Kopach O. Ye., Svan O. B., Boris R. M. Influence of receipt drinking water excess ions copper and zinc on antioxidant-prooxidant balance in the liver conditions and mechanical injury and effectiveness of antidote therapy. Journal of Education, Health and Sport. 2018;8(2):372-379. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1220406
http://ojs.ukw.edu.pl/index.php/johs/article/view/5427

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 01.02.2018. Revised: 05.02.2018. Accepted: 26.02.2018.

UDC 616.36-099: 546.56-008.1-06: 616-001.1

INFLUENCE OF RECEIPT DRINKING WATER EXCESS IONS COPPER AND ZINC ON ANTIOXIDANT-PROOXIDANT BALANCE IN THE LIVER CONDITIONS AND MECHANICAL INJURY AND EFFECTIVENESS OF ANTIDOTE THERAPY

[1]O. Ye. Kopach, [2]O. B. Svan, [3]R. M. Boris

[1]SHEI «Ternopil State Medical University by I. Ya. Horbachevsky of MPH of Ukraine», Ternopil, Ukraine
[2]"Kyiv Medical University" UAMM "Ministry of Health of Ukraine

Abstract

In the late period of a traumatic illness (14-28 days), after intensive skeletal trauma infliction, elevated liver tissue is the intensity of free radical lipid oxidation, signs of extinction of antioxidant defense are noted. Against her background heavy skeletal trauma and excessive intake of copper ions and zinc in comparison with animals without additional intoxication in these terms in liver tissue is higher level of lipid peroxidation, less - the activity of enzymes antioxidant protection. Introduction of unithiol to injured animals with excessive ions of copper and zinc within 14 days after application accompanied by severe skeletal trauma disorders significantly less traumatic disease markers studied as up to 28 days do not reach the level of animals without additional toxic effects.

Keywords: skeletal trauma, lipoperoxidation, antioxidant defense, salt of copper and zinc, unithiol.

Introduction: In modern conditions heavy metal salts belong to the main pollutants in the environment of Ukraine. If in the territory of the industrial regions of Ukraine (central, southern and eastern) in the soil and water dominated cadmium, lead and ^{88}strontium [14], then zinc and copper prevail almost all over Ukraine [4, 15]. Even in such an ecologically safe region as Ternopil, an excessive accumulation of copper and zinc ions in living organisms that inhabit reservoirs [16] is noted. Excess of maximum permissible concentration of copper ions in drinking water of Ternopil in 20 times and zinc ions in 3 times, as evidenced by the Program of environmental monitoring of Ternopil region for 2006-2010 [12].

In a number of publications, mechanisms of adverse effects on the body of accumulation of copper and zinc ions are presented. Significant interference of these ions in the system of peroxide oxidation of lipids / antioxidant protection with the intensification of oxidative processes, activation and subsequent inhibition of antioxidant defense, in particular reduction of superoxide dismutase activity, catalase, reduced glutathione. There are significant changes in the functional state of the liver, pancreas, muscles, transmembrane processes. [18-20]. In this regard, there was a hypothesis about the possibility of modifying the course of various pathological processes under conditions of accumulation of copper ions and zinc in the body, which required a special study.

As a model of pathology we used a severe skeletal trauma, whose frequency is constantly increasing year by year, and the effectiveness of treatment continues to be unsatisfactory [7]. In these conditions there is a traumatic disease that involves the pathological process of tissue and organs that are removed from the zone of direct damage, and accompanied by the development of multiple organ failure and insufficiency. The pathogenesis of traumatic disease is dominated by hypoxia with the intensification of lipid peroxidation processes [10] and the depletion of antioxidant defense [3].

The aim: To determine the effect of excess copper and zinc ions supply with drinking water on the antioxidant-prooxidant balance in the liver under mechanical trauma and the effectiveness of antidote therapy.

Materials and methods: Experiments were performed on 60 non-linear white male rats weighing 180-200g. Chronic intoxication with copper and zinc sulfates was performed by daily administration into the stomach through a probe of their solutions in a dose of 5 mg/kg^{-1} in terms of metal once a day [6]. After 14 days in terms of thiopental sodium anesthesia (40 mg/kg^{-1}·body weight) in the first group and research (20 individuals) simulated skeletal injury, caused by shock dosed closed fracture of both hips and additionally simulated blood loss (20-22% of the volume of circulating blood) by crossing the femoral vein which was

injected into the paranephral tissue for the formation of a hematoma [5]. In the second experimental group (20 individuals) after additional toxic effects, and starting from 1 day after trauma, unithiol was injected into the peritoneum - a universal antidote of heavy metal salts in a dose of 0.01 g per 100 g of animal weight [8], the third experimental group consisted of 20 animals that modeled the skeletal trauma itself. The control group included 6 intact animals.

Animals were extracted from the experiment under conditions of thiopental-sodium anesthesia (60 mg · kg $^{-1}$ body weight) after 1-4 and 28 days post-traumatic period by total blood flow from the heart. In animals liver homogenate were determined the content of TBC-active products of the LPO [1], the activity of superoxide dismutase [17] and catalase [11]

The received digital material was processed in the Department of System Statistical Studies of the State Health Research Center "Ternopil State Medical University by I. Ya. Horbachevsky of the Ministry of Health of Ukraine" in the STATISTICA software package (Stat Soft Inc., USA) using Mann-Whitney's non-parametric criterion.

Results and discussions: As we can see from the Table 1, 14 and 28 days after modeling severe skeletal injury liver tissue content of TBA-active products of lipid peroxidation remained significantly higher than in controls (at 108.2 and 62.3%, $p < 0.05$) decreasing to 28 days (by 22.0% compared to the previous observation period, $p < 0.05$).

Table 1 - The content of TBA-active products of LP in the liver tissue in the late traumatic period in rats with excessive intake of copper ions and zinc ions and their correction by unithiol (M ± m)

Experiment conditions	14 days	28 days	p
Control = (3,18 ± 0,12) mmol kg $^{-1}$			
Skeletal trauma		5,16±0,21*	< 0.05
The salts of copper and zinc + Skeletal trauma		8,16±0,35$^{*\#}$	<0.05
The salts of copper and zinc + Skeletal trauma and unithiol		6.54 ± 0.28 $^{*\#o}$	<0.05

Notes: Here and in table 2 and 3:
1. * - control differences are statistically significant ($p < 0,05$);
2. $^{\#}$ - differences in the group with skeletal trauma are statistically significant ($p < 0,05$);
3. ^{o} - differences in the group with skeletal trauma and excessive of copper and zinc ions are statistically significant ($p < 0,05$).

Against the background of severe skeletal trauma and excessive intake of copper and zinc ions, the rate was higher than in the control: after 14 days - 3.84 times ($p < 0.05$). After 28 days it decreased - by 33,1% compared with the previous term of observation, but 2,56 times

exceeded the control group (p <0,05). Following the use of unithiol, skeletal trauma was associated with an excessive flow of copper and zinc ions with less intensification of lipid peroxidation processes. After 14 days, the content of liver tissue of TBA-active products of LPP was 24.6%. It was lower than in the group of animals with the same trauma without correction of unithiol (p <0.05), and after 28 days - by 18.9% (p <0.05). In both of these terms, the indicator was significantly higher than in animals with heavy skeletal trauma (p <0,05).

The activity of SOD in liver tissue in the period of late manifestations of traumatic illness (Table 2) practically did not differ from the level of control (p> 0,05). In the background of severe skeletal trauma and excessive intake of copper ions and zinc, the index for all terms of observation was lower than in the control group: 48.6% and 31.4% respectively, which was statistically significant (p <0.05). During these periods of observation it was also much lower than in animals with only skeletal trauma (p <0.05).

Table 2 - Activity of SOD in liver tissue in the late period of traumatic illness in rats with excessive intake of copper and zinc and its correction with unithiol (M ± m)

Experiment conditions	14 days	28 days	p
To control = (0.70 ± 0.04) conditional units kg $^{-1}$			
Skeletal trauma	0,80 ± 0,03	0.68 ± 0.04	<0.05
The salts of copper and zinc + skeletal trauma	0,36 ± 0,02 $^{*\#}$	0,48 ± 0,03 $^{*\#}$	<0.05
The salts of copper and zinc + skeletal trauma and unithiol	0.46 ± 0.02 $^{*\#o}$	0,58 ± 0,02 $^{*\#}$	<0.05

After application of unithiol in comparison with the group of animals without correction, the index increased and in 14 days exceeded by 27,8% (p <0,05), after 28 days - by 20,8% (p <0,05), but did not reach of the control group and the group with the heavy skeletal trauma (p <0.

In turn, the activity of catalase in the liver tissue against the background of severe skeletal trauma (table 3) in 14 days was significantly lower than in the control (by 22.7%, p <0.05). After 28 days the index increased and reached the level control (p> 0.05). Simulation of severe trauma on the background of an excess of copper and zinc ions was accompanied by a significantly lower activity of the catalase in the liver tissue: after 14 days - by 43.3% (p <0,05), after 28 days - by 30,6% (p <0 , 05). As can be seen, the index up to 28 days increased in comparison with the previous observation period (by 22.4%), which turned out to be statistically significant (p <0.05).

After using of unithiol correction, the catalase activity disruption was less. After 14 and 28 days index was statistically significant greater than in the group of animals with severe skeletal trauma and excessive intake of copper and zinc ions (liability etc. but is 18.5 and 11.3%, p <0.05). It should be noted that in conditions of unithiol correction the index up to 28 days did not reach the level of animals with the skeletal trauma itself (p <0,05).

Table 3 - Activity of catalase in liver tissue in the late period of traumatic illness in rats with excessive intake of copper and zinc and its correction with unithiol (M ± m)

Experiment conditions	14 days	28 days	p
Control = (1,795 ± 0, 084) mkcat kg $^{-1}$			
Skeletal trauma	1,387 ± 0.047 *	1,586 ± 0,044	< 0.05
The salts of copper and zinc + skeletal trauma	1,017 ± 0.029 $^{*\#}$	1,245 ± 0.027 $^{*\#}$	
The salts of copper and zinc + skeletal trauma and unithiol	1,205 ± 0.039 $^{*\#o}$	1,386 ± 0.035 ^{*o}	<0.05

Thus, in the period of late manifestations of traumatic illness, the level of processes of lipid peroxidation, which drops to 28 days, remains, but does not reach the level of control. Under these conditions, the activity of SOD is normal. The activity of catalase increases from 14 to 28 days, reaching the level of control in this period. Consequently, in the late period of traumatic illness, the intensity of free radical oxidation of lipids remains high, signs of extinction of antioxidant defense are noted. The lack of normalization of the key markers of traumatic illness in the late period after the trauma is given in the works of other authors [2, 9], which indicates cascading and autocatalytic processes that occur in severe trauma.

In severe skeletal trauma, simulated against the background of excessive copper and zinc ions, all of the studied parameters are much worse. The content of TBA-active products of LP, the reduced activity of SOD and catalase remains in the clinker's tissue high. Indicators up to 28 days are improved, but do not reach the level of injured animals without excessive copper and zinc ions. This fact confirms the significant negative effect that ions of copper and zinc cause in the body against the background of injury [18]. They are able to modify the course of a traumatic illness, which confirms the above working hypothesis.

Application of unithiol from the moment of injury to 14 days posttraumatic period was accompanied by a pronounced positive effect. Compared to non-corrected animals, the content of TBA-active products in the liver tissue significantly decreased. SOD and catalase activity increased. However, they all did not reach the level of animals with the skeletal trauma without the addition of copper and zinc ions. Thus, unithiol significantly improved the

course of traumatic disease in the late period after injury. This is obviously due to its antidote properties in respect of ions of heavy metals: mercury, copper, chromium, bismuth and other substances. It contains in the molecule two sulfhydryl (SH) groups that can interact with many metal ions to form solid compounds. There is an opinion that unithiol has a high affinity for copper ions. The basis of the mechanism of action of unithiol is the interaction not only with the heavy metal ions in the blood, but also with those that have already entered into interaction with enzymes and other protein substances in the body. At the same time, previously released metal ions of the SH group of proteins are released and their functions are restored. Such a mechanism is due to the fact that the connection of unithiol with heavy metal ions is more durable than the bond of the same metals with SH-groups of proteins. Unithiol compounds with heavy metal ions are low-toxic, water-soluble and therefore easily excreted from the body with urine [8, 13].

The results obtained by us indicate the ability of heavy metal salts to deepen the mechanisms of violations that occur in severe trauma. They aim at the expediency of determining in the blood of the injured body the content of heavy metal ions and elevated levels, to be used in complex intensive therapy of unithiol, requiring further in-depth study.

Conclusion: 1. In the late period of a traumatic illness (14-28 days) after the application of severe skeletal trauma in the liver tissue, the intensity of free radical oxidation of lipids increases. Signs of extinction of antioxidant defense are noted.

2. In the period of late manifestations of traumatic illness against a background of severe skeletal trauma and excessive intake of copper ions and zinc in comparison with animals without additional intoxication in the liver tissue, the level of lipid peroxidation is higher, and the activity of antioxidant defense enzymes is lower.

3. The introduction of unithiol into traumatized animals with excessive intake of copper and zinc for 14 days after causing severe skeletal trauma is accompanied by fewer violations of the studied markers of traumatic illness that do not reach the level of animals without additional toxic effects up to 28 days

References

1. Andreeva L. I. Modification of the method for determining lipid peroxides in a test with thiobarbituric acid / L. I. Andreeva, L.A. Kozhemyakin, A.A. Kiskun // Lab. delo. - 1988. - No.11. - P. 41-43.

2. Bondarenko Yu.I. Dynamics of indicators of cytolysis and endogenous intoxication in the period of late manifestations of traumatic illness and their correction by thiotriazolin /

Yu. I. Bondarenko, S. M. Pridruga, R. M. Boris // Clinical and Experimental Pathology - 2013. - Vol. 12, No. 1. - S.42-45.

3. Boris R.M. Dynamics of the parameters of the enzyme level of antioxidant defense in the period of early and late manifestations of traumatic illness under conditions of experimental cranioscheletal trauma and its correction by cell therapy / R.M. Boris, A. M. Goltsev, A.I Gozhenko // Archive of clinical and experimental medicine.- 2013. - Vol. 22, No. 1. - P. 23-28.

4. Braginsky L.P. On the method of toxicological experiment with heavy metals on hydrobiontes / L.P. Braginsky, P.N. Linnik // Hydrobiol. journ - 2003. - Vol. 39, No. 1. - P. 92-104.

5. Volotovska N.V Features of apoptosis of hepatic macrophages under the influence of a mechanical trauma of varying severity in white rats / N.V Volotovska, A.A Hudyma // Clinical and Experimental Pathology. - 2012. - T 11, No. 3 (41), p. 1- P. 24-26.

6. Zasekin D.A. The use of laboratory white rats to create biological object with an increased content of heavy metals / D.A. Zasekin, I. V. Kalinin // Naukoviy Visnyk NAU.- 1999. - issue 19. - P. 21-24.

7. Selected aspects of pathogenesis and treatment of traumatic illness / V. N. Elsky, V. G. Klimovitsky, S. E. Zolotukhin[and others] - Donetsk: " Лебедь " LLC, 2002. - 360 p.

8. Kaminsky R. F. Oxidation-reduction processes as the basis for the development of pathological changes in the myocardium and an important link in the therapeutic effect of pharmaceuticals for chronic mercuric intoxication / R. F. Kaminsky, N. A. Kolesova, Yu. B. Tchaikovsky // Ukrainian Morphological Almanac.- 2010. - № 2, T. 8. - P. 81-84.

9. Kozak D.V. Dynamics of indices of endogenous intoxication under conditions of polytrauma / D.V. Kozak // Achievements of clinical and experimental medicine.- 2012. - No 1. - P. 69-71.

10. Kozak D.V. Features of indicators of peroxide oxidation of lipids in the dynamics of the early and late periods of polytrauma / D.V. Kozak // Actual problems of transport medicine 2012. - No. 3. - P. 103-106.

11. Korolyuk M.A Method for determining the activity of catalase / M.A Korolyuk, L. I. Ivanova, I. G. Mayorova, V. Y. Tokarev // Laboratory business. - 1988. - No 1. - P. 16-19.

12. Program of environmental monitoring of Ternopil region for 2006 - 2010 - Ternopil, 2005. - 22 p.

13. Prevention and intensive care of acute poisoning in children and adolescents /V.I Black, B.S. Sheiman, N.P. Grebnyak, A.N. Kolesnikov, A.Yu. Fedorenko - 2007 - 1010 p.

14. Lead in conditions of industrial cities: external exposure biomonitoring, markers of action and effect, prevention / I. M. Trahtenberg, E. M. Biletska, V. F Demchenko [and others] // Environment and health.- 2002. - No 3. - P. 10-12.

15. The state of low-molecular-weight sulfur-containing compounds of carp hepatopancreas contaminated by copper and zinc salts / O. B. Stolyar, V. Z. Kurant, V.A. Khomenchuk [and others] // Hydrobiol. journal. - 2003. - P. 39, No. 4. - P. 91 – 98

16. Stolyar O .B. The accumulation of metals and antioxidant protection in the tissues of Anodonta cygnea bivalve molluscs from the Ternopil pond / O. B. Stolyar, R. L. Mykhailiv, O. V. Mishchuk // Scientific notes of the Ternopil Pedagogical University. Series: Biology. - 2003. - No. 2 (21). - P. 94-97.

17. Chevari S. The role of superoxide dismutase in the oxidative processes of cells and the method of determining it in biological materials [Text] / S. Chevari, I. Chaba, J. Sokei // Lab. a business. - 1985. - No 11. - P. 678-681.

18. Alsop D. Metal absorption and acute toxicity in zebrafish: common mechanisms across multiple metals / D. Alsop, CM Wood //Aquat. Toxicol - 2011. - Vol. 105, No. 3-4. - P. 385-393.

19. Copper stress induces a global stress response in Staphylococcus aureus and represses sahe and agroexpression and biofilm formation / J. Baker, S. Sitthisak, M. Sengupta [et al.] //Appl. Environ Microb- 2010 - Vol. 76, №1 . - P. 150-160.

20. Toxicity of Ag, CuO and ZnO nanoparticles to selected environmental relevant test organisms and mammalian cells in vitro: a critical review / O. Bondarenko, K. Juganson, A. Ivask[et al.]// Arch. Toxicol Jul- 2013. - Vol. 87, No. 7. - P. 1181-1200.