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Protective Effect of Sodium Tetraborate on Chromium-induced Brain Damage in Rats

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ABSTRACT

Hexavalent chromium presents a particular threat due to its high toxicity. In this study showed the neuroprotective effect of sodium tetraborate in chromic intoxication. The experiment was performed on Wistar rats divided into 4 groups: 1 group - control; II group - single intraperitoneal injection of $K_2Ñr_2O_7$ in a dose of $0,5LD_{50}$, in the ²²² and ²V groups with drinking water received $Na_2B_4O_7$. Animals of the III group - 4 mg/kg of body weight, in IV group - 72 mg/kg of body weight daily for 10 days, the last administration of $Na_2B_4O_7$ was combined with a single intraperitoneal injection of $K_2Ñr_2O_7$ at a dose of 14 mg/kg ($0,5LD_{50}$).$Na_2B_4O_7$ in a dose of 4 mg/kg leads to a decrease in the level of MDA by 33%, an increase in the activity of catalase by 69%, superoxide dismutase by 21%, GR by 49%. $Na_2B_4O_7$ at a dose of 72 mg/kg increases the MDA content by 31% in comparison with the data of rats of chromic intoxication. $K_2Ñr_2O_7$ reduces the GSH level by 42%, the non-protein thiol by 36%. $Na_2B_4O_7$ at a dose of 4 mg/kg increased the GSH level by 53%, the non-protein thiol by 35%, and at a dose of 72 mg/kg reduced the GSH content by 23% (ñ<0.05), the non-protein thiol by 20% (ñ<0.05) in comparison with the data of rats exposed to $K_2Ñr_2O_7$.

Keywords: bichromate potassium, sodium tetraborate, brain damage, lipid peroxidation, antioxidant system.

INTRODUCTION

Pollution of the environment by heavy metals, as a result of high industrial activity in the late nineteenth and early twentieth centuries, has increased significantly throughout the world. Among heavy metal ions, chromium (VI) is a particular threat due to its high toxicity[1]. Ecopathogenic risk in the Western region of the Republic of Kazakhstan is associated with geochemical features - chromic

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biogeochemical region, oil and gas processing complex. The first in the countries of independent states and the world's second-richest chromites ore deposit, South-Kimpersai, is located in the Aktobe region, where a large enrichment plant, chrome compounds and chromium ferroalloys are concentrated. The expansion of these industries leads to an increase in the contingent of persons having professional contact with these compounds, as well as to increase emissions to the environment, which are dangerous for the population not only of the Aktobe region but also of the border regions of Western Kazakhstan in the South Urals[2].

Chromium exists in a medium in three stable oxidation states, Cr, Cr^{+3}, Cr^{+6}, which have different toxicity and transport characteristics[3]. Cr^{+6} is considered the most toxic form, because it has a high oxidation potential, high solubility and mobility across membranes in living (systems) organisms and in the environment[4]. The toxic effects of chromium are widely believed to be associated with the stimulation of free-radical processes, as well as the formation of intermediates during the reduction of Cr^{+6}, which have a high reactivity[5]. Once inside the Cr^{+6} cell, Cr^{+3} is reduced, generating active forms of oxygen (AFO), which cause oxidation of macromolecules such as DNA and lipids[6,7,8,9,10,11] and induce oxidative damage to tissues such as the liver, pancreas, kidney brain[12,13,14,15], which has a number of negative consequences for human health, including neurotoxicity, hepatotoxicity, nephrotoxicity, genotoxicity, carcinogenicity and immunotoxicity[16,17,18,19]. And the main role in the implementation of the damaging effect of oxidative stress is played by the hydroxyl radical. The damaging role of AFO is often associated not only with their direct action on cellular structures, but with the initiation of a cascade of processes leading to cell damage[20,21]. It is well known that the brain of mammals is rich in unsaturated fat cells, has a weak antioxidant defense system and is sensitive to damage to induced free radical oxidation (FRO), mediated lipid peroxidation (LPO). Generation of FRO and enhancement of LPO of neutron membranes can lead to oxidative damage to the brain[22,23,24,15]. Reducing antioxidant activity of the brain and increasing the AFO often causes neurotoxicity[25,26].

Bohr is widely recognized as an important dietary component with numerous beneficial effects on health. Quickly absorbed from the gastrointestinal tract into the bloodstream and in physiological quantities affects a wide range of metabolic processes[27,28,29], which is associated with antioxidant effects of boron[30]. Boron compounds have anti-inflammatory, hypolipidemic and antitumor actions[31,32], are non-genotoxic[33]; have a beneficial effect on the central nervous system[34]. Boric acid (BA) and borax are the most common form of boron in humans, which are water soluble and bioavailable[35]. According to a number of scientists[36.37.38.39] boric acid, borax have protective effects by modulating the indices of oxidative stress in aluminum-induced hepatotoxicity, titanium, aluminum caused by genotoxicity, thioacetamide induced hepatic insufficiency, and chloride-aluminum-induced neurotoxicity.

The results suggest that boron food supplies have beneficial effects on the central nervous system are among the most favorable assumptions that boron is a useful microelement for humans and give grounds for recommending the use of boron compounds in the violation of the CNS function[34,40]. In vivo and in vitro studies have shown that BA and boron compounds have a protective role against many cancers[35,41]. Boron carriers bind the catalytic site of the 26 S-proteasome and block its activity[42,35]. The proteasome works with a labeled protein called ubiquitin and forms the ubiquitin-proteasome pathway. This pathway plays an important role in the degradation of oxidized, mis-synthesized, damaged and unnecessary proteins in the cell-an important role in degradation of oxidized, incorrectly synthesized, damaged and unnecessary protein in the cell, and plays a key regulatory role in protein groups, involved in intracellular signaling cascades[43,44], and affect the transcription of intracellular signals of processes associated with the permeability and protection of cells and systems[45,46,47]. Consequently, the boron compounds influencing these processes can play the role of a neuroprotective agent. However, according to our information, there is no report on the effects of boron compounds on the brain under conditions of chromic intoxication, i.e. on chromium-induced neurotoxicity.

Based on the foregoing, the purpose of this study is to study the effect of sodium tetraborate on chromium-induced neurotoxicity and oxidative stress.

MATERIALS AND METHODS

The work was performed on 24 male Wistar rats weighing 170-190 g. The animals were in standard conditions in the vivarium of the Central Research Laboratory of the West Kazakhstan State Medical University named after Marat Ospanov (Aktobe, Republic of Kazakhstan) on a standard diet with free access to food and water. The experiments were carried out in accordance with the European Convention for the Protection of Vertebrates used for experimental and other purposes (Strasbourg, 1986). The program of the experiment was discussed and approved by the local ethics commission of the university.

Ten days after acclimatization, animals were randomly divided into 4 groups (6 rats each): Control group: untreated animals.

Experimental group 1

Animals were given a single intraperitoneal injection of potassium dichromate ($K_2Ñr_2O_7$ "Chemistry and Technology" Ltd., Kazakhstan) at a rate of $0,5LD_{50}$.

Experimental group 2

Animals received sodium tetraborate ($Na_2B_4O_7$ "Farmak" Ltd., Ukraine) in drinking water at a rate of 4.0 mg/kg body weight.

Experimental group 3

Animals received sodium tetraborate at a rate of 72 mg/kg of body weight for 10 days and were injected a single intraperitoneal injection of potassium dichromate at a rate of 14 mg/kg ($0,5LD_{50}$) at the last day.

The choice of the type of boron and chromium compounds, doses, methods of administration, and the duration of the experiment are justified by previous studies[48] and literature data[36,37,49]. Euthanization of animals in all groups was performed simultaneously 24 hours after the administration of potassium dichromate by the method of cervical instantaneous decapitation under light ether anesthesia in order to avoid stress.

The brain was rinsed in a cooled physiological solution, dewatered with filter paper, the soft marrow was removed from the blood vessels (cleaned), weighed, homogenized (10% weight/volume) in the appropriate buffer (pH-7,4) and centrifuged at 7000g for 20 minutes.

Peroxide oxidation and antioxidant status. The content of malonic dialdehyde (MDA) in the brain tissues was determined spectrophotometric by the method of Draper, Hedley[50]. The essence of the method: at a high temperature in an acidic medium, MDA reacts with 2-thiobarbituric acid, forming a colored complex with an absorption maximum at 532 nm. The molar extinction coefficient is $1,56*10^{-5}cm.Ì^{-1}$. The MDA level was expressed in nmol/g tissue.

Catalase activity (CAT) was measured according to Koroluk et al.[51]. The reaction is started by adding 2 ml of hydrogen peroxide to 10 ìl of the supernatant and after 10 min it is stopped by adding 1 ml of 4% ammonium molybdate. The sample absorption is measured at 410 nm. The activity of CAT is expressed in moles of H_2O_2 min/g of tissue.

The levels of non-protein thiol (NPSH) were determined by the Ellman method[52]. An aliquot of 500ìl supernatant was mixed with 10% trichloroacetic acid. After centrifugation, the protein precipitate was discarded and the free SH-groups in the clear supernatant were determined. An aliquot of 100 µl of supernatant was added to 850 µl of potassium phosphate buffer 1 M (pH=7.4) and 50 µl of 5,5-dithio-bis-2-nitrobenzoic acid – DTNB (10 mM). The colorimetric reaction was measured at 421 nm. The results were expressed as micromoles per gram of tissue (mmol/g tissue).

The level of glutathione (GSH) was determined by Ellman's method[52] in the modification of Jollow et al.[53] based on the formation of yellow staining, when DTNB (5,5-dithiobis 2-nitrobenzoic acid) is added to the sample containing SH groups. To 0.5 ml of homogenate is added to 3.0 ml of 4% sulfosalicylic acid. The mixture is centrifuged at 1600g for 15 minutes. To 0.05 ml (50 µl) of the supernatant is added the Ellmana reagent. After 10 minutes the extinction was measured at 412 nm. The amount is expressed in µg/g tissue.

The activity of glutathione reductase in the brain was determined by the method of Yawata Y., Tanaka R.[54]. Principle of the method: glutathione reductase (GR) catalyzes the reduction reaction of oxidized glutathione using as the restored equivalent of $NADPH_2$. Reduction of the level of the latter in the test sample is determined on a spectrophotometer at a wavelength of 340 nm. The molar extinction coefficient of NADPH at "$_{340}$= 6,22 ìl'"ñì$^{-1}$. GP activity is expressed in nmol of oxidized NADPH/min/mg protein - (nmol/min/mgPt).

The activity of superoxide dismutase (SOD) was determined by Chevari et al.[55], based on the reduction of nitrotetrazolium by superoxide radicals, which are formed by reaction with phenazinmetasulfate and the reduced form of nicotinamide adenine dinucleotide (NADH). The formation of nitroformazane, the nitrotetrazolium reduction product, is blocked in the SOD environment. The amount of nitroforman is directly proportional to the activity of SOD. The activity of SOD is expressed as a percentage. The protein content was determined by the Lowry et al method[56].

Statistical analysis

Statistical processing of data was carried out using the "Statistica 10" software package of StatSoft, Inc USA. Verification of the null hypothesis that there was no difference between the observed distribution was performed using the criterion of Shapiro-Wilk's W-Test. The differences between the samples were estimated: with a normal distribution of paired variables using Student's t-test and ANOVA in the case of multiple independent variables. The arithmetic mean values M±m of the quantitative indices, represented in the text as M±m, were calculated, where M is the average arithmetic mean, m is the error of the mean. In all statistical analysis procedures, significance level was assumed to be $p<0.05$.

Table 1: The effect of sodium tetraborate on the content of malonic dialdehyde, glutathione and non-protein thiol in the brain with chromium-induced neurotoxicity

Indicators	Groups of animals			
	Control	$K_2Cr_2O_7$	$K_2Cr_2O_7$+ $Na_2B_4O_7$ (4 mg/kg)	$K_2Cr_2O_7$+ $Na_2B_4O_7$ (72 mg/kg)
MDA, nmol/g	151±7.0	$252±16.0^{x}$	$170±14_{0}$	$330±19^{x}{}_{0}$
GSH, µg/g	161±8.0	$93±4.3^{x}$	$142±7.0^{x}{}_{0}$	$74±3.6^{\delta}{}_{0}$
NPSH, µmol/g	1.87±0.07	$1.20±0.09^{x}$	$1.62±0.11^{x}{}_{0}$	$0.96±0.08^{\delta}{}_{0}$

Units: x - $p<0.05$ in comparison with the control data; o - $p<0.05$ in comparison with the data of $K_2Cr_2O_7$

Table 2: Effect of $Na_2B_4O_7$ on the enzyme link of the antioxidant system of the brain with chromium-induced neurotoxicity

Indicators	Groups of animals			
	Control	$K_2Cr_2O_7$	$K_2Cr_2O_7$+$Na_2B_4O_7$ (4 mg/kg)	$K_2Cr_2O_7$+$Na_2B_4O_7$ (72 mg/kg)
CAT, ìmol/min/mg	106±8.0	$71±6.0^{x}$	$120±7.0_{0}$	$81±6.2^{x}$
SOD, %	36±1.4	33±1.2	$40±2.1_{0}$	30±2.0
GR, µmol/min/mg	9.3±0.6	$7.0±0.42^{x}$	$10.4±0.52_{0}$	$7.2±0.651^{x}$

Units: x - $p<0.05$ in comparison with the control data; o - $p<0.05$ in comparison with the data of $K_2Cr_2O_7$

RESULTS

Assessment of lipid peroxidation (LPO) and non-enzymatic link of antioxidant status in the brain. Analysis of the data showed that under the influence of $K_2Cr_2O_7$, there is a significant increase in the LPO in t he brain, as evidenced by MDA levels (Table 1). The content of the latter is increased by 67% in comparison with the data of the control group. Preventive administration of sodium tetraborate in low dose (4 mg/kg MT) leads to a significant decrease in MDA level in the brain by 33%, whereas the use of $Na_2B_4O_7$ in a high dose (72 mg/kg MT) increases the MDA content by 31% with the data of rats of chromic intoxication.

In addition, $K_2Cr_2O_7$ - intoxication significantly reduced the level in the brain GSH by 42%, non-protein thiol by 36%. Preventive use of $Na_2B_4O_7$ at a dose of 4 mg/kg of MT significantly increased the GSH level by 53% in the brain, a non-protein thiol by 35%, and the administration of sodium tetraborate at a high dose (72 mg/kg MT) reduced the GSH content by 23% (ñ<0.05), non-protein thiol by 20% (ñ<0.05) as compared to the data of rats exposed to $K_2Cr_2O_7$.

Enzymatic antioxidant status in the cerebrum

The parameters of the enzyme link of the antioxidant system of the control and experimental groups obtained during the experiments are presented in table 2. Chromium-induced brain damage was accompanied by a significant decrease in CAT activity by 33%, GR by 25%. The activity of SOD remained at the level of control group data. Preventive use of $Na_2B_4O_7$ in a low dose led to a significant increase in CAT activity by 69%, SOD by 21%, GR by 49%, whereas its use in a high dose before chromic poisoning will not cause significant changes in comparison with data of animals, chromium-induced damage to the brain.

DISCUSSION

Chromium causes a wide range of toxicological effects and physiological and biochemical dysfunctions, which involve serious health risks, including neurotoxicity[57,18,11,15]. The present study evaluated the possible consequences of different doses of sodium tetraborate (4 mg/kg and 72 mg/kg MT) on the central nervous system in rats exposed to potassium dichromate intraperitoneally (0,5LD_{50} -14 mg/kg ÌÒ). The brain has a relatively weak system of antioxidant protection, contains a high content of phospholipids, polyunsaturated fatty acids in comparison with other organs, consumes 20% of oxygen in the body, especially susceptible to oxidative stress and, as shown, in previous studies[58,59,13,15] Cr^{+6} can lead to increased LPO in the brain. As established in this study, in fact, the MDA level increased significantly under the conditions of chromic intoxication amid a significant decrease in the level of GSH and NPSH in the brain, reflecting consumption through oxidative stress. There are several ways of depletion of the level of the latter in the conditions of chromic intoxication. First, as shown by Hojo, Satomi[60], GSH can be the source of an electron donor for the conversion of Cr^{+6} to Cr^{+3}. Secondly, the sulfhydryl group of the cysteine of the glutathione fragment has a high potency to metals, forming thermodynamic stable mercaptoid complexes with several metals. Thirdly, GSH and NPSH can be oxidized by interaction with free radicals induced by $K_2Cr_2O_7$.

Preventive use of tetraborate in a low dose led to a significant decrease in MDA and an increase in the content of GSH and NPSH, whereas tetraborate in a high dose, on the contrary, increased the level of MDA. An increase in the level of GSH may be due to the effect of low doses of $Na_2B_4O_7$ on the synthesis of de novo GSH and its regulation, or both. Therefore, in order to counteract the progressive formation of free radicals arising and leading to damage and death of brain neurons when exposed to $K_2Cr_2O_7$, i.e. to prevent its neurotoxicity, it is necessary to maintain the levels of GSH and NPSH.

The damaging role of FRO under the influence of Cr^{+6} is associated not only with their effect on cellular structures, but with the initiation of a cascade of processes leading to damage to cells and cell membranes[20,21], including, can change the permeability of the hemoencephalic barrier (HEB) due to degeneration of endothelial cells and pericytes. On the other hand, the results obtained in this study showed that chromatin intoxication leads to a significant decrease in the activity of CAT, GR. Oral administration of $Na_2B_4O_7$ at low dose before exposure to $K_2Cr_2O_7$ is accompanied by an increase

in the activity of all studied enzymes and the level of GSH and NPSH, in comparison with the data of animals exposed to Cr^{+6}. Preventive use of $Na_2B_4O_7$ in a high dose does not lead to a significant increase in CAT, SOD, GR, and the content of GSH and NPSH, on the contrary, decreases in comparison with the data of rats with chromium-induced brain damage.

Thus, sodium tetraborate in low dose inhibits chromium-induced LPO in the brain, in high, on the contrary, stimulates the SRO lipids. Consequently, tetraborate in a low dose shows an antioxidant effect (neuroprotective effect). It is reported that the toxicity induced by vanadium[61], titanium[37], aluminum[38], arsenic[62] can be prevented by the addition of boron compounds. Recently, we have shown that boric acid (5 mg/kg) when combined orally with potassium dichromate (3 mg/kg) inhibits the development of chromium-induced oxidative stress by inhibiting LPO and increasing the power of antioxidant status[63]. A prophylactic use of sodium tetraborate at a dose of 4 mg/kg MT with chromic intoxication (0.33 LD_{50}) leads to a decrease in MDA level, increased activity of CAT and GSH level, preventing chromium-induced liver damage and genotoxicity of Cr(VI)[64].

Boron (its compounds) under these conditions exhibits an antioxidant property, due to the means to hydroxyl groups[65] and the ability to form diester bridges between cis-hydroxyl-containing molecules. Another mechanism that reduces the toxicity of chromium to neurons in the brain should be sought, obviously, in the increase in the level of GSH and NPSH and activation of antioxidant enzymes[66]. The third mechanism is probably related to the fact that sodium tetraborate in low dose has a stabilizing effect affecting the permeability of the BBB under these conditions[39].

Thus, the preventive use of $Na_2B_4O_7$ at a low dose reduces the peroxidation of brain lipids induced by $K_2Cr_2O_7$ - intoxication, and also increases the activity of antioxidant brain enzymes (SOD, KAT, GR) and increases the capacity of the non-enzyme link (GSH and NPSH) of the antioxidant system. The mechanisms by which sodium tetraborate has a protective effect under conditions of chromium-induced neurotoxicity may be associated with a decrease in the formation of free radicals induced by Cr^{+6}, an increase in the activity of antioxidant enzymes and a level of antioxidant substances, which in turn neutralize SR or through antioxidant effects of direct boron, as a result, the permeability of the blood-brain barrier also decreases.

The use of sodium tetraborate in high dose (72 mg/kg) did not show the expected positive effect; on the contrary, LPO in brain was intensified against a background of low active antioxidant enzymes (KAT, GR) and a low level of GSH and NPSH, which is consistent with the HuQ findings. et al (2014), who observed that a low concentration of boron plays a protective role in the development of the spleen, while a high concentration of boron can damage the organs and produce a toxic effect due to the accumulation of MDA and the development of oxidative stress[67]. Oxidative stress caused by an imbalance between pro- and antioxidant levels[63] can initiate several metabolic and functional dysregulation, which ultimately leads to cell death[68], i.e. oxidative neuronal necrosis.

It is known that FRO forming upon the reduction of Cr^{+6} in Cr^{+3} are mediators of cellular structures[69]. In this regard, it can be assumed that $Na_2B_4O_7$â at low doses, increasing antioxidant capacity, preserving the prooxidant-antioxidant status, inhibits LPO in the brain and thereby prevents (destroys) the destabilization and disintegration of neuronal membranes, stabilizes the permeability of HEB, and as a consequence shows neuroprotective effects. However, $Na_2B_4O_7$â in high dose (72 mg/kg MT) reduces the power of antioxidant status, disrupts pro and antioxidant balance, increases LPO, disintegrates and destabilizes membranes, increases the permeability of HEB, thereby increasing the neurotoxicity of $K_2Cr_2O_7$, i.e. cell membrane in neurons are destroyed and death of brain cells occurs.

CONCLUSIONS

A low dose of sodium tetraborate can protect the brain from chromium-induced damage in rats. However, a high dose of $Na_2B_4O_7$ does not prevent chromium-induced damage in the brain and even increases the neurotoxicity of Cr^{+6}.

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