Effects of Ethylthiosulfanylate and Chromium (VI) on the State of Glutathione Antioxidant System and Oxidative Stress Marker Content in Rat Kidneys

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Abstract

Hexavalent chromium (Cr(VI)) is a heavy metal and powerful toxicant with strong oxidative properties. Antioxidant defense system plays a key role in the processes of elimination and prevention of the negative effects of Cr(VI)-induced oxidative stress in biological systems. Prolonged action of Cr(VI)-induced oxidative stress leads to dysfunction of the antioxidant defense system and as a result provokes cell apoptosis. Ethylthiosulfanylate is synthetic sulfur-containing organic compound that belongs to the class of thiosulfonates. Structurally, thiosulfonates are synthetic analogues of natural organosulfur biologically active substances obtained from garlic, onion, cauliflower and broccoli. Thiosulfonates have antioxidant properties and activate the processes of reactive oxygen species (ROS) utilization. Therefore, the aim of this study was to examine the effect of ethylthiosulfanylate as a synthetic analogue of natural biologically active substances on the state of glutathione antioxidant system and oxidative stress marker content in the kidneys of rats under the condition of Cr(VI)-induced oxidative stress. Our results report that 14 days of ethylthiosulfanylate pretreatment (100 mg/kg body weight) caused attenuation of the intensity of Cr(VI)-induced lipid and protein peroxidation processes ($P < 0.05$). Moreover, previous impact of ethylthiosulfanylate prevented depletion of the total reduced glutathione (GSH) pool after 14 days of potassium dichromate action in kidneys of rats ($P < 0.05$). The present study indicates that ethylthiosulfanylate had antioxidant properties and partially inhibited Cr(VI)-induced oxidative damage in kidneys of rats. The obtained results may become a part of the background for the creation of effective methods of prevention and correction of the antioxidant and pro-oxidant states in kidneys affected by the action of Cr(VI)-induced oxidative stress.

Keywords: rats; antioxidant system; kidneys; oxidative stress; ethylthiosulfanylate; hexavalent chromium
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1. Introduction

Chromium is a silver-gray, lustrous metal that can be in eleven different oxidation states from – IV to + VI. The most common and persistent forms of chromium are Cr(III) and Cr(VI) [1]. Cr(III) is an important trace element for humans and animals and is widely distributed in nature in the form of chromium-containing compounds. Hexavalent Cr(VI) is a heavy metal with high toxicity, carcinogenicity, and mutagenicity, and it causes oxidative stress in the cells of living organisms [2]. Cr(VI) occurs in nature as chromium-containing compounds present in ultramafic rocks or in its ionic form in water. The main sources of hexavalent chromium compounds are anthropogenic activity and industry. Hexavalent chromium compounds are used for industrial purposes in the processes of leather tanning and wood preservatives and finds use in the production of textiles, stainless steel, refractory materials, anti-corrosion coatings, pigments for paints and plastics [1]. Hexavalent chromium is a powerful toxicant and has strong oxidative properties. Reduction of hexavalent chromium to its trivalent form generates a large number of hydroxyl radicals, superoxide anions, hydrogen peroxide, thiol radicals, and chromium salts and as a result activates the peroxidation processes of biomolecules [1]. The kidneys are very sensitive to Cr(VI)-induced oxidative stress. Intraperitoneal action of potassium dichromate at a dose of 20 mg/kg body weight causes a decrease of GSH content and an increase of lipid peroxidation process in rat kidneys [3]. Potassium dichromate exposure at a dose of 15 mg/kg body weight leads to an increase in serum creatinine and urea content, as well as an increase in malondialdehyde (MDA) concentration, myeloperoxidase activity and tumor necrosis factor content in rat kidney tissue. A similar dose of potassium dichromate causes a decrease in melatonin content and damage to renal tubules, and leads to negative histopathological changes in the kidneys of rats [2].

The antioxidant defense system plays a key role in the processes of elimination, prevention and mitigation of the negative effects of Cr(VI)-induced oxidative stress in biological systems [1, 4]. However, the prolonged and intense action of Cr(VI)-induced oxidative stress leads to depletion of antioxidant defense system enzymes and as a result provokes cell apoptosis [5, 6]. In recent years, an urgent task for scientists has been to find compounds with antioxidant properties that have the ability to prevent Cr(VI)-induced depletion of the antioxidant defense system. Biologically active substances with antioxidant, detoxifying and cytoprotective properties can effectively perform the elimination and prevention of the negative effects of Cr(VI)-induced oxidative stress [2, 3]. Vitamin E and atorvastatin pretreatment decreases the intensity of MDA and nitric oxide formation, reduces superoxide dismutase (SOD) activity in rat kidneys, and attenuates the intensity of rat serum creatinine and urea elevation under the action of potassium dichromate oxidative stress [2]. Pycnogenol attenuates the increase of thiobarbituric acid reactive substances (TBARS), MDA, carbonyl group of proteins (CP), and prevents depletion of GSH content and catalase (CAT) activity in rat kidneys under the condition of $K_2Cr_2O_7$ toxicity [7]. The literature also reports that carvedilol and extra virgin olive oil can effectively ameliorate potassium dichromate toxicity due to a decrease of TBARS level and through the restoration of GSH content and the enzymatic activity of glutathione peroxidase (GP), glutathione S-transferase (GST), SOD, and CAT in rat kidneys [8]. The antioxidant activity of ascorbic acid protects rat brain against $K_2Cr_2O_7$-induced peroxidation processes, GSH content depletion and glutathione reductase (GR) activity suppression [9].

Ethylthiosulfanylate is synthetic sulfur-containing organic compound. The ethylthiosulfanylate molecule has the structure RSO_2SR' in which R is an aniline residue and R' is an alkyl residue ($-C_2H_5$) [10]. Ethylthiosulfanylate belongs to the class of thiosulfonates (RSO_2SR'), which are synthetic analogues of organosulfur biologically active substances obtained from garlic, onion, cauliflower and broccoli. Thiosulfonates have antioxidant properties and activate the processes of detoxification and ROS and free radical utilization [11]. Thiosulfonates perform the processes of Nrf2-induced activation of the nqo1 and gstp1 gene promoters, which control the

activity of more than 200 genes responsible for the activation of antioxidant defense system enzymes and free radical scavenging [12]. Thiotaurine (2-aminoethane thiosulfonate), which belongs to the class of thiosulfonates, prevents increase in TBARS level and GSH content depletion in the blood plasma and liver of rats under the action of acetaminophen-induced oxidative stress. The thiotaurine thiosulfonate molecule has the structure RSO_2SR' in which R is an aminoethane residue and R' is a hydrogen atom (-H) [13].

Organosulfur natural analogues of thiosulfonates obtained from garlic extracts activate the mechanisms of hydroxyl radical scavenging and suppression of superoxide anion generation processes [14]. Allicin is one of the main organosulfur components of garlic extracts. Molecules of allicin regulate the activity of nuclear transcription factor (Nrf2), which is responsible for the antioxidant defense system gene expression. Allicin-induced stimulation of Nrf2 leads to the activation of enzymes such as SOD, glutathione GP, GST, NAD(P)H: quinone oxidoreductase 1 (NQO1), gamma-glutamylcysteine synthetase (γ-GCS), hemoxidase (HO)-1 and to the inhibition of Ang II-induced oxidative stress pathways [15, 16].

Thus, nowadays there is enough information describing the effects of thiosulfonates and their natural analogues on the state of the pro/antioxidant system of animals. However, there is little known about the antioxidant properties of thiosulfonates under the condition of heavy metals-induced oxidative stress in the tissues of animals. Due to the important role of thiosulfonates and their natural analogues in maintaining the antioxidant status in living organisms, the purpose of our studies was to investigate the effect of ethylthiosulfanylate as a synthetic analogue of natural biologically active substances on the state of glutathione antioxidant system and oxidative stress marker content in the kidneys of rats under the condition of Cr(VI)-induced oxidative stress.

2. Materials and Methods

2.1 Experimental design

The research was conducted in the vivarium of the Institute of Animal Biology of NAAS on white male Wistar laboratory rats (130-140 g), which were randomly divided into 7 groups with 5 animals per group. Animals of all groups were fed with standard compound feed for laboratory rats with free access to drinking water and feed.

Group I (intact control): were injected daily intraperitoneally with 150 µl of physiological saline solution for 7 days.

Group III/Group IV: received potassium dichromate ($K_2Cr_2O_7$) intraperitoneally at a dose 2.5 mg Cr(VI)/kg body weight per day for 7 days/14 days.

Group II: were injected daily intragastrally with 1000 µl of oil for 14 days («Oleina» oil, traditional: refined, deodorized, frozen; Producer of PJSC with II «DOEP»; certified according to State Standard of Ukraine 4492: 2017 and complies with ISO 14024) and then immediately after that injected daily intraperitoneally with 150 µl of physiological saline solution for 7 days.

Group V: were injected daily intragastrally with an oil solution of ethylthiosulfanylate at a dose of 100 mg/kg body weight for 14 days and then immediately after that were injected daily intraperitoneally with 150 µl of physiological saline solution for 7 days.

Group VI/Group VII: received intragastrally an oil solution of ethylthiosulfanylate at a dose 100 mg/kg body weight daily for 14 days and then immediately after that received $K_2Cr_2O_7$ intraperitoneally daily at a dose of 2.5 mg Cr(VI)/kg body weight per day for 7 days/14 days.

All procedures were made to minimize animal suffering following the guidelines of European Convention “For the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and “Common Ethical Principles for Animal Experiments”

(Ukraine, 2001). Permission to conduct research was obtained from the Committee on Bioethics of Institute of Animal Biology NAAS of Lviv (Protocol № 80). The effects of newly synthesized ethyl 4-aminobenzenethiosulfonate compound were studied on the rat body synthesized at the Department of Technology of Biologically Active Compounds, Pharmacy and Biotechnology of National University "Lviv Polytechnic" according to the protocol described in detail in Lubenets *et al.* [10, 17].

After decapitation of the animals, which occurred under thiopental anesthesia, the kidneys were collected. All procedures on kidneys were performed at 4°C. The research material was the kidney homogenates of rats, which were prepared on 0.05 M Tris-HCl buffer with pH 7.4 in the ratio 1 g of tissue and 9 ml of buffer (1:9, weight/volume) and then centrifuged for 15 min at 1000 g. After centrifugation, obtained supernatants were analyzed for GSH content, peroxidation product level, and antioxidant enzyme activity.

2.1.1 Groups of animals

The groups of animals were compared according to the following scheme (Figure 1). Group I was an intact control in relation to experimental groups III and IV, each of which did not receive an oil solution. Group II was a control in relation to experimental groups V, VI and VII, each of which received an oil solution. We recorded the percentage (%) change in indicators for III and IV experimental groups relative to the group I (intact control). We recorded also the % change in indicators for V, VI and VII experimental groups relative to group II (oil control). At the final stage, we analyzed % change in indicators of III/IV experimental groups relative to the group I (intact control) and compared it with % change in indicators of VI/VII experimental groups relative to the group II (oil control).

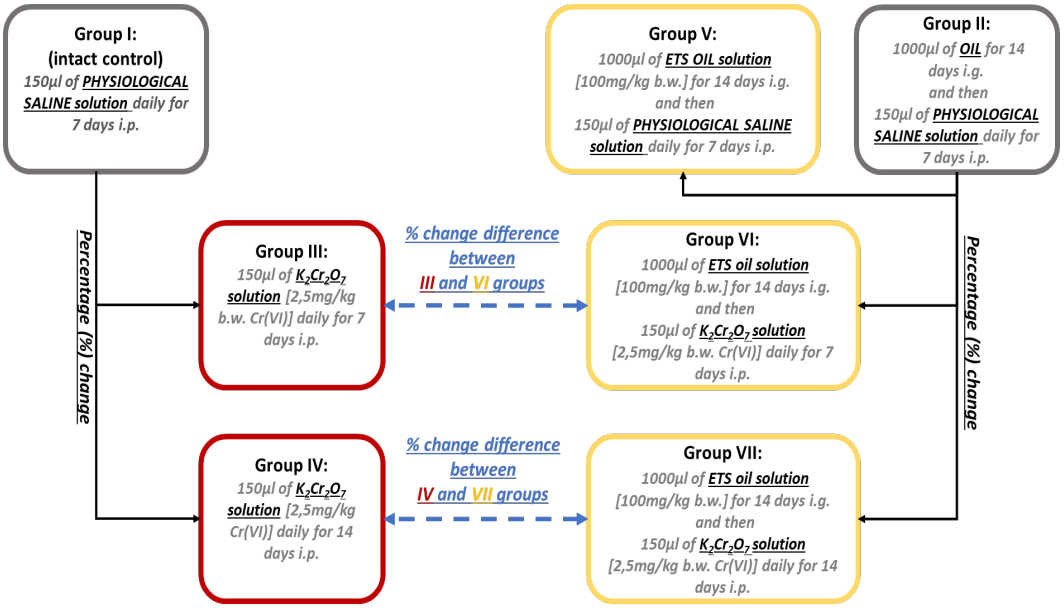

Figure 1. Groups of animals
ETS (ethylthiosulfanylate), i.p. (intraperitoneally), i.g. (intragastrally), b.w. (body weight)

2.2 Processing

2.2.1 Concentration of LHP

The content of LHP (lipid hydroperoxides) was determined according to the principle of precipitation of proteins with trichloroacetic acid solution and lipid extraction by ethanol action [18]. This method is based on the spectrophotometrically measurement of the level of colored product that forms the interaction of the experimental extracts (ethanol extracts of lipids) with ammonium thiocyanate. The absorption was measured spectrophotometrically at λ 480 nm. The concentration of LHP was determined by the difference between values of control and experimental samples and was expressed as SU/g tissue.

2.2.2 Concentration of TBARS

The content of TBARS (thiobarbituric acid reactive substances) in homogenates was determined by color reaction of MDA with thiobarbituric acid (TBA) [18]. The reaction was conducted at high temperature in an acidic environment. The level of colored product (colored complex of one MDA and two TBA molecules) was measured spectrophotometrically at λ 535 nm and λ 580 nm, and the values were expressed as nmol MDA/g tissue.

2.2.3 Concentration of CP

The content of CP (carbonyl group of proteins) was determined by the interaction of the carbonyl groups of amino acids with 2,4-dinitrophenylhydrazine (DNPH) with the formation of 2,4-dinitrophenylhydrazones [18]. The absorption was measured spectrophotometrically at λ 370 nm and the values were expressed in nmol CP/mg protein.

2.2.4 Activity of GP

GP (glutathione peroxidase, EC 1.11.1.9) activity was established by the rate of oxidation of GSH before and after incubation with tertiary butyl hydroperoxide [18]. The intensity of GSH oxidation was determined by the formation of colored product (dinitrophenyl anion) during the interaction of 5,5-dytiobis-2-nitrobenzoic acid (DTNBA) with SH-groups. The absorption was measured spectrophotometrically at λ 412 nm. The activity of GP was expressed in nmol GSH/min.×mg protein.

2.2.5 Activity of GR

GR (glutathione reductase, EC 1.6.4.2) activity was determined in the reaction medium which consisted of 2.5 ml of 0.15 M phosphate buffer (pH 7.4), 0.2 ml of oxidized glutathione (7.5 mM), 0.1 ml of tissue homogenate and 0.1 ml of NADPH (1.2 mM). The enzyme activity was determined spectrophotometrically at λ 340 nm for 1 min at 37°C. The GR activity was calculated using the molar absorption ratio for NADPH at the wavelength of 340 nm and expressed in μmol NADPH/min.×mg protein. The intensity of reaction depends on the tempo of extinction decrease. The principle of this method is based on determining the rate of glutathione reduction in the presence of NADPH [18].

2.2.6 Concentration of GSH

The content of GSH (reduced glutathione) in kidney homogenates was determined according to the principle of measurement of the level of formation of colored product – thionitrophenyl anion as described by Rosalovsky *et al*. [19]. The thionitrophenyl anion formation process is based on the interaction between DTNBA and SH-groups of GSH molecules. The absorption was measured spectrophotometrically at λ 412 nm. The content of GSH was expressed in mmol GSH/g tissue.

2.2.7 Protein concentration

The concentration of total protein in the tissue homogenates was measured by the Lowry method [20] using "Simko LTD" kits (Ukraine, Lviv). The measurements of all absorbance values were performed on a spectrophotometer “Unico” 1205 (USA).

2.3 Statistical analysis

Statistical evaluation of the results was performed using mean values (M) ± standard error (S.E.M), and the variances between groups were tested for significance using one-way ANOVA, followed by Tukey-Kramer test. The differences were statistically significant at $P < 0.05$. All calculations were performed using Microsoft Excel software.

3. Results and Discussion

3.1 Oxidative stress markers

Our results reported that Cr(VI) administration caused an increase in oxidative stress marker levels in rat kidney tissue (Table 1). The content of LHP in the kidneys of animals of III (Cr(VI) 7 days) and IV (Cr(VI) 14 days) experimental groups was significantly increased in comparison with group I (control) by 48 and 57%, respectively. The level of kidney CP under the action of Cr(VI) was significantly elevated by 62% (group III) and 97% (group IV) compared to the group I. We also observed a significantly higher concentration of TBARS in rat kidneys of groups III (Cr(VI) 7 days) and IV (Cr(VI) 14 days) compared to the group I by 15 and 23%, respectively. The content of LHP and CP was significantly elevated in the rat kidney tissue of groups III (Cr(VI) 7 days) and IV (Cr(VI) 14 days) compared to the group II by 48 and 57% (LHP) and 93% (CP), respectively. Transition metals and especially Cr(VI) are catalysts in the processes of oxidative destruction of biological macromolecules. A wide range of ROS (hydroxyl radical, superoxide anion, hydrogen peroxide) are generated during the Cr(VI) reduction to Cr(V), Cr(IV) and Cr(III). The result of ROS generation is an increase of peroxidation processes of lipids and proteins in the kidney tissue. Cr(VI)-induced activation of peroxidation processes causes the formation of oxidative stress products, such as LHP, TBARS and CP [3, 7].

Administration of ethylthiosulfanylate during the 14 days caused decrease of the LHP level in the kidney tissue of rats of group V relative to the group II by 9%. Ethylthiosulfanylate pretreatment by the next action of Cr(VI) led to significant increase of LHP content in the rat kidneys of VI and VII experimental groups compared with the group II by 9 and 22%, respectively. However, the increase of LHP content in the rat kidneys of groups VI (9%) and VII (22%) relative to the group II was by 39 and 35% lower than the percentage increase of LHP concentration in the kidneys of animals of groups III (48%) and IV (57%) compared to the group I.

Table 1. The content of indicators of oxidative stress in kidneys of rats (M±S.E.M., n=5)

Groups of animals	I – Control	II – Oil	III – Cr 7 days	IV – Cr 14 days	V – Ethylth.	VI – Ethylth. + Cr 7 days	VII – Ethylth. + Cr 14 days
LHP. SU/g tissue	0.23±0.004	0.23±0.03***	0.34±0.02***	0.36±0.005***	0.21±0.01***,#	0.25±0.01***,#	0.28±0.01***,#
TBARS. nmol/g tissue	6.29±0.31	6.27±0.13**	7.22±0.58**	7.76±0.16**	5.71±0.25**	5.94±0.73**	6.01±0.05**
CP. nmol/mg prot.	0.39±0.09	0.40±0.03***	0.63±0.08***	0.77±0.07***	0.42±0.03***,#	0.43±0.01***,#	0.56±0.05***,#

Note: Significant difference of II, III, IV, V, VI, VII groups compared to the group I (control) is: **–*** ($P < 0.01 - P < 0.001$); significant difference of V, VI, VII groups compared to the group II is: # ($P < 0.05$).

After 14 days of ethylthiosulfanylate exposure, CP content was not changed in the kidneys of rats of group V compared to the group II. The level of CP by previous influence of ethylthiosulfanylate and the next action of $K_2Cr_2O_7$ was statistically elevated in the kidneys of rats of groups VI and VII in comparison with group II by 8 and 40%, respectively. However, the increase of CP content in the rat kidneys of groups VI (8%) and VII (40%) relative to the group II was by 54 and 57% lower than the percentage increase of CP concentration in the kidneys of animals of groups III (62%) and IV (97%) compared to the group I. The level of LHP and CP was significantly decreased in the rat kidneys of group VI (Ethylth. + Cr(VI) 7 days) compared to the group III (Cr(VI) 7 days) by 26 and 32%, respectively. Significant decrease of LHP and CP concentration was also observed in the kidneys of animals of group VII (Ethylth. + Cr(VI) 14 days) compared to the group IV (Cr(VI) 14 days) by 22, and 27%, respectively.

This may indicate that ethylthiosulfanylate has antioxidant properties. It is known that polysulfides are secondary antioxidants (peroxide scavengers), which are involved in the mechanisms of reduction of lipid hydroperoxides to alcohols by inhibition of the chain reactions of autoxidation. Organosulfur polysulfides have the ability to transform first into sulfoxides and then into sulfenic acid during the processes of lipid hydroperoxide reduction. Sulfenic acid is capable of interacting with lipid hydroperoxides and decomposing them by dehydration [21, 22]. According to the literature, polysulfides and organosulfur compounds have the ability to transform into other sulfur-containing molecules and thiols, such as GSH molecules. [23, 24]. In turn, GSH molecules play an important role in non-enzymatic pathway of hydrogen peroxide scavenging and inhibition of the processes of LHP and CP formation [25].

Therefore, Cr(VI)-induced oxidative stress arises after intraperitoneal potassium dichromate injections and leads to increase of the content of LHP, TBARS and CP (groups III and IV) in rat kidney tissue. Previous intragastric exposure of ethylthiosulfanylate partially inhibits the processes of lipid and protein peroxidation and suppressed the intensity of the formation of LHP and CP in the kidney tissue of animals (groups VI and VII) under the action of Cr(VI).

3.2 Glutathione antioxidant system

The action of Cr(VI) at 7 (groups III) and 14 days (groups IV) led to a significant increase of GP activity in the kidneys of animals compared to the control (group I) by 128 and 43%, respectively (Table 2). The literature data report that Cr(VI) activates the processes of hydrogen peroxide and hydroxyl radical formation, which in turn stimulates lipid peroxidation processes [3, 7]. Kotyzova *et al*. [26] suggest that the increase of LHP level and hydrogen peroxide content from Cr(VI)-induced oxidative stress leads to GP activation in the kidneys of rats. However, GR activity after Cr(VI) exposure in the rat kidneys of III (Cr(VI) 7 days) and IV (Cr(VI) 14 days) experimental groups was significantly decreased compared to the group I by 37 and 41%, respectively. The GSH content after 7 days of potassium dichromate administration was statistically higher by 7% in the kidney tissue of animals of group III in comparison with the group I. This may indicate that the accumulation of cellular GSH in rat kidneys reduces the concentration of ROS and LHP under the condition of Cr(VI) induced oxidative stress after 7 days of $K_2Cr_2O_7$ injection [27]. However, after 14 days of potassium dichromate administration, the level of GSH was statistically lower by 23% in the rat kidneys of group IV in comparison with group I. GSH content was also significantly lower in the kidneys of animals of III and IV groups compared to the group II by 30 and 50%, respectively.

The literature data report that GSH plays a key role in a non-enzymatic process of Cr(VI) reduction to Cr(V) and Cr(IV). The reduction of Cr(VI) by GSH generates GS-Cr(V)/Cr(VI) complexes, which can subsequently be transformed into solids. Utilization of GSH by Cr(VI) reduction may be the reason for the GSH content depletion [1]. The processes of ROS neutralization also cause an active decrease in the level of the GSH molecules [8, 28]. The pathway of new GSH molecules biosynthesis is ATP-dependent [29], and ATP deficiency may cause a decrease in the GSH content in cells [30]. Heavy metal-induced oxidative stress leads to dysfunction of the mitochondrial respiratory chain and as a result inhibition of the processes of mitochondrial ATP synthesis [25]. GR catalyzes the process of GSH recovery in the presence of NADPH. Cr(VI) toxicity leads to decrease in the content of NADPH [1], and this could be the reason for the decrease in the efficiency of the reduction process of GSSG to GSH [31, 32]. GR induces the enzymatic process of NADPH-dependent Cr(VI) reduction to Cr(V) [1]. Cr(VI) action leads to NADPH oxidase (NOX) activation and as a result provokes the more intensive use of NADPH molecules with NOX participation [32]. The reason for the GR depletion under the action of $K_2Cr_2O_7$ may be the disruption of GR enzymatic activity during Cr(VI) reduction and Cr-induced depletion of the GSH pool and NADPH content [9]. Prolonged Cr(VI)-induced loading of GR enzymatic activity may cause depletion of the corresponding enzyme activity. GR induces the enzymatic pathway of Cr(VI) reduction to Cr(V) in the presence of NADPH. The products of Cr(VI) reduction are NADPH-Cr(V) complexes, which can also be transformed into solids [1]. Mehany *et al*. [2] suggest that potassium dichromate exposure inhibits the activity of many antioxidant enzymes. Cr(VI) is able to bind to the SH group of the active site of the enzyme. Cr(VI) action leads to displace of cofactors-metals from the active site and disrupts the activity of antioxidant enzymes and this may be the reason of GR activity depletion under the action of Cr(VI)-induced oxidative stress.

After ethylthiosulfanylate pretreatment for 14 days, the GSH level was significantly increased by 36% in the kidneys of animals of group V compared to group II. The previous impact of ethylthiosulfanylate on the next actin of Cr(VI) for 7 and 14 days also led to significant increase of GSH content in the rat kidneys of VI and VII experimental groups relative to group II by 39 and 38%, respectively. The GSH content was significantly higher in the kidneys of animals of group VI (Ethylth. + Cr(VI) 7 days) compared to the group III (Cr(VI) 7 days) and in the rat kidney tissue of group VII (Ethylth. + Cr(VI) 14 days) compared to the group IV (Cr(VI) 14 days) by 100 and 176%, respectively.

Table 2. Indicators of glutathione antioxidant system in kidneys of rats (M±S.E.M., n=5)

Groups of animals	I – Control	II – Oil	III – Cr 7 days	IV – Cr 14 days	V – Ethylth.	VI – Ethylth. + Cr 7 days	VII – Ethylth. + Cr 14 days
GP. nmol/min.× mg prot.	52.6±1.27	48.02±2.69***	120.05±9.59***	75.36±1.07***	65.68±9.92***	91.77±16.82***	66.08±15.21***
GR. μmol/min.× mg prot.	3.13±0.39	3.19±0.42**	1.96±0.09**	1.87±0.28**	3.05±0.32**	2.78±0.21**	2.12±0.27**
GSH. mmol/g tissue	0.43±0.06	0.66±0.07***	0.46±0.11***	0.33±0.02***	0.9±0.05***,#	0.92±0.09***,#	0.91±0.07***,#

Note: the statistically significant difference II, III, IV, V, VI, VII groups compared to the group I (control) is: **–*** ($P < 0.01 - P < 0.001$); the statistically significant difference V, VI, VII groups compared to the group II is: # ($P < 0.05$).

Thiosulfonates are responsible for the Nrf2-dependent activation of antioxidant defense gene expression that encodes γ-GCS and glutathione synthetase (GS) [15, 16, 29]. The main function of these enzymes is carrying out the processes of GSH molecule biosynthesis. At first stage, γ-GCS induces the synthesis of gamma-glutamylcysteine from L-glutamate and cysteine. Then, GS catalyzes the second stage of GSH molecule biosynthesis from gamma-glutamylcysteine. These two steps are ATP-dependent and play an important role in maintaining the pool of cellular GSH. During biochemical processes, thiosulfonates have the ability to transform into other sulfur-containing compounds, which can be further used as materials for the synthesis of GSH molecules [33].

Therefore, Cr(VI)-induced oxidative stress represents a high load on the glutathione defense system activity and induces an intense activation of GP (III and IV groups), depletion of GR resource (III and IV groups) and decrease of GSH content (group IV) in the rat kidney tissue. The effect of ethylthiosulfanylate without Cr(VI) action, on the contrary, leads to the accumulation of renal GSH (group V). Ethylthiosulfanylate pretreatment partially offsets the negative effect of $K_2Cr_2O_7$-induced oxidative stress and prevents the depletion of the GSH pool in the kidneys of animals (groups VI and VII) under the action of Cr(VI).

4. Conclusions

It is known that biologically active substances with antioxidant properties (atorvastatin, pycnogenol, carvedilol, extra virgin olive oil, ascorbic acid, vitamin E) have the ability to ameliorate Cr(VI)-induced toxicity. Even though there has been little known about the antioxidant properties of thiosulfonates, there is enough information describing the protective properties of thiosulfonates against Cr(VI) toxicity in tissues of animal organism. Our results indicate that ethylthiosulfanylate pretreatment may be effective in correcting Cr(VI)-induced oxidative stress. We assume that further studies of the antioxidant properties of ethylthiosulfanylate are important for the better understanding of the role of thiosulfonates in the mechanisms of prevention of heavy metals-induced oxidative stress.

In general, the obtained results report that potassium dichromate causes Cr(VI)-induced oxidative stress and leads to disbalance of glutathione antioxidant defense system mechanisms. Pretreatment with ethylthiosulfanylate partially eliminates the negative effects of Cr(VI)-induced oxidative stress, attenuates the intensity of peroxidation processes and prevents depletion of GSH content under the condition of Cr(VI)-induced oxidative stress. Ethylthiosulfanylate administration also leads to accumulation of the total GSH pool in rat kidneys.

We hypothesize that a possible mechanism for preventing Cr(IV) toxicity may be related to ethylthiosulfanylate-induced GSH accumulation in rat kidneys. GSH accumulation is very important under the condition of Cr(VI)-induced oxidative stress. GSH molecules initiate the reduction of toxic Cr(VI) compounds to a non-toxic form – Cr(III). GSH molecules are also involved in the enzymatic reduction of LHP and non-enzymatic neutralization of ROS. Therefore, it can be assumed that the ethylthiosulfanylate-induced accumulation of GSH may contribute to inhibition of peroxidation processes, suppression of ROS formation, and stimulation of Cr(VI) reduction processes under the condition of $K_2Cr_2O_7$ toxicity. We hypothesize also that a possible mechanism for preventing Cr(IV)-induced oxidative stress may be related to ethylthiosulfanylate-induced decreasing in the intensity of lipid peroxidation processes in rat kidneys. Literature data report that thiosulfonates are able to react directly with free radicals. Organosulfur compounds and polysulfides are involved in the processes of LHP reduction and decomposition. It is possible that the above antioxidant properties of organosulfur compounds can be used to explain the possible mechanisms for the prevention of Cr(VI)-induced oxidative stress with the participation of ethylthiosulfanylate.

In the following studies, we plan to determine the additional indicators needed to better understand the antioxidant properties of ethylthiosulfanylate. In particular, we plan to investigate the effect of ethylthiosulfanylate on the enzymatic activity of γ-GCS (gamma-glutamylcysteine synthetase), GS (glutathione synthetase), GST (glutathione S-transferase), SOD and CAT. We are also interested in the measuring of H_2O_2 concentration and level of Cr(VI) accumulation in rat tissues. Furthermore, we plan also to combine the action of ethylthiosulfanylate with other effective protectors against Cr(VI) toxicity. It is known that vitamin E has a positive antioxidant effect and reduces the intensity of Cr(VI)-induced oxidative damage. Perhaps the combined effect of ethylthiosulfanylate with vitamin E will increase the effectiveness of counteraction of Cr(VI)-induced oxidative stress.

Our results indicate that ethylthiosulfanylate pretreatment partially stabilized Cr(VI)-induced disturbance in the mechanisms of the antioxidant defense system action in rat kidneys. Moreover, the results of our study may become a part of the background for creation of effective methods of prevention and correction of the antioxidant and pro-oxidant states in kidneys affected by the action of Cr(VI)-induced oxidative stress.

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