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EFFECT OF $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ ON SURVIVAL, AVERAGE LIFE AND HEMATOLOGICAL INDICATORS IN ANIMALS WITH BURNS III DEGREE

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ABSTRACT — Taking into account the properties of copper: normalization of the endocrine system, participation in the process of hematopoiesis, participation in the synthesis of collagen and elastin, neutralization of toxins, reduction of inflammatory reactions — we used a number of copper chelates to treat burn injuries under experimental conditions.

Before discovering possible healing properties for burns, we quantified the toxicological properties of the substance. For this, the average lethal dose LD50/7LD50/7 which is the dose of a substance at which 50% of animals die within 7 days (after subcutaneous injection of the substance), was calculated by the integration method of H. Behrens in experiments on rats.

In the second series, we caused grade III burn lesions on the backs of epilated animals, 30% of the body surface. The organometallic complex $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ was injected into the animals intraperitoneally 30 min after the burn injury (for the first time at a dose of 50 mg/kg). From day 2, chelate was administered at a dose of 20 mg/kg. The injection was done once every 2 days for 10 days until the scab falls off.

According to the experimental results, the $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ metal complex is a compound with low toxicity. When administered subcutaneously in the dose range from 100 mg/kg to 300 mg/kg, behavioral disturbances and death of animals were not observed within 24 hours. We studied 5 hematological indicators: blood clotting time (BCT), the number of leukocytes, erythrocytes, the level of hemoglobin and platelets.

Survival and average life expectancy in the group (*burn*) were, respectively, 40% and 18.8 days, and in the group *burn + metal complex* — 60% and 19.2 days, which indicates a positive effect of this metal complex on the body. There was a significant difference in clotting time, leukocyte count and platelet count compared to the control group ($p<0.05$), which indicates the healing properties of the Cu complex in case of burns.

KEYWORDS — thermal burns, blood clotting time, white blood cells, platelets, hemoglobin, red blood cells, metal complexes.

INTRODUCTION

The copper biological role is diverse. 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. 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, reduces inflammatory reactions.

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. According to literary sources [7-9,11] and also our early studies [2-6,10,12], several copper-based complexes are of low toxicity and expressed radioprotective properties. In order to identify a possible positive effect on burns, we studied a number of compounds when used internally.

MATERIALS AND METHODS

All experiments were carried out on sexually mature, outbred white rats (a total of 60 animals: 10 rats for each series) weighing an average of 170–190 g.

Before discovering the healing properties in case of burns, we had to carry out quantitative assessments of the toxicological properties of the substance. Determination of the toxicological properties of $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ is performed to quantitatively evaluate the dose-effect relationship. The

toxicological properties of the Cu complexes are characterized by the calculation of the LD50/7 parameter. LD50/7 is the dose of a substance in case of which the 50% of the animals die within 7 days (after subcutaneous injection of the substance).

For this purpose, the substance was given to the animals in gradually increasing doses, from the maximally ineffective to LD100/7 (i.e. the minimum dose that is absolutely lethal to 100% of the animals within 7 days). The mean lethal dose of LD50/7 was calculated using the integration method of H. Berens in experiments on rats [1].

In the second series, we caused grade III burn lesions on the backs of epilated animals, 30% of the body surface. The organometallic complex $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ was injected into the animals intraperitoneally 30 min after the burn injury (for the first time at a dose of 50 mg/kg). From the second day, chelate was administered at a dose of 20 mg/kg. The injection was done once every 2 days for 10 days until the scab falls off.

The data obtained from animals subjected to a burn followed by injection of a chelate were compared with those obtained in the control group (animals exposed only to burn lesions).

Blood samples were taken from the tail vein of animals on days 3, 7, 14 and 30 for hematological examination. Also, visual monitoring of the condition of burn wounds in 2 groups of experimental rats (*burn* and *burn + metal complex*) was carried out. The examinations continued for 3 months until the wounds were completely healed (up to hair growth).

RESULTS

According to experimental results, $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ metal complex is a compound with low toxicological properties. In case of subcutaneous injection (100–300 mg/kg), no behavioral disorder or animal death was observed within 24 hours. Only in case of injection of extra high doses (1675 mg/kg and more) did the animals show weakness and inhibited motor functions, often immediately after the injection, which was leading to animal death within a few hours.

Hematological examinations (at a dose of 270 mg/kg) were performed in parallel (5 indicators were studied: blood clotting time (BCT), white blood cells, red blood cells, hemoglobin and platelet levels), the results of which are presented in Table 1, aaccording to which, on the 7th day of the experiment, a significant difference in almost all indicators was observed between the animals that received a large dose of the $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ substance, versus the control group (normal, intact animals).

After carrying out a quantitative assessment of the toxicological properties of the substance, we studied the following 2 groups of animals: those subjected to burn injuries followed by injection of a chelate were compared with the data obtained from the control group (animals exposed only to burn injuries).

Made the determination of the survival rate and average life expectancy in these groups. The dynamics of the survival of animals from the groups *pure burn* (1) and *burn + metal complex* (2) was described by regression curves and equations, respectively: $y_1 = 83.67–26.48\lg(x)$, $y_2 = 84.07–21.15\lg(x)$, where y is the percentage of surviving animals, and x is the day of the experiment (and in absolute terms, the survival rate and average life expectancy in subgroup (1) was 40% and 18.8 days, respectively, and in subgroup (2) 60% and 19.2 days), which speaks of the positive effect of this metal complex on the body. This was also confirmed by the dynamics of changes in blood parameters during the entire period of the experiments.

According to the data obtained, the $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ complex exhibited effective properties, since throughout the entire period and until the end of the experiment, blood counts in animals with a clean burn and in rats that received burns lesions followed by complex injections were significantly different. There was a significant difference in clotting time, leukocyte count and platelet count compared to the control group ($p < 0.05$), which indicates the healing properties of the Cu- complex in case of burns.

Thus, leukocytosis, usually found after a burn, is not only facilitated by this compound in the early stages of experimental work ($14.1\pm1.05 \cdot 10^9$/l — only with burn lesions, $8.8\pm0.7 \cdot 10^9$/l — with burn lesions with subsequent injections of the drug), but also after 30 days ($12.53\pm0.23 \cdot 10^9$/l — only for burn injuries, $5.93\pm0.6 \cdot 10^9$/l — for burn injuries with subsequent injections of the compound).

The results of other hematological evaluations (groups *burn* (indicated in parentheses) and *burn + chelate*) are presented in Table 2.

On the 80th day after the complete healing of the wound, a visual examination showed that the rejection of the scab was noted at approximately the same time within 12–16 days after the application of the burn. As for wound healing, epithelialization and hair growth, these processes are more intense in the *burn + metal complex* group than in the burn group, which also speaks of the positive effect of this metal complex on the burned body.

CONCLUSION

Based on the results of assessing the survival rate, life expectancy and hematological param-

Table 1. *Hematological indicators during the evaluation of toxicity*

indicator / day	Norm	7	14	30
Blood clotting time (sec)	311.0±19.00	501.0±30.39 (*)	476.0±32.34 (*)	322.0±21.00
White Blood Cells (N/μL)	11500.0±420.0	15400.0±529.15 (*)	14360±591.27 (*)	13500.0±520.0
Platelets (N/μL)	520000±18230	505000±18708.3	534000±22271.06	526000±19430
Hemoglobin (g/L)	138.1±5.82	139.0±1.18	146.0±1.38	139.1±2.82
Red Blood Cells (N/μL)	5823000±278800	5254000±113075.2 (*)	6538000±126427.8 (*)	5852000±217800

Table 2. *Changes in hematological parameters in "burn" (indicated in parentheses) vs "burn+chelate" effect*

day / indicators	3	7	14	21	30
Blood clotting time (sec)	265.0±17.27 (415,0±7,63) (*)	321.25±21.85 (327,5±32,5)	279.38±15.39 (360,0±34,64) (*)	238.33±14.81 (338.5±43.55) (*)	276.67±23.33 (316,7±52,47)
Platelets (N/μL)	531666.67±23190.04 (588333,3±44378,42)	545000.0±22598.0 (637500.0±2500.0) (*)	491250.0±17132.62 (495000.0±66583,28)	503333.3±34801.02 (600000±58291.5)	643333.3±69841.09 (705000±50000)
Hemoglobin (g/L)	131.87±1.65 (134,6±6,06)	140.75±1.29 (136,5±5,5)	131.88±2.7 (163,3±10,13) (*)	142.3±1.45 (162.3±1.61) (*)	147.37±6.39 (161,3±1,76) (*)
Red Blood Cells ($\times 10^{12}$/μL)	4.77±0.15 (5,92±0,13) (*)	5.4±0.31 (3,13±0,1) (*)	5.85±0.22 (6,56±0,18) (*)	5.84±0.35 (6.47±1.125)	6.33±2.6 (6,38±1,9)

*— $p<0.05$

eters, it can be concluded that the studied complex $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ has significant therapeutic properties in case of burns.

Based on the results obtained, it can be assumed that the newly synthesized complex $Cu(II)_2(3,5\text{-}DIPS)_4(H_2O)_3$ has therapeutic properties for burn injuries. The results of this study allow us to continue researching new compounds with effective radioprotective and therapeutic properties with the expectation of discovering new highly effective compounds.

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