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Influence of Boron Compounds on Chromium-induced Hemorheology Disorders in Rats

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ABSTRACT

The authors of this study evaluated the protective influence of boric acid on chromium-induced blood rheology disorders. The experiment was carried out on Wistar rats, which were divided into three groups: Group 1 - the control group; Group 2 - rats with simulated chromium-induced hemorheology disorders; Group 3 - rats, in which boric acid was administered throughout 10 days on the background of chromium-induced hemorheology disorders. In rats with simulated chromium-induced hemorheology disorders, one could observe decrease in deformability of red blood cells and in hematocrit level on the background of increased aggregation, peroxide hemolysis and osmotic fragility of red blood cells. In the third group, corrective application of boric acid inhibited development of blood rheology disorders, i.e., its protective action was revealed.

Keywords: Potassium dichromate, Boric acid, Hemorheology, Rat, Protective action**.**

INTRODUCTION

Quantitative and qualitative changes in blood rheology occur under the impact of industrial chemicals and these changes reflect their chemical profile (Krivokhizhina, *et al*., 2006). Since red blood cells make 93% of formed elements, changes in their physico - chemica properties hinder implementation of their main function - transport of oxygen in microvasculature, which leads to the development of tissue hypoxia (lack of oxygen transfer or termination of its delivery, decrease in redox activity, development of structural and qualitative changes in cell membranes (Khetsuriani and Kipiani, 2002; Lukyanova, 2003)), increased risk of cardio - vascular diseases and relevant complications (Lowe, *et al*., 1997) as well as ischemic cerebrovascular diseases (Szapary, *et al*., 2004). Rheological properties of blood depend on many factors (Ormotsadze and Nadareishvili, 2002); they may change under the impact of various stress factors (Gyawali, *et al*., 2015) and chemicals (Zairova *et al*., 2006; Kotelnikov and Kotelnikova, 2005; Zimetti *et al*., 2006; Pagano and Faggio, 2015).

Chromium is a self-existing microelement. Its valence Cr^{+3} or Cr^{+6} influences absorption. The oxidation degree and solubility of chromium

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compounds determine their toxicity. The impact of hexavalent chromium Cr^{+6} has a number of negative effects, including neurotoxicity, hepatotoxicity, cardiotoxicity, renal toxicity, genotoxicity, carcinogenicity, immunotoxicity as well the development of microcytic hypochromic anemia (Kawanish *et al*., 2002; O'Brien *et al*., 2003; Bazarbekova, 2002; Sudha *et al*., 2011; Stout *et al*., 2009). Once inside the cell, Cr^{+6} is restored to Cr^{+3}; this is accompanied by the generation of reactive oxygen intermediates, which cause oxidation of macromolecules such as DNA and lipids (Aruldhas *et al*., 2005; Wise *et al*., 2008; Wang *et al*., 2011; Iztleuov, 2003; Iztleuov *et al*., 2011) and induce tissue damages in a number of organs, such as liver, pancreas, kidneys and the blood-vascular system (Stout *et al*., 2009; Solis-Heredia *et al*., 2000; Bagchi *et al*., 2002; Fatima *et al*., 2005). Different people are exposed to high concentrations of Cr^{+6} professionally, ecologically or internally (Mamyrbayev, 2012).

Boron is a conditionally self-existing element. Naturally, it exists in the form of borates. Physiological concentrations of boron compounds affect a wide range of metabolic processes (Hunt, 1998), which is "apparently" associated with their antioxidant effects (Turkez *et al*., 2007; Hu *et al*., 2014). Boron compounds have anti-inflammatory, antitumor and hypolipidemic properties (Barronco *et al*., 2008). Besides, these compounds are not genotoxic (Ornat and Konur, 2004; Oto *et al*., 2015).

The damaging impact of Cr^{+6} - induced oxidative stress is caused mainly by a hydroxyl radical, which damages macromolecules, forms protein crosslinks promoting protein denaturation and aggregation. Besides, it causes formation of secondary radicals by reacting with low-molecular compounds (Iztleuov, 2004). Oxidative stress develops when the content of antioxidants is reduced (Tapiero *et al*., 2004). Antioxidants can protect cells from free radicals in the presence of metal-induced oxidative stress (Valko *et al*., 2005). Once inside the body, boric acid (boron compounds) enhances the prooxidant - antioxidant balance (Bolanos *et al*., 2004; Turkez, 2008) and increases activity of antioxidant enzymes, thereby neutralizing reactive oxygen intermediates, eliminating and preventing oxidative damage of cell membranes in macromolecules. However, protective action of boron compounds in the presence of chromium-induced hemorheology disorders.

The aim of this study was to evaluate the protective influence of boric acid on chromium-induced blood rheology disorders.

MATERIALS AND METHOD

Experiments were performed on 24 male Wistar rats weighing 190 – 220 g. The animals were kept in plastic cages in observance of a certain light regime (12-hour light / 12-hour dark periods) at a temperature of 23 - 25^{0}C, with free access to food and water. Experiments were conducted in the morning hours (9 AM - 12 PM). All manipulations were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg: Council of Europe, 1986). The program of this experiment was discussed and approved by the regional ethics committee of the West Kazakhstan Marat Ospanov State Medical University.

Ten days after acclimatization, the animals were randomly divided into three groups (eight rats in each group): the first group comprised intact rats (control), the second and the third group comprised animals with simulated chromium-induced hemorheology (diselementosis) caused by a single intraperitoneal injection of potassium dichromate $K_2Cr_2O_7$, purchased from LLP "Chemistry and Technology" (Kazakhstan) at the rate of 14 mg / kg of body weight (0,5LD_{50}). In contrast to the second group, boric acid H_3BO_3 purchased from OJSC "Farmak" (Ukraine) was administered orally after $K_2Cr_2O_7$ in rats from the third group - 5.0 mg / kg of body weight during 10 days. Selection of doses, routes of administration and duration were substantiated in the previous studies (Iztleuov *et al*., 2011) and chosen according to relevant literature data (Moore, 1997; Pahl *et al*., 2005).

Experimental animals were under ether anesthesia. Blood was collected from the heart using siliconed needles. Rheological properties of red blood cells was assessed by erythrocyte deformability index (EDI), erythrocyte aggregation coefficient (EAC), by erythrocyte peroxide hemolysis

(EPH), hematocrit (Ht) as well as by erythrocyte osmotic fragility (EOF).

Erythrocyte deformability index was determined using the method developed by Zakharova N.B., Tselik N.I., and Klyachkin M.L. (1989). This method implies preparing the 60% erythrocyte suspension and 0.02 µl (20 µl) of the suspension is applied onto a filter paper with pore diameter of 4±1 µl. After 60 seconds, the stain diameter (D_1) is measured. Next, after 60 seconds, 20 uL (0.02 µl) of a 60% suspension is applied again to the center of the obtained spot and the spot diameter (D_2) is measured again. Erythrocyte deformability index is calculated using the following formula:

$EDI=D_1/D_2$
where
D_1 and D_2 – diameters of stains.

Erythrocyte aggregation coefficient (EAC) was determined using the method developed by V.A. Lapotnikov and L.M. Kharash (1982).

This method implies the following: erythrocyte aggregates in a blood sample are fixed by formalin. The difference in weight of erythrocyte aggregates fixed by formalin and single cells causes changes in erythrocyte sedimentation rate (ESR) as compared to the control sample without formalin. In order to determine erythrocyte aggregation, the authors used 0.1M phosphate buffer with pH 7.4, 0.077 M EDTA (ethylenediaminetetraacetate) 4% clarified formalin. These reagents were used to prepare working solutions: solution No.1 - 3 ml of 0.077 M EDTA, 5.0 µl of 4% clarified formalin, 12.0 µl of 0.1M phosphate buffer, pH - 7,4. Solution No.2 - 3,0 µl of 0.077 M EDTA, 17 µl of 0.1 M phosphate buffer, pH - 7,4. Sequence of procedures: using siliconed needles, blood by 0.5 µl (500 µl) is poured in two centrifuge tubes containing 2 µl of solution No. 1 and No. 2, respectively. After blood was mixed with both solutions, ESR was determined in each sample using the incubator at 37^0C.

Determination of peroxide hemolysis of erythrocyte membranes was performed according to the method developed by A.A. Pokrovskiy and A.A. Abrarov (1964), which is a modified macromethod developed by Gyorgy P., Cogan G., Rose C.S (1952).

Modification implied spectrophotometric (l = 543 nm) determination of erythrocyte content, hemolyzed in standard conditions under the impact of hydrogen peroxide (H_2O_2). The use of ultra-microanalysis was a specific feature of this modified method, which significantly reduced the volume of blood (20 µl, 0,02 µl) required for this experiment.

The level of hematocrit was measured by using the automated hematological analyzer CELL-DYN Ruby (Abbott, USA).

Erythrocyte osmotic fragility was determined by the Daisy method (Todorov, 1968).

Sequence of procedures: basic solution (NaCl -. 180,0 g, Na_2HPO_4 -27,31 g, NaH_2PO_4 "" $2H_2O$ - 4,86 g, Aquae dest -. 2000,0 ml (2,0 l), pH -7,4) was used to prepare dilutions corresponding to 0.85; 0.55; 0.5; 0.45; 0.4; 0,35 and 0,3% of NaCl. 5.0 µl of each dilution was put in a number of centrifuge tubes, and then 50 µl (0.05 µl) of blood was added, mixed up and left for at least 30 minutes at room temperature. Afterwards, these dilutions were centrifuged during 5 min at 2000 rpm (700 g). Optical density of the supernatant was measured at a wavelength of 543 nm by using a "Genesys -5" spectrophotometer (USA).

Cell hemolysis was calculated percentagewise as related to 100% hemolysis induced by 0,1% NaCl solution.

RESULTS AND DISCUSSION

Analysis of the obtained data shows blood rheology disorders in the presence of chromium-induced diselementosis. This is displayed by significant reduction of erythrocyte deformability index and hematocrit level against the backdrop of increase in erythrocyte aggregation coefficient, erythrocyte peroxide hemolysis (Table 1) and in erythrocyte osmotic fragility (EOF). EOF is significantly increased ($p < 0,05$) in the presence of 0.4; 0.45; 0.5; 0,55 and 0,85% NaCl (Table 2). Intake of boric acid on the background of chromium-induced hemorheology disorders leads to the improvement of blood rheological properties (pd" 0,05).

Chromium causes a wide range of toxicological effects and biochemical dysfunctions that imply serious health risks (Mamyrbayev, 2012; Bielicka *et al*., 2005). Some studies show that Cr^{+6} and its compounds do not directly generate free radicals; however, reduction of Cr^{+6} to Cr+3, as well as the effect of Haber - Weiss and Fenton mechanisms (Lloid *et al*., 1998), imply the emergence of different radicals that cause damages characteristic of oxidative stress (Pritchard *et al*., 2000; Barrera *et al*., 2003). Consequently, one of the possible basic approaches used for prevention (correction) of $K_2Cr_2O_7$ - induced damage implies using agents (elements) with powerful antioxidant properties. Recent studies have shown that boron and its compounds displayed significant protective effects against damages induced by metals, such as aluminum and arsenic (Turkez *et al*., 2011; Kucukkurt *et al*., 2015). Erythrocytes are sensitive to the impact of heavy metals, including chromium compounds (Ryspekova *et al*., 2013); they present a convenient model to assess cytotoxicity of chemicals. Hemolysis and its value may serve as a stability test for cell membranes of erythrocytes (Pagano and Faggio, 2015). The latter are characterized by certain rheological properties (deformation and flow), due to which they have a lenticular shape, high flexibility, elasticity and deformability. Deterioration of their rheological properties trigger the occurrence of hemorheology disorders in the presence of various diseases (Muraviov and Tikhomirova, 2009; Sharapova, 2012; Baev *et al*., 2013), including the cardio - vascular (Vaya *et al*., 2013) and cerebrovascular diseases (Szapary *et al*., 2004); this may complicate the course of these diseases (Plotnikov *et al*., 2005). This experiment was conducted with a view to show the impact of potassium dichromate on blood rheology and to explore the impact of boric acid on the morpho-functional disturbances of erythrocyte membranes,

Table 1: The impact of boric acid on some chromium-induced blood rheology disorders in rats

Indices Groups	Hematocrit, %	EDI	EAC	EPH, %
Group 1	41.7±3.0	0.707±0.02	0.93±0.06	1.30±0.11
Group 2	33±2.7*	0.337±0.03*	2.63±0.13*	1.86±0.21*
Group 3	42±3.3	0.61±0.024*	1.23±0.06*	1.2±0.09

Note: some parameters refer to both Table 1 and Table 2 – Group 1 – intact; Group 2 – rats with simulated chromium-induced hemorheology disorders; Group 3 – rats, in which boric acid was administered throughout 10 days on the background of chromium-induced hemorheology disorders; EDI - erythrocyte deformability index; EAC - erythrocyte aggregation coefficient, EPH - erythrocyte peroxide hemolysis; significant differences (@<0,05): asterisk – in relation to the control group; bold – in relation to data obtained from Group 2

Table 2: Protective impact of boric acid on erythrocyte osmotic fragility

Indices Groups	Erythrocyte osmotic fragility, %						
	0,3 % NaCl	0.35 % NaCl	0.40 % NaCl	0.45 % NaCl	0.50 % NaCl	0.55 % NaCl	0.85 % NaCl
Group 1	88±6.3	81±4.8	66±4.2	30±2.6	21±1.7	12±1.2	3.3±0.42
Group 2	96±7.0	90±8.0	87±7.0*	63±4.1*	43±3.0*	24±2.1*	7.2±1.0*
Group 3	87±8.0	84±6.3	72±5.3	45±3.3*	26±1.8*	15±2.0	3,6±0.6

induced by hexavalent chromium ($K_2Cr_2O_7$). In this study, EDI decrease and EAC increase, peroxide hemolysis and erythrocyte osmotic fragility reflects deterioration of blood rheological properties. Intake of boric acid in the presence of chromium-induced diselementosis improves blood rheological properties (EDI, Ht, EAC, EPG and EOF). Perhaps, changes in blood rheological properties is associated with antioxidant activity of boron compounds (indirect action). Thus, a number of scientists found that boron compounds (boric acid, borax, etc.) could be used for correcting metal-induced (arsenic, bismuth, cadmium, mercury, lead) oxidative stress effects (Turkez *et al*., 2007; Kucukkurt *et al*., 2015; Pawa and Ali, 2006). Indeed, oxidative stress occurs at the decreased level of antioxidants (Tapiero *et al*., 2004). Boric acid intake contributes to the preservation of pro-oxidant - antioxidant balance (Bolanos *et al*., 2004; Turkez, 2008; Inse *et al*., 2010).

Possibly, there is another mechanism – the direct and immediate impact, which implies hematopoiesis disorders under the influence of boron compounds, which, in turn, influence physical and chemical blood properties (Oto *et al*., 2015). Boron in low doses (40 and 80 mg / l) can stimulate erythropoiesis and hemoglobin synthesis (Feng *et al*., 2014) having protective effect in the presence of metal-induced hemorheology disorders (Turkez *et al*., 2012). Boron in high doses (160 - 640 mg / l) can inhibit hematopoiesis and hemoglobin synthesis; i.e., it can be toxic (Feng *et al*., 2014).

Thus, the authors of the present study first found that boric acid in the presence of chromium-induced hemorheology disorders prevents (hinders) blood rheology disorders in rats (showing protective action). Apparently, taken in certain doses, boric acid is a promising remedy in the case of chromium-induced hemorheology disorders and suggests new dimensions of subsequent studies related to the biological effects of boron compounds. In the presence of chromium-induced disorders (diselementosis) in rats, one can observe changes in blood rheological properties – erythrocyte deformability index and the level of hematocrit are reduced on the background of increase in EAC, peroxide hemolysis and erythrocyte osmotic fragility. Oral administration of boric acid on the background of chromium-induced changes in the blood rheological properties inhibits the development of blood cell disorders (protective action).

CONCLUSION

Implications and recommendations for future studies are as follows: Some studies show that Cr^{+6} and its compounds do not directly generate free radicals; however, reduction of Cr^{+6} to Cr^{+3}, as well as the effect of Haber - Weiss and Fenton mechanisms (Lloid *et al*., 1998), imply the emergence of different radicals that cause damages characteristic of oxidative stress (Pritchard *et al*., 2000; Barrera *et al*., 2003). Consequently, one of the possible basic approaches used for prevention (correction) of $K_2Cr_2O_7$ - induced damage implies using agents (elements) with powerful antioxidant properties. Recent studies have shown that boron and its compounds displayed significant protective effects against damages induced by metals, such as aluminum and arsenic (Turkez *et al*., 2011; Kucukkurt *et al*., 2015). Erythrocytes are sensitive to the impact of heavy metals, including chromium compounds (Ryspekova *et al*., 2013); they present a convenient model to assess cytotoxicity of chemicals. Hemolysis and its value may serve as a stability test for cell membranes of erythrocytes (Pagano and Faggio, 2015). The latter are characterized by certain rheological properties (deformation and flow), due to which they have a biconcave shape, high flexibility, elasticity and deformability. Deterioration of their rheological properties trigger the occurrence of hemorheology disorders in the presence of various diseases (Muraviov and Tikhomirova, 2009; Sharapova, 2012; Baev *et al*., 2013), including the cardio - vascular (Vaya *et al*., 2013) and cerebrovascular diseases (Szapary *et al*., 2004); this may complicate the course of these diseases (Plotnikov *et al*., 2005). This experiment was conducted with a view to show the impact of potassium dichromate on blood rheology and to explore the impact of boric acid on the morpho-functional disturbances of erythrocyte membranes, induced by hexavalent chromium ($K_2Cr_2O_7$). In this study, EDI decrease and EAC increase, peroxide hemolysis and erythrocyte osmotic fragility reflects deterioration of blood rheological properties. Intake of boric acid in the presence of chromium-induced diselementosis improves blood rheological properties (EDI, Ht, EAC, EPG and EOF).

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