changes in hematological parameters in the "burn" and "burn + [Cu(acac)$_2$]" groups.

Days	Norm	3-rd	7-th	14-th	21-st	30-th
Blood clotting Time (sec)	311,0 ±19,00	372,3 ±25,96 (415.0 ±7.63) (*)	248,7 ±24,18 (327.5 ±32.5) (*)	167,6 ±8,38 (360,0 ±34,64) (*)	178,8 ±15,05 (338,5 ±43,55) (*)	179,6 ±10,13 (316,7 ±52,47) (*)
Leukocytes (N/μL)	11500,0 ±420.0	13222,2 ±1371,72 (8300,0 ±590,0) (*)	19810,0 ±726,7 (10200,2 ±200,0) (*)	19755,6 ±1165,13 (8800 ±700,0) (*)	17725,0 ±1362,74 (7360,0 ±650,0) (*)	16450 ±1033,54 (5930,0 ±600,0) (*)

Platelets (N/μl)	520000 ±18230	381666,7 ±26114,07 (588333,3 ±44378,42) (*)	423500,0 ±21187,65 (637500,0 ±2500,0) (*)	493333,3 ±27233,56 (495000,0 ±66583,28)	487857,1 ±50034,00 (600000,0 ±58291,5)	425625,1 ±25167,95 (705000 ±50000) (*)
Erythrocytes (N/μL)	5823000 ±278800	5480000,0 ±424980,39 (5920000,0 ±130000,0)	4571000,0 ±226634,56 (3130000,0 ±100000,0) (*)	4855555,6 ±274302,71 (6560000 ±180000,0) (*)	474125,0 ±292156,01 (6470000,0 ±1125000,0) (*)	5436500,0 ±244682,52 (6380000 ±1900000)
Hemoglobin (g/L)	138,1 ±5,82	143,4 ±6,11 (134,6 ±6,06)	139,5 ±6,16 (136,5 ±5,5)	145,9 ±4,69 (163,3 ±10,13)	139,9 ±4,8 (162,3 ±1,61) (*)	149,9 ±3,3 (161,3 ±1,76) (*)
Hematocrit	43,8 ±2,1	41,5 ±1,68 (47,6 ±1,4) (*)	34,6 ±2,58 (49,1 ±0,91) (*)	39,0 ±1,98 (47,3 ±1,11) (*)	41,3 ±1,81 (48,3 ±1,13) (*)	41,7 ±2,02 (44,5 ±1,71)

** p<0,05*

It can be seen from the table that in the early stages after the burn and injection of the complex in animals injected with [$Cu(acac)_2$], there is a significant decrease in BCT and hematocrit, an increase in the number of leukocytes (this trend persists until the end of the experiment). There is also a significant decrease in the levels of platelets, erythrocytes and hemoglobin. Changes in cytogenetic parameters for "clean burn" and "burn + [$Cu(acac)_2$]" are presented in Table 4.

Table 4. Cytogenetic parameters for "clean burn" and for "burn + [$Cu(acac)_2$]"

Indicators	**Intact**	**Burn**	**Burn + [$Cu(acac)_2$] (30 days)**
Mitotic index (%)	20,35±2,8	14,2±1,9	18,4±0,2 (*)
Chromosomal aberrations (%)	2,6±0,26	4,2±0,3	3,8±0,42
Polyploid cells (%)	0,5±0,08	3,3±0,36	1,5±0,04 (*)

** p<0,05*

The study of cytogenetic parameters showed that the injection of the copper(II) complex [$Cu(acac)_2$] after a burn had a beneficial effect on the body of rats (in particular, when comparing the "burn" and "burn + [$Cu(acac)_2$]" groups, a significant difference was found in the MI and PC indicators). After several series of experiments, it can be argued that this complex has a therapeutic effect on the burn.

CONCLUSION

Thus, the copper(II) complex [Cu(acac)2] turned out to be a low-toxic compound: the LD50/7 value was 1815 mg/kg, which allowed its use after a burn and the study of the therapeutic effect. The study of survival, blood counts and cytogenetic parameters showed a beneficial effect of the copper complex [Cu(acac)2].

Studies have shown that in the early stages after the burn and the injection of [Cu(acac)2] in animals, there is a significant decrease in BCT and hematocrit, an increase in the number of leukocytes. There is also a significant decrease in the levels of platelets, erythrocytes and hemoglobin. By the end of the experiment, there was a trend towards normalization of hematological parameters.

The dynamics of the survival of animals from the group "burn + injections of the metal complex" with the corresponding regression curve and an equation that allows using extrapolation to predict the change in the percentage of survival in the long term of the experiment is given.

The processes of wound healing, epithelialization, hair growth in the "burn + [Cu(acac)2]" group are more intense than in the pure burn group, which indicates the beneficial effect of this copper complex on the burnt organism.

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REFERENCES

1. Karapetyan A.G., Dallakyan A.M., Petrosyan Zh.G., Arutyunyan N.K., Porchia M., Santini C. Evaluation of peripheral blood parameters and cytogenetic parameters when using compounds of copper-organic complexes after a burn. Medical Science of Armenia NAS RA, 2020, v. LX, 1, pp. 46-54, ISSN: 0514-7484.
2. Okladnikova N.D., Khokhryakov V.V., Shevkunov V.A., Pesternikova V.S., Shadilov A.E. Clinical and cytogenetic description of a case of high radionuclide incorporation. Radiobiology and radioecology, 2004, v. 44, 4, pp. 115-118.
3. Loginov L.P., Smirnov S.V., Khvatov V.B. and others. Blood transfusions in burnt patients in the process of restoring the skin. Sat. scientific tr. II Congress of Combustiologists of Russia, Moscow, 2008, 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, 4, pp. 37-41. PMID: 9412028
5. Gubler E.V., Khrebtovich V.N., Subbota A.G. Thermal burns and burn disease. In: Disease Modeling. Ed. S.V. Andreeeva. M., 1972, pp. 46-59.
6. Zinoviev E.V. etc. New St. Petersburg medical records. 2006, 3, pp. 73-74.
7. 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.
8. Ermini ML, Summa M, Zamborlin A,V, Mapanao AK, Mugnaioli E, Bertorelli R, Voliani V. Copper nano-architecture topical cream for the accelerated recovery of burnt skinю Nanoscale Adv. 2023 Feb 14; 5(4): 1212–1219. doi: 10.1039/d2na00786j
9. Fernelius W.C., Bryant B.E. Preparation of Metal Derivatives of 1,3-Diketones. Inorganic Syntheses, 1957, v. 5, pp. 105-113. https://doi.org/10.1002/9780470132364.ch29
10. Starikova, Z.A., Shugam, E.A. Crystal chemical data for inner complexes of β-diketones. Journal of Structural Chemistry, 1969, v. 10, pp. 267-269.
11. Behrens, H. u.P. D. C. Kley: Organische mikrochemische Analyse, 1922.
12. Kishkun A.A. Guide to laboratory diagnostic methods, M., 2007.

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