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Protective Action of Sodium Tetraborate on Chrom-induced Hepato- and Genotoxicity in Rats

IZTLEUOV YERBOLAT MARATOVICH[1]*, KUBENOVA NURGUL NARIMANOVNA[2], ISMAILOVA IRINA VLADIMIROVNA[3] and IZTLEUOV MARAT KAPENOVICH[4]

[1]Department of Radiation diagnostics, Faculty of Medicine,
West–Kazakhstan Marat Ospanov State Medical University, Aktobe, Kazakhstan.
[2]Department of Stomatology, Faculty of Medicine,
West–Kazakhstan Marat Ospanov State Medical University, Aktobe, Kazakhstan.
[3]Department of General medical practice, Faculty of Medicine,
West–Kazakhstan Marat Ospanov State Medical University, Aktobe, Kazakhstan.
[4]Department of Natural sciences, Faculty of Medicine,
West–Kazakhstan Marat Ospanov State Medical University, Aktobe, Kazakhstan.
*Corresponding author E-mail: ermar80@mail.ru

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ABSTRACT

The protective effect of sodium tetraborate on chromium-induced hepato- and genotoxicity was investigated. The experiment was performed on Wistar rats divided into 4 groups: I - control, II - during 5 days received sodium tetraborate (4.0 mg/kg/day) orally, III - once intraperitoneally dichromate potassium (0.33 LD_{50}), IV - preliminarily during five days sodium tetraborate orally and the last administration was combined with a single intraperitoneal injection of potassium dichromate (0.33 LD_{50}). The introduction of potassium dichromate increases the activity of liver marker enzymes in the blood serum, the number of polychromatophilic erythrocytes (PCE) with micronuclei (MN) in the bone marrow, the malonic dialdehyde in the liver tissues, and decreases the catalase activity and glutathione content in the hepatic tissue. In the group receiving sodium tetraborate there is a tendency to decrease in the blood serum the activity of marker liver enzymes, the number of micronuclei in PCE, inhibition of lipid peroxidation (LP) and activation of the antioxidant status in the liver. In the fourth group, the preventive use of sodium tetraborate inhibited the development of cytolysis, cholestasis, LP and had a hepatoprotective, antimutagenic effect.

Keywords: Bichromate potassium, Sodium tetraborate, Cytogenetic disorders, Lipid peroxidation, Antioxidant system, Hepatoprotective action.

INTRODUCTION

Chromium (Cr) is an essential microelement and daily comes to the human body with food in the amount of 50-200 ¼g/day. The valence of chromium (Cr^{+3} or Cr^{+6}) affects the degree of absorption. Cr^{+6} are adsorbed through the lungs and the gastrointestinal tract more easily and intensively than Cr^{+3}. The degree of oxidation and solubility of chromium compounds determines their toxicity. Potassium dichromate ($K_2Cr_2O_7$) (hexavalent form) is widely used in metalworking, leather, textile, chemical, paint and varnish, ceramic, match and pyrotechnic industries[1,2].

The effect of Cr^{+6} on the body has a number of negative consequences, including neurotoxicity, hepatotoxicity, nephrotoxicity, genotoxicity, carcinogenicity and immunotoxicity[3,4,5,6].

Getting inside the cell Cr^{+6} is restored to Cr^{+3} occur, generating active forms of oxygen that cause the oxidation of macromolecules such as DNA and lipids[7,8,9,10,11,12] and induce tissue damage such as liver, pancreas, cerebellum and kidney[13,14,15]. People are professionally, ecologically or internally[16] exposed to high concentrations of Cr^{+6}. The main role in the realization of the damaging effect of oxidative stress is played by the hydroxyl radical, which damages the macromolecules, forms protein crosslinks, facilitates the aggregation and denaturation of proteins, causes the formation of secondary radicals as a result of interaction with low-molecular compounds[12,17]. Boron is a conditionally essential element[18]. In nature it occurs in the form of borates. Boron compounds are used to saturate the surfaces of steel products, in the construction of nuclear reactors, rockets, in the glass and chemical industries, in agriculture, medical institutions, in many cosmetics and personal care products. In medicine, boron compounds (boric acid, borax) have long been used. Boron is rapidly absorbed from the gastrointestinal tract into the bloodstream and in physiological quantities affects a wide range of metabolic processes[19,20,21], which is probably due to the antioxidant effects of boron compounds[22]. Boron compounds possess anti-inflammatory, hypolipidemic and antitumor actions[23], are non-genotoxic[24]. However, the facts of gonadotropic and embryotropic action of boron are noted[25,26,27]. Boron preparations have a therapeutic effect in osteoporosis, arthritis and bone fluorosis. Boron is prescribed at the initial stages of epilepsy development.

Particularly boron compounds compose scientific interest because of an ambiguous and relatively unknown action (mechanism), a role in the treatment of various pathologies. Compounds of boron (boric acid, borax), according to a number of scientists[28,29,30], have protective effects by modulating the indices of oxidative stress in aluminum-induced hepatotoxicity, titanium, aluminum induced genotoxicity and thioacetamide-induced liver failure.

As for as we know, the protective effects of boron compounds in chromium-induced damage of organs and systems, in particular hepatotoxicity, cytogenetic disorders (genotoxicity) have not been studied.

MATERIALS AND METHODS

The work was performed on 24 male rats "Wistar" weighing 170-220 g. The animals were kept in standard conditions in the vivarium of the Central Research Laboratory of the West–Kazakhstan Marat Ospanov State Medical University (Aktobe, Republic of Kazakhstan). The experiments were carried out in accordance with the European Convention on the Protection of Vertebrate Animals used in the experiment[31].

The program of the experiment was discussed and approved by the regional ethics commission of the university.

Animals 10 days after acclimatization were randomly divided into 4 groups (six rats each): Control group: intact animals. Experimental group 1: Animals with drinking water received sodium tetraborate ($Na_2B_4O_7$ "Farmak" Ltd., Ukraine, Kiev, Frunze Street 63) at a rate of 4.0 mg/kg body weight during 5 days. Experimental group 2: Animals were given a single intraperitoneal injection of potassium dichromate ($K_2!r_2O_7$ "Chemistry and Technology" Ltd., Kazakhstan, Almaty, L.Chaikin street 14) at a rate of 0.33 LD50. Experimental group 3: Animals on day 5 of sodium tetraborate at a rate of 4.0 mg/kg of body weight were injected with a single intraperitoneal injection of potassium dichromate at a rate of 9.24 mg/kg bw. (0.33 LD_{50}).

The choice of doses, the methods of administration and the duration of the experiment are justified by the earlier study[32] and according to the literature[28,33]. Euthanasia of animals in all groups was carried out simultaneously 24 hours after the administration of the studied substances by the method of cervical instantaneous decapitation under light ether anesthesia in order to avoid stress.

The blood was collected in EDTA test tubes (Vacutainer tubes from BD Franklin Lakes NJ USA) and centrifuged at 3000 g for 10 minutes. Collected serum samples were stored at -20 ° C until analysis. The liver was washed from the blood, repeatedly perfusing it with a chilled saline solution using a 10 ml thick needle and syringe. The washed liver was placed on an ice-standing Petri dish and ground with

scissors, the homogenate was prepared using 0.1 M potassium phosphate buffer pH 7.4 and centrifuged.

All procedures were performed in a cold room at 0-4 ° C. The hindlimbs of the animals, together with part of the pelvic bones, were separated from the body. The distal part of the femur was selected, leaving the marrow canal closed. Through the proximal part of the bone marrow canal, the contents of the canal were taken with a syringe and mixed with 0.2 ml of serum of the IV (Rh) group, centrifuged (1000 g, 5 min). The supernatant was removed, the pellet was resuspended, and the suspension was used to prepare cytogenetic preparations[34].

Mutagenic and antimutagenic activity was assessed using the method of micronuclei (MN) counting in polychromatophilic erythrocytes (PCE) of bone marrow of rats in vivo, according to the generally accepted method[35]. A smear from the suspension prepared for cytogenetic preparations is stained using Papenheim's method using a May-Grunwald fixation, Giemsa paint[34]. The resulting preparations (two from each animal) are encrypted and subjected to microscopic cytogenetic analysis: 3000 PCE are analyzed from each animal. The positive result obtained is an increase in the number of PCE with micronuclei indicating that the test substance induces chromosomal damage and/or disturbances in the mitotic apparatus of cells in experimental animals[36]. The antimutagenic effect (AME) was calculated by the formula: $AME=(M_1-M_2)/M_1*100$, where M_1 is the number of cells with micronuclei under the action of a mutagen - $K_2Cr_2O_7$; M_2 - the number of cells with MN under the action of $Na_2B_4O_7+K_2Cr_2O_7$.

Biochemical examination

The activity of liver marker enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltranspentidase (GGT) and content of total bilirubin (TB) on the biochemical analyzer "Architect" C 4000 was determined using a standard set of reagents in serum (Abbott, USA).

Peroxide oxidation of lipids and antioxidant status. The content of malonic dialdehyde (MDA) in liver tissues was determined spectrophotometrically by the method of Draper, Hadley[37]. Essence of method a high temperature in an acidic medium, the MDA reacts with 2-thiobarbituric acid to form a colored complex with an absorption maximum at 532 nm. The molar extinction coefficient is $1.56*10^{-5}$ cm $*M^{-1}$. The MDA level was expressed in nmol/g tissue.

Catalase activity (CAT) was measured according to the method[38]. The reaction is started by adding 2.0 ml of hydrogen peroxide to 10 ¼l of the supernatant and after 10 minutes it is stopped by adding 1.0 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 level of glutathione (GSH) was determined by the Ellman method[39] in the modification of Jollow et al.[40] based on the formation of yellow staining, when DTNB (5,5-dithiobis 2-nitrobenzoic acid) is added to a sample containing SH groups. The homogenate in an amount of 0.05 ml is added to 3.0 ml of 4% sulfosalicylic acid. The mixture is centrifuged at 1600 g for 15 minutes. To 0.05 ml (50 ¼l) of the supernatant is added Ellmana reagent. After 10 minutes the optical density of the samples is measured at 412 nm. The amount was expressed as ¼mol/g tissue.

Statistics

Statistical processing of data was carried out using the software package "STATISTICA 10.0" by StatSoft, Inc. USA. Verification of the null hypothesis the absence of no difference between the observed distribution was performed using the Shapiro-Wilk's W-test. Estimation of differences between the samples was carried out: with a normal distribution of paired variables using the Student's t-test and ANOVA in the case of multiple independent variables.

The arithmetic mean values of the quantitative indicators represented in the text in the form M±m were calculated, where M - is the arithmetic average, m - is the mean error. In all statistical analysis procedures, significance level was taken to be pd"0,05.

RESULTS

The data obtained during the experiments indicate that liver damage $K_2!r_2O_7$ is accompanied by the development of the syndrome of cytolysis and cholestasis (Table 1).

Under the influence of $K_2!r_2O_7$, the activity of membrane-bound enzymes in the blood serum increases: AST increases by 182.4%, ALT - by 312.3%, ALP-by 84.4%, GGT-by 91.5%, total bilirubin increases in 2.3 times compared to the data of intact rats. Oral administration of $Na_2B_4O_7$

Table 1: Effect of sodium tatraborate on biochemical indices in the serum of rats with chromium-induced liver damage

Indicators	Groups of animals			
	Control	$Na_2B_4O_7$	$K_2Cr_2O_7$	$K_2Cr_2O_7$ + $Na_2B_4O_7$
AST, U/L	142±9.27	130±7.2	401±10.7*	252±14*$_+$
ALT, U/L	50.2±2.5	47±1.7	207±10.5*	126±5,2*$_+$
ALP, U/L	430±21	400±25	793±52*	585±43*$_+$
GGT, U/L	1.06±0.045	0.9±0.057	2.03±0.09*	1.32±0.007*$_+$
Bilirubin, μmol/l	6.3±0.36	6.0±0.3	14.4±0.7*	9.8±0.63*$_+$

Units: * - p<0,05 in comparison with the control data; + - p<0,05 in comparison with the data of $K_2Cr_2O_7$

Table 2: Effect of $Na_2B_4O_7$ on the content of malonic dialdehyde and the state of the antioxidant system of rats with chromium-induced liver damage

Indicators	Groups of animals			
	Control	$Na_2B_4O_7$	$K_2Cr_2O_7$	$Na_2B_4O_7$+ $K_2Cr_2O_7$
MDA nmol/g	278±16	220±13*	724±37*	464±22*$_+$
CAT mol/g/min	1370±52	1562±54*	873±52*	1187±52*$_+$
GSH mcmol/g	4.5±0.25	5.2±0.33*	3.15±0.21*	3.99±0.3$_+$

Units: * - p<0,05 in comparison with the control data; + - p<0,05 in comparison with the data of $K_2Cr_2O_7$

Table 3: The protective effect of $Na_2B_4O_7$ on chromium-induced cytogenetic disorders of bone marrow cells

Groups	Indicators		
	Number of cells analyzed	Number of cells with micronuclei, ‰	AME, %
Control	3000	2.34±0.21	
I	3000	2.0±0.17	
II	3000	10.33±0.7*	
III	3000	5.13±0.52*$_+$	50.34

Units: * - p<0,05 in comparison with the control data; + - p<0,05 in comparison with the data of $K_2Cr_2O_7$

during 5 days does not cause significant changes in the studied parameters. Whereas, preventive use of sodium teraborate with drinking water for 5 days before intraperitoneal administration of $K_2!r_2O_7$ leads to a decrease in hepatotoxicity. The activity of AST decreases by 37%, ALT by 39%, ALP by 26%, GGT by 35%, content of TB by 32% compared to the data of the group receiving $K_2!r_2O_7$.

Analysis of antioxidant status in rats of experimental groups (Table 2) shows that oral administration of $Na_2B_4O_7$ for five days is accompanied by a significant decrease in the liver of the MDA level by 20.5% against a background of a significant increase in CAT activity. The amount of GSH is increased unreliably (r>0,05). With chromium-induced damage, the MDA content increases 2.6 times, the catalase activity in the liver decreases by 36.3%, the GSH content by 30% compared to the control data. Preventive use of $Na_2B_4O_7$ before chromic intoxication leads to activation of the antioxidant status: the amount of MDA is reduced by 35.8%, the activity of CAT is increased by 36%, and the amount of GSH is increased by 26.7% compared to the data of the group receiving $K_2!r_2O_7$.

The study of the effect of $Na_2B_4O_7$ on chromotopic genotoxicity (Table 3) shows that, oral administration of $Na_2B_4O_7$ for 5 days is accompanied by an inaccurate decrease in the amount of PCE with micronuclei in the bone marrow to 2.0±0.17‰, @e+0.05.

Single parenteral administration of $K_2!r_2O_7$ at the rate of $0.33LD_{50}$ is accompanied by the induction of cytogenetic disorders in the cells of the bone marrow, which is manifested by an increase in the frequency of MN in the PCE of the bone marrow by 4.4 times in comparison with the control data. With the preventive use of $Na_2B_4O_7$ before the introduction of $K_2!r_2O_7$, the amount of PCE with micronuclei significantly decreases and corresponds to 5.13±0.52‰.

This statistically statistically significantly differed from the mutagenic effect of $K_2!r_2O_7$ (@<0,001), and the reduction of the latter was 50.34%.

DISCUSSION

Chromium causes a wide range of toxicological effects and biochemical dysfunctions that involve serious health risks[1,5,12,16]. The results of our experiment on increasing the activity of membrane-binding enzymes (AST, ALT, ALP, GGT) in blood serum and increasing the concentration of total bilirubin testify to liver damage with the development of cytolysis and cholestasis syndrome in animals exposed to potassium dichromate intraperitoneally. A preventive use with drinking water sodium tetraborate reduces the hepatotoxic effects of potassium dichromate, inhibits the leakage of marker enzymes (AST, ALT, ALP, GGT) of the liver, and thereby limits chromium-induced liver damage (hepatoprotective effect).

In this research a significant increase in the level of MDA in the liver against the background of a decrease in hepatic GSH and catalase activity in animals with chromium-induced damage in comparison with the control data indicate the development of oxidative stress, LPO and damage to cellular structures[10,12,14]. Preventive administration of sodium tetraborate led to a significant (pd"0.05) decrease in the amount of MDA in the liver and an increase (pd"0,05) in the concentration of GSH and catalase activity in the liver tissues. Consequently, sodium teeroborate inhibits chromium induced lipid peroxidation activity (antioxidant effect). It was reported that toxicity (oxidative stress) induced by vanadium[41], titanium[29], arsenic[42] can be prevented by addition of boron compounds. Recently, we have shown that boric acid when combined with oral administration with potassium dichromate inhibits the development of chromium-induced oxidative stress by inhibiting LPO and increasing the power of antioxidant status[43]. Boron under these conditions exhibits an antioxidant property due to its affinity for hydroxyl groups[44] and the ability to form diester bridges between cis-hydroxyl-containing molecules. Another mechanism that reduces the toxicity of chromium to liver hepatocytes is evidently in the activation of antioxidant enzymes[45]. Oxidative stress develops when the level of antioxidants is lowered[46], and antioxidants can protect cells from free radical attacks in metal-induced oxidative stress[47]. A positive correlation between antioxidant and antimutagenic

properties of a number of natural compounds was established[48]. At the present time, a sufficient amount of information has been collected on the importance of free radicals (lipid peroxidation) in the mechanisms of induced mutations (cytogenetic effects).

In the present research, the mutagenic and antimutagenic activity of potassium dichromate and sodium terrobate in somatic cells was assessed by the method of micronucleation in PCE of bone marrow of rats in vivo.

The method is recommended as the main one for screening mutagens and antimutagens of the medium, pharmacological and chemical compounds [36] and is included as mandatory in studies of the countries of the European Economic Community and Japan[49].

And we found that under the conditions of preventive use sodium tetraborate causes modulation of chromium-induced mutagenesis in MN in PCE of bone marrow (pd"0,001) and reduction of antimutagenic effect was 50.34%.

The results obtained agree with the data of the authors[30,50] who showed that boron compounds (boric acid, borax, etc.) can reduce genotoxicity under conditions of aluminum-induced and cyclophosphamide-induced oxidative stress. Oxidative stress caused by an imbalance between pro- and antioxidant levels[43] can initiate several metabolic and functional dysregulation, which ultimately leads to cell death[51]. Oxidative stress can be caused either by increased production of free radical oxidation, or by suppression of antioxidant protection. Under the conditions of our study, oxidative stress (chromium-induced) develops through both mechanisms.

When hexavalent chromium is reduced to trivalent, as well as by the mechanisms of Haber-Weiss and Fenton[52], various radicals appear, such as superoxidanion, peroxynitrite, nitric oxide and hydroxyl, which cause damage characteristic of oxidative stress[53], activate LPO, lead to Destabilization and disintegration of cell membranes. Therefore, one of the possible basic approaches used to prevent (correct) chromium-induced ($K_2!r_2O_7$) damage is the use of substances with strong antioxidant properties. Our study shows that it is possible to reduce hepatotoxicity, genotoxicity of chromium compounds by preventive administration of sodium tetraborate.

CONCLUSIONS

Thus, for the first time in the present experiment, it was established for us that sodium tetraborate, upon preliminary administration for 5 days, had a hepatoprotective, antioxidant and antimutagenic effect in chromium-induced liver injury and cytogenetic disorders. This is evidenced by an improvement in the functional state of the liver, inhibition of LPO, activation of antioxidant protection, and a decrease in cytogenetic effects in the body. Preventive use of $Na_2B_4O_7$ reduces the phenomena of cytolytic and cholestatic syndromes, reduces the amount of PCE with micronuclei in the bone marrow. We established that the hepatoprotective and antimutagenic effect of sodium tetraborate in chromium-induced liver damage and the genetic apparatus is due to the inhibition of LPO and the increase in the power of the antioxidant system. This leads to stabilization of hepatocyte membrane structures and improved functioning of membrane-bound enzyme systems of the liver, reduction of cytogenetic disorders in the genetic apparatus of somatic cells of the body.

Consequently, in certain doses, sodium tetraborate is a promising means of preventing (correcting) chromodyne effects in workers of chromium production and population of ecologically unfavorable regions. Our study suggests new discoveries for further study of the biological effects of boron compounds.

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