Biol Trace Elem Res (2016) 174:387–391
DOI 10.1007/s12011-016-0733-0

The Therapeutic Role of Glutathione in Oxidative Stress and Oxidative DNA Damage Caused by Hexavalent Chromium

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Received: 31 March 2016 / Accepted: 2 May 2016 / Published online: 11 May 2016

Abstract Hexavalent chromium Cr (VI) causes various toxic and carcinogenic effects. The main carcinogenic effect is observed in the pulmonary system through inhalation route. Reduction of Cr (VI) to Cr (V, IV, and III) reactive intermediates within the cells by intracellular reducing agents such as glutathione is an important event leading to oxidative stress and oxidative DNA damage. This study evaluated the effects of intraperitoneal administration of Cr (VI) and GSH on total oxidant status (TOS), total antioxidant capacity (TAC), oxidative stress index, and oxidative DNA damage by evaluating the level of 8-hydroxy-2-deoxyguanosine (8-OHdG) in Swiss-Albino mice. Seventy two mice were divided into 6 groups and treated intraperitoneally as follow: control (saline), group GSH (30 mg/kg GSH) groups of Cr-20 (20 mg/kg, $K_2Cr_2O_7$), Cr-30 (30 mg/kg $K_2Cr_2O_7$), Cr-20 + GSH (20 mg/kg $K_2Cr_2O_7$ + 30 mg/kg GSH), Cr-30 + GSH (30 mg/kg $K_2Cr_2O_7$ + 30 mg/kg GSH). Total oxidant capacities of Cr-20 and Cr-30 were increased compared to control, Cr-20 + GSH, and Cr-30 + GSH. TOS levels in Cr-20 + GSH and Cr-30 + GSH were lower than in Cr-20 and Cr-30. No difference in TAC was observed among the groups. 8-Hydroxy-2-deoxyguanosine levels were increased in groups Cr-20 and Cr-30 compared with control and groups Cr-20 + GSH and Cr-30 + GSH. No difference was determined in 8-OHdG levels among control, groups GSH, Cr-20 + GSH and Cr-30 + GSH. Results indicate that Cr (VI) given i.p. route causes increased oxidative stress and oxidative DNA damage in the blood of Swiss-Albino mice. Administration of GSH via i.p. route protects from oxidative stress and DNA damage.

Keywords Hexavalent chromium · GSH · Oxidative stress · DNA damage

Introduction

Hexavalent chromium [Cr (VI)] is an environmental toxicant and carcinogen [1, 2]. The most common form of chromium is the trivalent form [Cr (III)] which is mainly found in chromite ore and occurs naturally. Hexavalent chromium is mainly produced as a result of industrial activities including chrome plating, stainless steel machining, welding, and leather tanning. Cr (III) is considered to have beneficiary effects in the biological system due to its role in glucose and lipid metabolisms [3]. However, the next stable oxidation state of Cr (VI) is the toxic form and has been associated with a number of toxicities including respiratory toxicity, hepatotoxicity, dermatitis, gastroenteritis, reproductive, and renal toxicity [1, 4, 5]. A great deal of epidemiological and clinical studies has also indicated that Cr (VI) compounds are associated with the high incidence of lung cancer among workers exposed to Cr (VI) by inhalation [3, 6]. The carcinogenic effect of Cr (VI) in the respiratory system has been well established, and Cr (VI) was classified as a class I carcinogen by the International Agency for Research on Cancer [7]. Ingestion of Cr (VI) through oral route has been also reported to induce gastro intestinal carcinogenicity in mice and rats [8, 9]. A recent meta-analysis also indicates that Cr (VI)

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is also a stomach carcinogen in humans [10]. The difference in the mechanism of toxicity between Cr (III) and Cr (VI) is attributed to the fact that Cr (VI) compounds are found as an oxyanion $(CrO_4)^{-2}$ that closely resembles cellular sulfate and phosphate anions, and therefore, it is actively transported into cells via non-specific anion transporters. Inside the cell, Cr (VI) is reduced to Cr (V), Cr (IV), and ultimately to Cr (III) by intracellular reducers such as ascorbic acid, glutathione (GSH) and cysteine. In contrast to Cr (VI), Cr (III) cannot readily cross the cellular membranes. However, reduced forms of chromium compounds inside the cell serve as reactive intermediates leading to generation of reactive oxygen species such as free radicals, superoxide anions, and hydroxyl radical in a Fenton-like reaction with hydrogen peroxide [11–14]. The reactive intermediates and free radicals are capable of reacting with cellular proteins and lipids leading to cytotoxicity. Interaction of ROS with cellular DNA could result in DNA damage, genotoxicity, and mutagenicity. Types of alterations in DNA include formation of DNA adducts, DNA-strand breaks, DNA-protein cross-links, oxidized bases, and DNA-inter and intra cross-links [15–18]. 8-Hydroxy-2-deoxyguanosine (8-OHdG) which is a widely accepted indicator of oxidative DNA damage was found to be high in various Cr (VI) toxicity studies [7].

Various toxic effects including carcinogenic and genotoxic effects of Cr (VI) are reported to be associated with generation of reactive oxygen species due to intracellular reduction by cellular reducers such as ascorbic acid, glutathione (GSH), and cysteine [15, 19, 20]. It has been reported that thiol containing amino acids and peptides may play a role in chromium genotoxicity and carcinogenity by forming Cr (III)-GSH-DNA adducts [7, 14, 20]. However, the exact role of reducing agent in Cr (VI) toxicity is not yet fully understood. Furthermore, toxic effects of Cr (VI) could vary with route of exposure. Due to extracellular reduction of Cr (VI) to Cr (III) by bodily fluids such as gastric juice, saliva, and intestinal bacteria, oral exposure may not be as toxic as inhalation routes. Inhalation of Cr (VI) causes lung cancer and neurotoxicity, while oral exposure may lead to gastrointestinal, dermatological, and hematological and biochemical adverse effects [3]. In addition, the toxic effects of Cr (VI) were mostly carried out in vitro cell cultures or in common route of exposure such as inhalation or oral routes.

In this study, the effects of intraperitoneal administration of GSH on acute Cr (VI) toxicity were studied in Swiss-Albino mice. The level of oxidant and antioxidant status as well as oxidative DNA damage were evaluated by measuring total oxidant status (TOS), total antioxidant capacity (TAC), GSH, and 8-OHdG levels.

Material and Methods

In this study, 3–5-month-old 72 female Swiss-Albino mice weighing 30–35 g were used as the animal material. Animals were maintained at normal room temperature (20–25 °C) and 12/12 h night/day cycle for the duration of the experiment. Animals were fed with standard pelleted diet ad libitum. Permission for the use of animals was obtained from the Kafkas University Local Ethics Committee (Permission No. 2012–79). In the study, animals were divided into 6 groups each containing 12 mice. All applications were performed intraperitoneally (i.p.). Hexavalent chromium was applied as potassium dichromate ($K_2Cr_2O_7$) to the corresponding groups via i.p. route. Groups were designed as follows: control (% 0.9 saline, 0.1 ml), group Cr-20 [20 mg/kg of Cr (IV)], group Cr-30 [30 mg/kg of Cr (IV)], group Cr-20 + GSH (20 mg/kg + 30 mg/kg GSH), and group Cr-30 + GSH (30 mg/kg + 30 mg/kg GSH). Reduced glutathione was injected to corresponding groups 3 days before the application of $K_2Cr_2O_7$. Twenty four hours after administration, blood samples from the animals in groups were taken for the determination of GSH, TAC, TOS, and oxidative stress index (OSI) as well as 8-hydroxy-2-deoxyguanosine (8-OHdG) levels. Anti-coagulated blood samples from the tubes were centrifuged for 10 min at 3000 rpm. The plasma samples were separated and put into the polyethylene tube and stored at −20 °C, until the analyses are performed. Plasma glutathione (GSH) was determined (Cayman Chemical Company, USA, Glutathione assay Kit Cat No: 703002), and 8-OHdG was determined by commercial kit (Cayman Chemical Company, USA, DNA/RNA oxidative damage ELISA Kit Cat No: 589320) and total antioxidant capacity and total oxidant status were determined (Rel Assay Diagnostics, Turkey) by spectrophotometry using a commercial kit. Oxidative stress index for each group was calculated using TAC and TOS values in the groups. Initially, the unit of TAC, millimoles of Trolox per liter, was converted to micromoles per liter, and the OSI value was calculated by dividing TOS to TAC, and the resulting value was multiplied by 100.

Statistical Analysis

The data obtained from the analyses were expressed as mean ± SEM. Statistical analyses of data were performed using SPSS 12.0. Data were initially tested for normality by Kolmogrov Smirnov test. Then the data were tested with ANOVA which is followed by post-hoc Tukey test. *P* values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

To determine the effects of Cr (VI) and role of GSH on Cr (VI) toxicity, Cr (VI) at 2 different doses (20 and 30 mg/kg) was given via i.p. route to Swiss-Albino mice. Total oxidant status in group Cr-20 and Cr-30 were significantly increased compared to that of control. There was no significant difference between group Cr-20 and group Cr-30 in terms of total oxidant capacity. In groups treated with both Cr and GSH (groups Cr-20 + GSH and Cr-30 + GSH), level of total oxidant capacity was found to be similar to that of control, but TOS in groups Cr-20 + GSH and Cr-30 + GSH were significantly lower than in groups Cr-20 and Cr-30. There was also no difference in TOS between groups Cr-20 + GSH and Cr-30 + GSH. When total antioxidant capacities were compared, no significant difference was determined among the groups. OSI values in groups Cr-20 and Cr-30 were higher than the other groups, while OSI values in Cr-20 + GSH and Cr-30 + GSH were not different from control. The results of TAC, TOS levels and OSI index obtained from the groups were presented in Table 1.

Except for group GSH, blood GSH levels among the groups were not significantly different and were found to be similar. For the evaluation of oxidative DNA damage induced by Cr (VI), 8-hydroxy-2-deoxyguanosine (8-OHdG) levels in the groups were also determined. 8-Hydroxy-2-deoxyguanosine levels were significantly increased in groups Cr-20 and Cr-30 compared with control and groups Cr-20 + GSH and Cr-30 + GSH. No significant difference was determined in 8-OHdG levels among control, groups GSH, Cr-20 + GSH, and Cr-30 + GSH. GSH and 8-OHdG levels obtained from the groups were presented in Table 1.

Discussion

The purpose of this study was to evaluate the effects of intraperitoneal injection of Cr (VI) and GSH on oxidant and antioxidant status as well as oxidative DNA damage in Swiss-Albino mice. Results indicated that Cr (VI) at 2 different doses (20 and 30 mg/kg) enhanced oxidative response as evidenced by increased blood total oxidant capacity in Cr (VI) only groups (at 20 and 30 mg/kg) compared to control, Cr-20 + GSH, and Cr-30 + GSH groups. On the other hand, GSH treatment in groups Cr20 + GSH and Cr30 + GSH normalized the TOS levels to the control levels indicating that GSH treatment prevents the Cr (VI) induced oxidative stress as evidenced by lower TOS levels in these groups. Total antioxidant capacity and blood GSH levels in all groups (except for GSH level of group GSH) were similar and showed no difference. TAC is suggested to be an appropriate parameter to evaluate antioxidant status of serum, and measuring TAC level is more advantageous than measuring individual antioxidant elements since TAC gives the measurement of all known and unknown antioxidants and their synergistic interaction in the biological sample studied [21]. Oxidative stress index calculated from TAC and TOS levels together is also reported to be a better indicator for oxidative stress [22], and calculated OSI obtained from the groups shows similar results to TAC and TOC comparisons among the groups. Oxidative stress results from excessive production of reactive oxygen species and free radical generation. Normally, ROS produced during cellular events are scavenged by cellular antioxidant system. However, antioxidant defense system could be overwhelmed by increased generation of ROS as a result of toxic compounds. The antioxidants could be used up by cellular oxidants and decrease the level of antioxidant available [23, 24]. It is well known that Cr (VI) is easily taken up by the cells via anion exchangers and reduced to reactive intermediates Cr (V), (IV) and ultimately Cr (III) radicals by cellular reducers within the cell [14, 25]. These reactive intermediates could directly cause oxidative damage and generate reactive oxygen species via Fenton-like reaction with H_2O_2 leading to hydroxyl radical production [26]. Earlier studies demonstrated that oral administration of Cr (VI) caused increased lipid peroxidation and malondialdehyde (MDA) level along with GSH depletion [11, 27]. Wang et al. reported that both short-term and long-term exposure to Cr (VI) resulted in rapid increase in ROS in Beas-2 B cells [28]. Similarly, acute and subacute in vivo Cr (VI) toxicity studies showed that Cr (VI) lead to increased superoxide anion production, MDA level, but it caused decreased level of catalase in the rat testis and several other antioxidant enzymes in the rat liver [4, 29]. There was no difference in TOS levels between Cr-20 and Cr-30 groups. It

Table 1 Effects of Cr (VI) and GSH on blood total oxidant status (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), glutathione (GSH) and 8-hydroxy-2-deoxyguanosine (8-OHdG) levels in Swiss-Albino mice

Groups	TOS (μmol H_2O_2 equiv/L)	TAC (mmol Trolox equiv./L)	OSI (arbitrary unit)	GSH (μmolar)	8-OHdG (pg/ml)
Control	8.6 ± 0.6^{a}	0.72 ± 0.04^{a}	1.30 ± 0.14^{a}	24.94 ± 0.47^{a}	2733.20 ± 111.32^{a}
GSH	9.5 ± 0.9^{ab}	0.73 ± 0.03^{a}	1.45 ± 0.15^{a}	26.82 ± 0.46^{b}	2951.35 ± 27.94^{a}
Cr-20	13.4 ± 1.7^{bc}	0.65 ± 0.05^{a}	2.41 ± 0.27^{b}	25.15 ± 0.22^{a}	3993.30 ± 127.43^{b}
Cr-30	14.6 ± 1.7^{c}	0.60 ± 0.08^{a}	2.75 ± 0.42^{b}	24.90 ± 0.23^{a}	3566.86 ± 106.00^{b}
Cr-20 + GSH	7.1 ± 0.7^{a}	0.69 ± 0.04^{a}	1.11 ± 0.15^{a}	24.65 ± 0.21^{a}	3040.98 ± 68.09^{a}
Cr-30 + GSH	8.5 ± 0.9^{a}	0.61 ± 0.02^{a}	1.42 ± 0.20^{a}	24.72 ± 0.21^{a}	3034.09 ± 24.12^{a}

Different letters within the same column indicate significant differences (p 0.05) appears that administration of Cr (VI) at 20 and 30 mg/kg for 3 consecutive days makes no difference in oxidant status. This may be due to short duration of administration (as in this study 3 days). It could differ if the duration of exposure to the toxic substance is increased.

In the present study, we evaluated the effect of i.p. injection of GSH and Cr (VI) on oxidative DNA damage by determining 8-OHdG levels among the groups. 8-Hydroxy-2-deoxyguanosine is a widely accepted biomarker of oxidative DNA damage and can be also used to evaluate the degree of oxidative stress [30]. It was determined in this study that Cr (VI) at 2 different doses increased 8-OHdG levels in groups Cr-20 and Cr-30 compared to control, Cr-20 + GSH, and Cr-30 + GSH, indicating Cr (VI)-induced oxidative DNA damage. No difference was observed between Cr-20 + GSH, Cr-30 + GSH and control in 8-OHdG levels. It appears that GSH given i.p route has a protective role against Cr (VI) treatment. It is considered that extracellular reduction of Cr (VI) by extracellular reducers is a main detoxification event when given via oral route in the stomach leading to decrease in toxicity and carcinogenicity [3]. GSH given via i.p route may be involved in the extracellular reduction of Cr (VI) to Cr (III) which is unable to enter cell due to weak permeability of the cell membranes to this trivalent form. On the other hand, chromium in its hexavalent form can easily enter the cells and be reduced to Cr (III) by intracellular ascorbic acid, GSH or cysteine. It has been reported that cellular reducers such as GSH, ascorbic acid, and cysteine may enhance DNA-protein cross-links and cytotoxicity induced by Cr (VI) [6, 15]. However, increased or decreased GSH level in the cells can change the genotoxicity, carcinogenicity, and cytotoxic effects. Buthionine sulfoximine induced depletion of GSH enhances the nephrotoxicity in mice, but protects from Cr (VI)-induced mutagenesis in mice and also protects from nephrotoxicity in rat [7, 31, 32]. Stearns et al. reported that the reactive intermediates of Cr (VI) were different in different buffers indicating that the buffer used in vitro could alter the reactive intermediates and depend on their intracellular metabolism [18]. Izzotti et al. reported that Cr (VI) exposed rats showed increased DNA fragmentation, nucleotide modifications and elevated 8-OHdG level, and treatment of rats with cysteine and a GSH precursor, N-acetyl cysteine, prevented these effects [33]. Reactions of Cr (VI) with ascorbate and GSH result in different reaction rate and reactive intermediates. Ascorbate is responsible for the 80–90 % reduction of Cr (VI) in vivo. It is kinetically favored reducing agent compared to GSH and shows 10-times faster reducing capacity than GSH. More importantly, the type of reducing agent in the reduction process of Cr (VI) prominently affects the formation of reactive intermediates produced. Reduction of Cr (VI) by ascorbate yields Cr (IV) intermediate via two-electron reaction, while reduction of Cr (VI) by GSH generates Cr (V) intermediate via one or two electron reaction [3]. Although these intermediates are ultimately reduced to Cr (III), reactions by each reactive intermediate of Cr (IV) and Cr (V), and Cr (III) with cellular ligands show differences [6, 34]. Cr (V) generated via GSH reduction could directly oxidize DNA bases (guanine) to GH and SH by electron abstraction and addition of O_2 leading to 8-oxodG, oxidized bases as well as lesions including DNA adducts, DNA single strand breaks and abasic sites and suggested to be the major DNA damaging agent with genotoxic and carcinogenic form of chromium [7, 35]. It has been reported that increased intracellular level of GSH reduces the DNA strand break, but depletion of intracellular GSH reduces DNA strand break indicating that the levels of Cr (VI)-induced DNA strand breaks are correlated with the levels of Cr (V) produced [36]. Moreover, generation of hydroxyl radicals and amount of oxidants is reported to be associated with Cr (V) during the intracellular reduction of Cr (VI) by GSH [14, 37]. Wong et al. have shown that ascorbate reduction of Cr(VI) generated 25–80 times lower yields of oxidants than GSH indicating that reduction of Cr(VI) by ascorbate produces low amounts of oxidants compared to reactions with GSH [37]. Sugiyama et al. reported that pretreatment of Chinese hamster V79 cells with ascorbate in Cr (VI) toxicity decreased the level of Cr (V) but increased the Cr (III) intermediates. Ascorbic acid also decreased alkali-labile sites, but it enhances DNA-protein cross-links and cytotoxicity [38]. Altogether, chromium toxicity could be affected by many factors such as exposure route, amount of exposure, type, and cellular level of reducer [3, 13].

In conclusion, findings of the present study indicate that Cr (VI) given as $K_2Cr_2O_7$ via i.p. route causes increased oxidative stress and oxidative DNA damage in the blood of Swiss-Albino mice. Administration of reduced glutathione via i.p. route appears to protect from oxidative DNA damage.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interests, and that this publication has been approved by all co-authors.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of Kafkas University or practice at which the studies were conducted. All animal testing was conducted after permission for the use of animals was obtained from Kafkas University Local Ethics Committee (permission no. 2012–79).

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