The Effect of Chromium and Boron on the Lipid Peroxidation and Antioxidant Status (In Experiment)

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Abstract

Background: Modern ecological situation is characterized by significant impairments as a result of adverse human influence. In this connection, it becomes necessary to study the negative effects of substances on living organisms and ways to reduce them.

Objective: This article evaluated the impact of potassium dichromate ($K_2Cr_2O_7$) and boric acid (H_3BO_3) on the status of lipid peroxidation (LPO) and antioxidant system (AOS).

Methods: The work is performed on 24 male rats of Wistar weighing 180 g to 220 g, were grown in the vivarium of the Central research laboratory of West Kazakhstan state medical University named after Marat Ospanov (Aktobe, Republic of Kazakhstan).

Results: An experiment on Wistar rats: 1st-warning; 2nd-received $K_2Cr_2O_7$; 3rd-H_3BO_3; 4th-$K_2Cr_2O_7$ and H_3BO. The introduction of $K_2Cr_2O_7$ intensified LPO and reduced activity of catalase, glutathione peroxidase and superoxide dismutase, the number of sulfhydryl groups. In the group treated with H_3BO_3-LPO inhibition, activation of AOS. Combined injection of $K_2Cr_2O_7$ and H_3BO_3 reduces the content of malondialdehyde, increases the number sulfhydryl groups compared to the group "$K_2Cr_2O_7$".

Conclusions: The results of the study allow asserting that the H_3BO_3 in the conditions of joint use with $K_2Cr_2O_7$ has an antioxidant effect, which leads to inhibition of LPO and activation of antioxidant system. Therefore, we first established the antioxidant effect of boric acid in the combined effects of potassium dichromate and boric acid. Our study shows that boric acid when co administered orally with potassium dichromate inhibits the development of chromium-induced oxidative stress in the tissue blood. Based on its efficacy are inhibition of lipid peroxidation and increase antioxidant defense in the blood.

Keywords: Antioxidant enzymes; Boric acid; Lipid peroxidation; Potassium dichromate; Sulfhydryl groups

Abbreviations: LPO: Lipid Peroxidation; DC: Diene Conjugates; UOD: Units of Optical Density; LHP: Lipid Hydroperoxides; MDA: Malondialdehyde; TBA: Thiobarbituric Acid; SH: Sulfhydryl Groups; EDTA:

Ethylendiaminetetraacetate; CAT: Catalase; SOD: Superoxide Dismutase; NBT: Nitro Blue Tetrazolium; GPO: Glutathione Peroxidase.

Introduction

Because of the increasing and widespread in the environment many anthropogenic factors [1], the actual evaluation of the toxicity of a chemical or biological agent, is the study of combined action. Understudied assessment of combined effects at low levels of active agents that occur in the home and in professional conditions.

Therefore, it is important to examine the processes of lipid peroxidation (LPO). The intensification of LPO is one of the main causes of damage to cell membranes and membranes of cellular organelles in terms of exposure to chemicals. The mechanisms of their effects are realized through a biological effect, determined by the nature of the combined interactions. Reaction of biological oxidation is accompanied by formation of free radicals-particles having on the outer valence orbitals of the unpaired electron. This results in a high reactivity of these radicals.

Chromium is an element with a global dispersion in the air. The content of chromium in the earth's crust is estimated at 0,0083% and is one of the most toxic chemical compounds because of its high level in the biosphere, and the most common pollutants of aquatic and terrestrial ecosystems [2]. In nature chromium exists mostly in two valence States: hexavalent chromium-Cr^{+6} trivalent chromium Cr^{+3}. Oxidation state and solubility of chromium compounds determine their toxicity. Potassium dichromate (Cr^{+6}) is widely used in metallurgy, chrome plating, textile manufacture, and other fields of human activity [3]. The effect of Cr^{+6} can lead to a number of pathological conditions: nephrotoxicity, dermatotoxicity, genotoxicity, Carcinogenicity, and immunotoxicity [3-5]. Compounds of Cr^{+6} are the most toxic, as can easily pass through the biomembrane through nonspecific transporters of anions phosphate and sulfate [6]. Immediately after penetration inside the cell, Cr^{+6} is restored to Cr^{+3}, which is accompanied by generation of reactive oxygen species and induces damage to cellular structures, including genetic [7-9].

Boron is essential for plants, animals and humans [10]. Boron compounds are used for the saturation of surface of steel products; glass and chemical industries; in agriculture, aerospace, medical institutions; in many cosmetics and personal care products [11,12]. Boron is absorbed from the gastrointestinal tract into the blood and in physiological amounts, affects a wide range of metabolic parameters in animals [13], which is probably related to the antioxidant effects of compounds of boron [14]. Boron compounds have anti-inflammatory, hypo-lipid and antitumor actions [15] and do not exert genotoxic effects [16].

As far as we know, the influence of combined action of chromium and boron on the LPO and AOS in the blood have not been studied. The study of interaction between chromium and boron as factors of "low intensity" LPO processes in the rats' blood makes it possible to identify the final effect of their action, and the nature of the changes at the level of prooxidant-antioxidant systems that determine the degree of adaptive changes in the body.

Materials and Methods

The work is performed on 24 male rats of Wistar weighing 180 g to 220 g, were grown in the vivarium of the Central research laboratory of West Kazakhstan state medical University named after Marat Ospanov (Aktobe, Republic of Kazakhstan). The animals were kept in vivarium with natural light and maximum standardization of temperature and food regimes with free access to food and water. The study was conducted in the first half of the day (9-12 hrs). All manipulations were conducted in accordance with the European Convention for the protection of vertebrate animals used in the experiment [17]. The program of the experiment was discussed and approved by the regional ethics Committee of the University.

Animals after 10 days of acclimatization were randomly divided into 4 groups (by six rats): 1st group-intact rats (control); 2nd group-group "chrome" along with drinking water received potassium dichromate ($K_2Cr_2O_7$) purchased from LLP "Chemistry and Technology" (050020, Kazakhstan, Almaty, L. Chaikina str., 14), at the rate of 3.0 mg/kg of body weight; 3-rd group-group "forest" together with water boric acid (H_3BO_3), purchased from JSC "Farmak" (04080, Ukraine, Kiev, Frunze str., 63), at the rate of 5.0 mg/kg of body weight; 4-th group-the group "the combination" animals together with the water received $K_2Cr_2O_7$ (3 mg/kg), and H_3BO_3 (5 mg/kg). The total duration of the experiment was 21 days. The choice of doses, modes of administration and the duration of the experiment, proved previously performed studies [18-24]. Euthanasia of animals was performed by the method of instant decapitation under light ether anesthesia.

Biochemical study. Blood was collected in tubes with EDTA (Vacutainer firm "BD Franklin Lakes N J", USA) and centrifuged at 2200 g for 10 min and the Collected plasma samples were stored at -20°C until analyzed. Erythrocytes obtained from blood samples were washed three times with 5 volumes of buffered phosphate saline (PBS; 150 mmol/l NaCl, 1.9 mmol/l NaH_2PO_4, pH 7.4), centrifugating at 1500 rpm.

Lipid peroxidation. Definition of diene conjugates (DC) in the plasma was carried out according to UV spectrum the primary oxidation products of polyunsaturated lipids with an absorption maximum at 233 nm; the molar extinction coefficient is equal to $2,2*10^{-5}M^{-1}cm^{-1}$. The contents of the DC expressed in units of optical density (UOD) per ml (D_{233}/ml).

The concentration of lipid hydroperoxides (LHP) in the studied sites was determined by iron-rhodanate method. The method is based on the ability to translate the LHP Fe^{+2} to Fe^{+3} which give a colored complex with ammonium thiocyanates at the absorption maximum 480 nm. The number of LHP UOD expressed per mg lipid (D_{480}/mg of lipid).

The contents of malondialdehyde (MDA) in plasma was determined using thiobarbituric acid (TBA) by the modified method of Andreeva et al. [25]. The principle of the method: at high temperature in an acidic medium, MDA reacts with 2-TBK, forming a colored trimethine complex with an absorption maximum at 532 nm. The molar ratio of extinction is equal to $1.56*10^{-5}M^{-1}cm^{-1}$. MDA level was expressed in μmol/L.

The system of antioxidant protection of blood. The content of sulfhydryl groups (SH) in plasma was determined by the method of Ellman [19]. To 1.5 ml of phosphate buffer pH of 7.6 (t = -25°C) we added 0.5 ml of dithionitrobenzene (3.6 mg 5.5-dithiobis (2-nitrobenzoic) acid (TNBC) in 10 ml of 0.01 M phosphate buffer), 0.1 ml of a solution ethylendiaminetetraacetate-EDTA (100 mg EDTA*$2H_2O$ per 10 ml of buffer) and 0.2 ml of plasma. The amount of formed

thionitrophenyl anion in the sample is directly proportional to the amount of SH-groups, reacted with TNBC. After 40 min, we measured the optical density of the samples spectrophotometrically at 412 nm. The number of SH-groups were expressed in µmol/L.

The activity of catalase (CAT) in erythrocytes was measured by a reaction. The reaction was started by adding 2.0 ml of hydrogen peroxide to 10 µl of the hemolysate and after 10 minutes was stopped by adding 1.0 ml 4% ammonium molybdate. After that, we measured the absorbance of the sample at 410 nm as opposed to a control sample, in which 2.0 ml of distilled water was added instead of hydrogen peroxide. Enzyme activity was expressed in units of activity per mg of protein (U min/mg). One unit of catalase activity was defined as the activity necessary to decompose 1 µmol of hydrogen peroxide per 1 minute (60 sec).

The activity of superoxide dismutase (SOD) in erythrocytes was determined assessing the speed of recovery of nitro blue tetrazolium (NBT) in the presence of the restored nicotinamide adenine dinucleotide and phenazine methosulfate. As a unit of SOD activity, we took the amount of enzyme required to inhibit NBT reduction by 50%, and activity was expressed as units act/mg of protein (U /mg Pt).

The activity of glutathione peroxidase (GPO) was determined by oxidation of NADP*H_2 in a coupled glutationreductase reduction reaction on a spectrophotometer at 340 nm. The activity calculation was performed considering the coefficient of molar extinction of the restored NADP*H_2 equal to 6.22 mM/cm. Results are expressed in nmol of oxidized NADPH min/mg of protein or U min/mg of protein. Total lipids were determined using a set of reagents "Lachema" (Czech Republic); the content of protein-by the method of Lowry et.al. [20], using bovine serum albumin as a standard. The optical density of the samples was measured using spectrophotometer "Genesys 5" (USA).

Results

Analysis of the results of the study of the LPO in blood and SH-groups in blood plasma shows that in animals treated orally with $K_2Cr_2O_7$ is activation of the LPO processes: the level of MDA, DC and LHP significantly increased on the background of decreasing the content of SH-groups (TABLE 1).

TABLE 1. **The effect of the combined action of $k_2Cr_2O_7$ and h_3bo_3 on the level of SH groups and peroxidation products in blood of rats.**

Indicators Groups	DC (D_{233}/ml)	LHP (D_{480}/mg of lipid)	MDA (Mmol/L)	SH-group (Mmol/L)
Control	1.20 ± 0.043	0.21 ± 0.013	1.22 ± 0.061	366 ± 16.17
$K_2Cr_2O_7$	$1.52 \pm 0.06^*$	$0.27 \pm 0.016^*$	$1.66 \pm 0.07^*$	$300 \pm 14.71^*$
H_3BO_3	$0.98 \pm 0.04^*$	$0.14 \pm 0.008^*$	$0.89 \pm 0.05^*$	$501 \pm 32.71^*$
$K_2Cr_2O_7+H_3BO_3$	$1.10 \pm 0.06_+$	$0.19 \pm 0.009_+$	$0.99 \pm 0.06^*_+$	$462 \pm 19.40^*_+$

In the group of animals, which were injected H_3BO_3 observed opposite changes: the amount of MDA, DC and LHP in plasma decreased significantly, and the level of SH-groups is increased by 36.9% compared with the control group. This indicates the deceleration of the LPO in blood at admission H_3BO_3 with drinking water. Probably due to the acceleration of degradation of the peroxide and TBA-products or inhibiting the synthesis, in the first place, MDA and ketones.

Combined injection of $K_2Cr_2O_7$ and H_3BO_3 animal is accompanied by a decrease in levels of LPO products in comparison with the data of a group of animals that received only $K_2Cr_2O_7$: the contents of MDA, DC and LHP in the blood plasma falls, the number of SH-groups of blood increases by 54%. The level of MDA was significantly lower ($p<0.05$), SH-groups of blood higher ($p<0.05$) compared with control, and the number of DC and LHP in plasma were not significantly different from the level of the intact.

The results indicate inhibition of LPO processes in the blood compared to animals treated with $K_2Cr_2O_7$. This is to some extent consistent with literature data on the inhibitory effects of boron compounds on oxidative stress caused by impacts of heavy metal compounds-arsenic trioxide, colloidal subcitrate of bismuth, chlorides of cadmium, mercury and lead [14] and thioacetamide.

Explanation of the mechanisms of the inhibitory effect of H_3BO_3 on the processes of generation of free radicals should obviously be sought in the activation of antioxidant enzyme systems, which prevent the development of free radical reactions, the accumulation of super oxidation and peroxide, maintain a high activity of redox processes, provide the ultimate elimination of oxygen metabolites.

The results of the research activity of antioxidant enzymes in red blood cells show that in animals treated orally with $K_2Cr_2O_7$ activity of SOD, CAT and GPO is reduced in comparison to the control (TABLE 2). With the introduction of drinking water H_3BO_3, on the contrary, in animals there is an increase in activity of the investigated antioxidant enzymes.

TABLE 2. **The activity of antioxidant enzymes of blood of rats, which were injected $K_2Cr_2O_7$, H_3BO_3 and ($K_2Cr_2O_7$+ H_3BO_3).**

Indicators Group	SOD (U/mg Pt)	CAT (U min/mg Pt)	GPO (U min/mg Pt)
Control	2.80 ± 0.15	70.2 ± 2.7	14.0 ± 1.03
$K_2Cr_2O_7$	2.40 ± 0.14	$60.0 \pm 1.9*$	$9.0 \pm 0.80*$
H_3BO_3	3.10 ± 0.19	$90.0 \pm 3.0*$	$25.0 \pm 1.60*$
$K_2Cr_2O_7$+H_3BO_3	$3.0 \pm 0.22_+$	$77.0 \pm 2.3_+$	$11.7 \pm 0.90_+$

Each value represents the mean $\pm$ standard deviation from 6 animals; data of the groups $K_2Cr_2O_7$, H_3BO_3 and $[K_2Cr_2O_7+H_3BO_3]$ are treated in comparison with the control group: x $p<0.05$. Group $[K_2Cr_2O_7+H_3BO_3]$-in comparison with the data of the group $K_2Cr_2O_7$: + $p<0.05$.

In a joint application, i.e. in the group ($K_2Cr_2O_7$+H_3BO_3), there is the leveling influence of $K_2Cr_2O_7$: the activity of SOD, CAT and MPO in the red blood cells are in the range of fluctuations of the control ($p>0.05$) and compared with a group of

$K_2Cr_2O_7$ increased respectively to 25 and 30 in 28.3%. This means that boric acid and some borates can enhance antioxidant ability by enhancing activity of enzymes in erythrocytes and provide protection from metal-induced damage (arsenic, bismuth, cadmium, mercury, lead) in vitro cultured erythrocytes thanks to the antioxidant ability.

Discussion and Conclusion

Thus, oral administration of $K_2Cr_2O_7$ is activation of LPO processes on the background of reducing the activity of antioxidant enzymes and enzymatic antioxidant-SH groups. When we receipt H_3BO_3, we can observe the inhibition LPO and increased activity of the antioxidant system and SH groups in the blood. Joint oral administration of H_3BO_3 and $K_2Cr_2O_7$ leads to the leveling of processes, characteristic for exposure to $K_2Cr_2O_7$. The results of the study allow asserting that the H_3BO_3 in the conditions of joint use with $K_2Cr_2O_7$ has an antioxidant effect, which leads to inhibition of LPO and activation of antioxidant system. Therefore, we first established the antioxidant effect of boric acid in the combined effects of potassium dichromate and boric acid.

Cr^{+6} and its compounds do not directly generate free radicals, however, this recovery of Cr^{+6} to Cr^{+3} as well as on the mechanism of the Haber-Weiss and Fenton [21], there are various radicals, such as superoxide anion, peroxynitrite, nitric oxide and hydroxyl that cause damages characteristic of oxidative stress [22], activate the LPO and lead to the destabilization and disintegration of cell membranes. In the present study after exposure to $K_2Cr_2O_7$, we observed a significant increase in the content of DK, LHP and MDA, the decrease in the level of SH–groups in blood and the activity of antioxidant enzymes in erythrocytes [18]. In the group $[K_2Cr_2O_7+H_3BO_3]$ we found a decrease in oxidative stress that was accompanied by a decrease in DC, LHP, MDA, and increased activities of antioxidant enzymes in erythrocytes, increase in SH-groups of blood. In fact, oxidative stress develops when the levels of antioxidants lowered. Moreover, antioxidants can protect cells from free radical attack while metal-induced oxidative stress. In works of some scientists, it was shown that catalase reduces the toxicity of Cr^{+6} in tumor cells [23] and the content of hydroxyl radicals [24]. In addition, it was found that overexpression of glutathione peroxidase protects cells from the toxicity of Cr^{+6} by reducing the number of HO^o-hydroxyl radicals [25]. Boric acid when applying to the body, apparently, contributes to the preservation of prooxidant-antioxidant balance.

Our study shows that boric acid when co administered orally with potassium dichromate inhibits the development of chromium-induced oxidative stress in the tissue blood. Based on its efficacy are inhibition of lipid peroxidation and increase antioxidant defense in the blood. Hence, boric acid, probably in certain doses is a promising means of protection against chromium-induced effects and allows to assume about opening up new directions for further study of the biological effects of boron compounds.

Implications and Recommendations

1) Oral administration of potassium dichromate in the organism of rats causes an increase in the level of malondialdehyde, diene conjugate and lipid hydroperoxide in blood on the background of reducing the activity of superoxide dismutase, catalase, glutathione peroxidase in erythrocytes and the content of sulfhydryl groups in blood plasma, i.e. leads to activation of lipid peroxidation and inhibition of the activity of antioxidant system.

2) Intragastric administration of boric acid in the organism of male rats is accompanied by a decrease of malondialdehyde, diene conjugate and hydroperoxides of lipids on the background of increasing the activity of superoxide dismutase, catalase, glutathione peroxidase and content of sulfhydryl groups in the blood, indicating inhibition of lipid peroxidation and increase the capacity of the antioxidant defense system.

3) The combined use of potassium dichromate and boric acid is accompanied by a decrease in the levels of products of lipid peroxidation and increase the capacity of the antioxidant system in comparison with the data group "chrome", i.e. boric acid in the conditions of combined effect provides an antioxidant effect.

Thus, the results of the study can be used to find substances that reduce the toxicity of Cr^{+6} for the human body.

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