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Beneficial Effects of N-Acetyl-L-cysteine or Taurine Pre- or Post-treatments in the Heart, Spleen, Lung, and Testis of Hexavalent Chromium-Exposed Mice

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Abstract

Hexavalent chromium[Cr(VI)] compounds may induce toxic effects, possibly via reactive intermediates and radicals formed during Cr(VI) reduction. In this study, we probed the possible effects of N-acetyl-L-cysteine (NAC) and taurine pre- or post-treatments on Cr(VI)-induced changes in lipid peroxidation and nonprotein thiols (NPSH) in mice heart, lung, spleen, and testis tissues. The mice were randomly assigned to six groups, consisting of control, Cr(VI)-exposed (20 mg Cr/kg, intraperitoneal ,ip), NAC (200 mg/kg, ip) as pre-treatment and post-treatment, and taurine (1 g/kg, ip) pre-treatment and post-treatment groups. Lipid peroxidation and NPSH levels were determined and the results were compared with regard to tissue- and antioxidant-specific basis. Exposure to Cr(VI) significantly increased lipid peroxidation in all tissues as compared to the control ($p < 0.05$); and consistent with this data, NPSH levels were significantly decreased ($p < 0.05$). Notably, administration of NAC and taurine, either before or after Cr(VI) exposure, was able to ameliorate the lipid peroxidation ($p < 0.05$) in all tissues. In the case of NPSH content, while the decline could be alleviated by both NAC and taurine pre- and post-treatments in the spleen, diverging results were obtained in other tissues. The effects of Cr(VI) on the lung thiols were abolished by pre-treatment with NAC and taurine; however, post-treatments could not exert significant effect. While thiol depletion in the heart was totally replenished by NAC and taurine administrations, NAC pre-treatment was partially more effective than post-treatment. In contrast with lipid peroxidation data, NAC treatment could not provide a statistically significant beneficial effect on NPSH content of the testis, whereas the effect in this tissue by taurine was profound. Thus, these data highlight the importance of tissue-specific factors and the critical role of administration time. Overall, our data suggest that NAC and taurine may have potential in prevention of Cr(VI)-induced toxicity in the heart, lung, spleen, and testis tissues.

Keywords Hexavalent chromium · N-Acetyl-L-cysteine · Taurine · Heart · Spleen · Lung · Testis

Introduction

Chromium (Cr) is a transition metal with several oxidation states. Trivalent chromium-Cr(III) and hexavalent chromium-Cr(VI) compounds differ with respect to toxicokinetics and toxicity [1]. From a toxicological perspective, since Cr(VI) is highly reactive in biological systems and far more toxic than Cr(III), Cr(VI) compounds have been accepted more important. Cr(III) had been postulated as an essential element for many years; however, EFSA Panel concluded that there is no convincing evidence for a possible role in specific biological functions in healthy humans and animals [2]. Moreover, the safety of Cr(III) compounds has been extensively investigated [3]. Cr(VI) compounds are occupational carcinogens associated with increased lung cancer risk [4]. Toxicity due to occupational [5], environmental [6], and rarely accidental [7] or intentional [8] exposure to Cr(VI) are well established. Toxic effects of Cr(VI) compounds have been shown in diverse targets including the liver [9], kidney [10], heart [11], lungs [12], spleen [13], and reproductive organs [14, 15].

Cr(VI)-induced cytotoxicity mechanism has yet to be fully illustrated; however, extensive evidence has shown the involvement of oxidative stress [16, 17] and reactive species

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generated during Cr(VI) reduction [18, 19]. Additionally, regarding the role of reduced glutathione (GSH), it has been proposed that Cr(VI) toxicity may be enhanced via reactive Cr(V) generation in low GSH concentration, and that high GSH content may enhance formation of Cr(III)-GSH complex [20]. The beneficial effects of various agents including ascorbic acid [21], NAC [22], taurine [9], and others [23, 24] against Cr(VI)-induced toxicity have been highlighted, underlining this mechanism of toxic effect.

NAC is primarily used in the clinic mainly as a mucolytic agent in treatment of pulmonary diseases [25], as well as in other indications including acetaminophen overdose [26] and contrast-induced nephropathy [27]. NAC is a favorable source of L-cysteine which has been suggested to regulate diverse pathways, e.g., oxidative stress via the production of GSH and taurine [28]. As compared with cysteine, NAC has advantages such as tolerability, water solubility, and less susceptibility to oxidation [29].

Taurine (2-aminoethanesulfonic acid)—synthesized from cysteine—is a semi-essential amino acid for human, and is distributed in various tissues with diverse cytoprotective activity including regulation of oxidation and calcium homeostasis [30]. Interestingly, oxidative stress induced by Cr(VI) in welders has been suggested to promote urinary excretion of taurine and some other metabolites [31]. Its beneficial effects against cardiotoxicity induced by iron [32], cisplatin [33], and cyclophosphamide [34] have been documented. Additionally, studies have shown protective effects of taurine in the lung [35], spleen [36], and testis [37].

In this preliminary study, our aim was to investigate the effect of acute Cr(VI) exposure in mice heart, lung, spleen, and testis tissues regarding lipid peroxidation and nonprotein sulfhydryls (NPSH), and to evaluate the possible attenuation of these parameters by pre- or post-treatment of NAC and taurine. In this vein, content of thiobarbituric acid reactive substances (TBARS) as a reflection of lipid peroxidation was investigated, and nonprotein thiols (NPSH) level as a constituent of non-enzymatic antioxidants was probed. The findings were compared especially in terms of pre- and post-treatments of NAC and taurine as well as tissue-specific data and discussed with current literature.

Materials and Methods

Chemicals

Potassium dichromate ($K_2Cr_2O_7$), NAC, taurine, 2-thiobarbituric acid, 1,1,3,3-tetraethoxypropane, trichloroacetic acid (TCA), Ellman's reactive, and GSH were obtained from Sigma Co. (St. Louis, MO, US). All other chemicals were of the highest grade (Merck Co., Darmstadt, Germany).

Experimental Design

For care and use of animals, all applicable international and national guidelines were followed, and experimental design was approved by Ankara University Local Committee for Ethics. The experimental animals (male Swiss Albino mice, weighing 30–32 g, 5–6 weeks of age) were maintained under controlled laboratory conditions (ambient temp. 22 ± 2 °C, relative humidity 50–55%, 12:12-h light–dark). During acclimatization, standard laboratory diet and water were provided ad libitum. The randomized assignment into six experimental groups, each containing six mice, was summarized in Table 1. Cr(VI) exposure [38], NAC [39, 40], and taurine [41, 42] treatments were selected based on relevant literature. Briefly, Cr(VI) exposure was designed to induce acute toxicity, whereas doses of NAC and taurine were selected to achieve beneficial effect rapidly, without obvious side effects. Potassium dichromate, NAC, and taurine were all administered as saline solutions via intraperitoneal (ip) injection.

Tissue and Homogenate Preparation

All experimental procedures were explained in detail in our previous work [9]. Briefly, after 12 h following the last administration to each group, mice were dissected in accordance with guidelines. The heart, lung, spleen, and testis tissues were immediately removed and rinsed in saline (ice-cold) and kept at − 80 °C until use. All homogenates were prepared in ice cooling using a Heidolph Diax900 homogenizer (Heidolph Instr., Germany). Tissues were homogenated in 0.1 M KCl solution for measurement of TBARS level, and 0.02 M Na_2-EDTA was used for NPSH content determination.

Analytical Procedures

Lipid Peroxidation

Lipid peroxidation was measured using a modified assay [43], based on spectrophotometric measurement of TBARS level. The calibration curve was constructed using a series of concentrations of malondialdehyde (MDA) prepared by hydrolyzing 1,1,3,3-tetraethoxypropane. Results were expressed as nmol MDA/g tissue.

Nonprotein Sulfhydryl Groups

For determination of NPSH content, the method described by Sedlak and Lindsay [44] was followed. To obtain NPSH content, tissue homogenates in Na_2-EDTA were added 10% TCA and were centrifuged at 3000*g* for 15 min and carefully collected clear supernatants were utilized. For preparation of calibration curve, a series of GSH solutions were used and data were presented as μmol GSH/g tissue.

Table 1 Exposure/treatment details in experimental groups

Group	Exposure details
Control	Saline, ip injection
Cr(VI)-exposed	$K_2Cr_2O_7$ (20 mg Cr/kg), single ip injection [38]
NAC pre-treatment	1) NAC (200 mg/kg), single ip injection [39, 40]
	2) 1 h later: $K_2Cr_2O_7$ (20 mg Cr/kg), single ip injection
NAC post-treatment	1) $K_2Cr_2O_7$ (20 mg Cr/kg), single ip injection
	2) 1 h later: NAC (200 mg/kg), single ip injection
Taurine pre-treatment	1) Taurine (1 g/kg), single ip injection [41, 42]
	2) 1 h later: $K_2Cr_2O_7$ (20 mg Cr/kg), single ip injection
Taurine post-treatment	1) $K_2Cr_2O_7$ (20 mg Cr/kg), single ip injection
	2) 1 h later: taurine (1 g/kg), single ip injection

Statistical Analysis

The study results were expressed as mean ± standard error of data from six samples in each group, and each measurement covered two replicates. All analyses were performed using GraphPad Prism (7.01, La Jolla, CA, USA). Differences between experimental groups were evaluated using ordinary one-way ANOVA, followed by Tukey as post-hoc test. The level of significance was set at $p < 0.05$.

Results

Lipid Peroxidation

Lipid peroxidation levels were assessed in the experimental groups which were arranged and treated as summarized in Table 1. TBARS levels in the $K_2Cr_2O_7$ administered group were significantly higher as compared to the control ($p < 0.05$) in all tissues (Fig. 1). The highest increase was found in the heart (~88% vs control), followed by the testis, spleen, and lung, respectively (63.3%, 46.4%, 27.3% vs control values). As shown in Fig. 1, Cr(VI)-induced lipid peroxidation was ameliorated in all tissues by pre-treatment or post-treatment of NAC and taurine, in a similar manner ($p < 0.05$). The differences observed between NAC and taurine as well as pre- and post-treatments were not statistically significant.

Nonprotein Sulfhydryl Groups

Contents of NPSH in all tissues were significantly decreased following Cr(VI) exposure ($p < 0.05$). The thiol levels decreased in the order of lung > spleen > testis > heart (Fig. 2). NAC and taurine pre- and post-treatments appeared to restore this decline; however, the issue needs to be examined in view of some tissue- and antioxidant-specific differences. For example, the NPSH content in the spleen tissue, reduced by 28.4% in Cr(VI) group, was alleviated with effect of these antioxidants ($p < 0.05$), and both NAC and taurine treatments as well as pre- and post-treatments acted in a similar pattern. Regarding the heart, reduction of NPSH level by Cr(VI) exposure (21.3%) was alleviated by both NAC and taurine treatments; however, while taurine pre- and post-treatments were comparable, NAC pre-treatment appeared slightly more effective as compared to post-treatment (with no statistically significant difference between pre- and post-treatment). On the other hand, the distinctive NPSH decline as a result of Cr(VI) exposure in the lung tissue (44.5%) was restored by pre-treatments of NAC and taurine ($p < 0.05$), whereas the improvement presented by post-treatments was statistically insignificant. Interestingly, Cr(VI)-induced thiol depletion in the testis (23.9%) was normalized by taurine pre-and post-treatments ($p < 0.05$), whereas no significant alteration could be presented by NAC.

Discussion

Toxic effects related to Cr(VI) are of interest especially in occupational exposure [5]; however, these compounds also remain as uncommon but serious cause of acute poisonings [45]. Generation of reactive species, resulting in diverse effects including lipid peroxidation and thiol depletion, has been suggested as a possible mechanism contributing to Cr(VI) toxicity [46]. Therefore, in this study, to help predict the potential of pre- or post-treatment of NAC and taurine on Cr(VI)-induced oxidative stress, we investigated lipid peroxidation and thiol contents in the heart, lung, spleen, and testis of acute Cr(VI)-exposed mice.

In accordance with previous experimental data representing the detrimental effects of Cr(VI) exposure in the lung [12], spleen [13], heart [47], and testis [48], our data show that lipid peroxidation was induced in these tissues (Fig. 1). Challenging these results, Garcia-Niño et al. suggested that Cr(VI) exposure caused no oxidative or histological injury in the heart and spleen, except a slight increase in

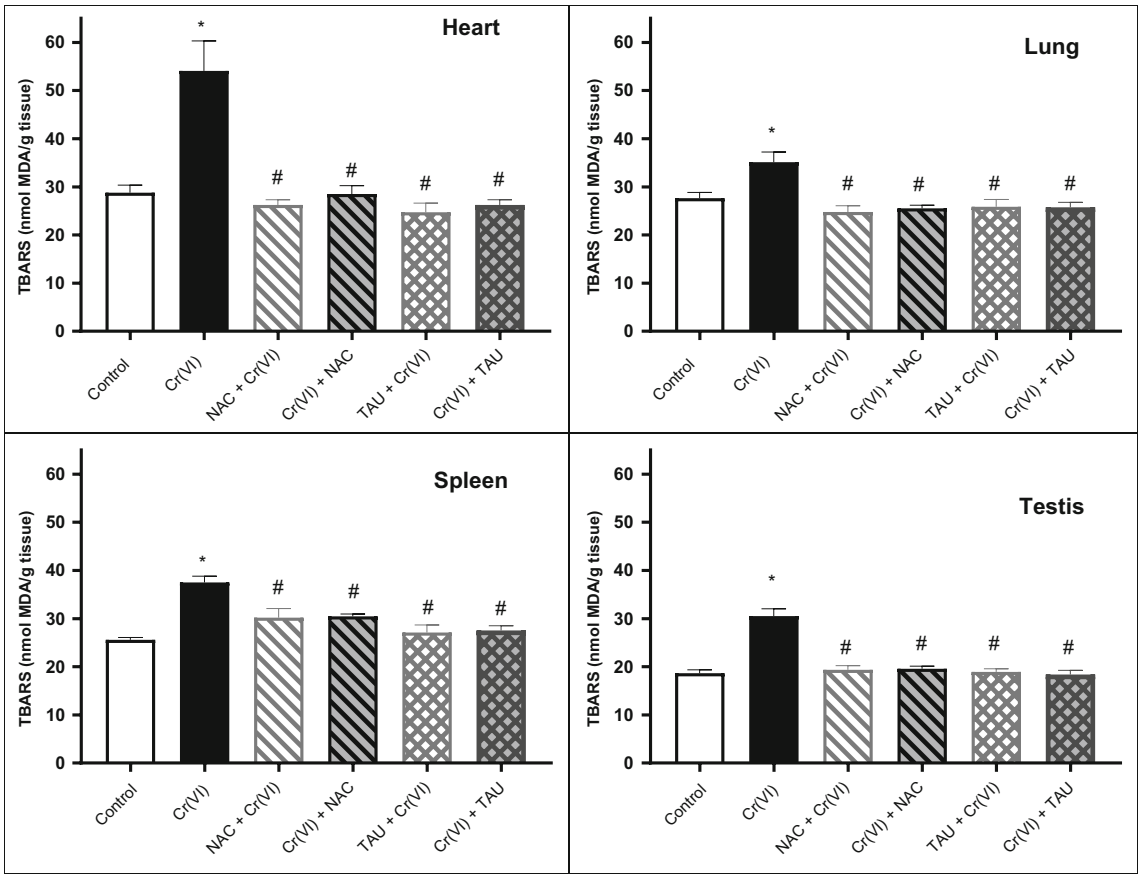

Fig. 1 Effects of Cr(VI) exposure and administration of NAC or taurine on lipid peroxidation in mice heart, lung, spleen, and testis. Each result is given as mean ± standard error ($n = 6$ per group), $^{*}p < 0.05$ vs control, $^{\#}p < 0.05$ vs Cr(VI)-exposed group

lipid peroxidation in the lung [49]. Our lipid peroxidation measurements showed that NAC or taurine treatments before or after exposure were able to revert Cr(VI)-induced lipid peroxidation effectively in all tissues examined (Fig. 1). These data are in line with previous studies reporting the beneficial effects of NAC against oxidative damage in the cardiac tissue by cisplatin [50], and myocardial injury induced by isoprenaline [51], as well as the protection conferred by taurine in iron-mediated myocardial oxidative stress [32], and cisplatin-induced cardiotoxicity [33]. Similarly, clinical utility of NAC in pulmonary diseases are well established [25], and experimental evidence reveals potential promise of taurine in attenuation of lung injury [35]. On the other hand, while taurine has been shown to protect the spleen against oxidative damage in endotoxemia, it may act as a prooxidant when administered alone [36]. Additionally, low taurine intake in rabbits has been suggested to result in a stimulatory effect on the spleen, whereas high concentrations have been shown to decrease organ function [52]. Regarding beneficial effects on reproductive system, NAC pre-treatment against Cr(VI) toxicity [53] and taurine treatment against acute in vivo exposure to aluminum chloride [37], endosulfan [54], and sodium fluoride [55] have been presented.

Notably, our results indicated that differences between NAC and taurine regarding mitigation of lipid peroxidation were not statistically significant; moreover, these effects were achievable regardless of whether NAC or taurine was administered before or after Cr(VI) exposure. NAC not only serves as a precursor for GSH synthesis but also acts as a direct scavenger of free radicals [56]; some additional mechanisms are also suggested as improving mitochondrial energy metabolism, increasing hypotaurine content [40], or chelating metals including Cr [57]. It has been shown that $K_2Cr_2O_7$ reacts with cysteine [58] and cysteine-containing compounds confer a protective effect against Cr(VI)-induced toxicity, possibly via scavenging radicals [59, 60]. The mechanism through which taurine managed to mitigate lipid peroxidation may be related with diverse cytoprotective activity, including its antioxidant role [61]. Interestingly, Kuo et al. reported that urinary excretion of taurine in Cr(VI)-exposed welders was increased possibly due to high oxidative stress [31]; in this context, reversal of taurine loss may be suggested as a viable strategy against Cr(VI)-induced toxicity.

In the case of NPSH levels, consistent with previous reports [9, 22], Cr(VI) exposure led to significant reductions in all tissues (Fig. 2). It has been shown that reduced glutathione

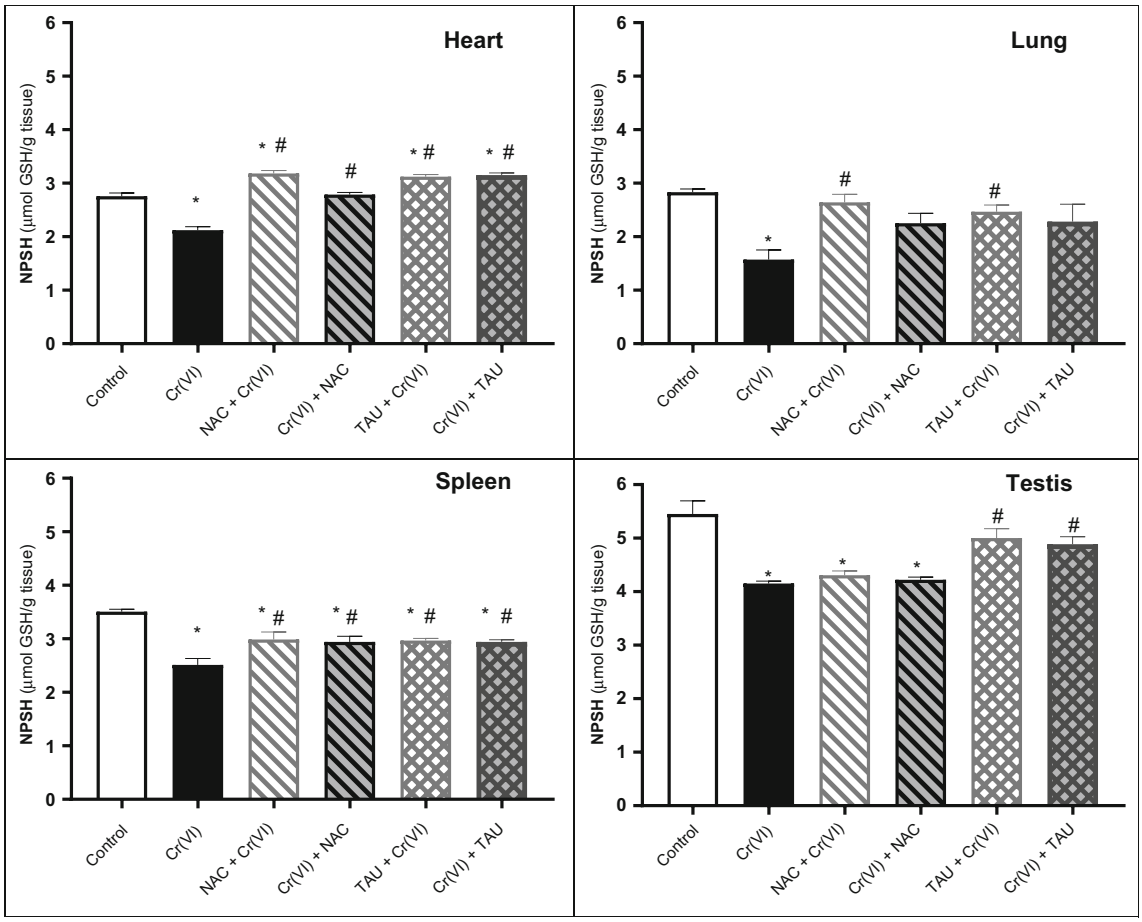

Fig. 2 Effects of Cr(VI) exposure and administration of NAC or taurine on NPSH content of mice heart, lung, spleen, and testis. Each result is given as mean ± standard error ($n = 6$ per group), $^{*}p < 0.05$ vs control, $\#p < 0.05$ vs Cr(VI)-exposed group

(GSH) level was decreased in Cr(VI)-exposed workers [62, 63], and GSH decline was recovered in experimental animals supplemented with GSH sources [20]. GSH comprises most of the cellular NPSH, and it takes part in vital functions such as scavenging free radicals, regenerating ascorbic acid, and providing substrate for related enzymes [64], as well as in protein activity modulation [65]. Thus, limitation of GSH synthesis due to several causes, e.g., insufficient L-cysteine [66], may result in susceptibility of cells to reactive species, causing a potency to cell injury and death [67]. Our data indicates that NPSH decline in the heart was totally reversed by all antioxidant treatments (Fig. 2); interestingly, NAC pre-treatment and taurine pre-/post-treatments resulted in higher levels than those observed in the controls. Nevertheless, we observed that the heart was the most influenced tissue regarding lipid peroxidation (Fig. 1), possibly related to higher perfusion rate; however, this dramatical increase was effectively reversed by both NAC and taurine. Hence, it may be suggested that to overcome the oxidant insult, GSH level in this tissue might have been upregulated. By the same token, the profile of reversal in lipid peroxidation appears concomitant to that of GSH levels (Figs. 1 and 2). It is known that intracellular cysteine availability is rate limiting for GSH synthesis, and NAC participates in GSH synthesis under oxidative stress conditions [68]. NAC has been reported to enhance the ability of Cr(VI) reduction in rat alveolar macrophages [69]. Additionally, the therapeutic role of taurine in the heart is well established [30].

Since Cr(VI) particles can accumulate in the lungs and exert cytotoxic effects therein, the results regarding the lung were of special interest. Among the organs examined, we recognized that the lung had the highest NPSH depletion and the least lipid peroxidation change in Cr-exposed group, which may underline the critical protective role of reductants. Extracellular reduction of Cr(VI) to Cr(III) in body fluids may govern low chromium uptake to the cells, thus act as an efficient detoxification mechanism [70]. The difference of Cr(III) and Cr(VI) in geometry and size explains the distinct character of their abilities in permeation through cell membranes. While only minute amount of Cr(III) can be taken to cells, the chromate anion readily crosses membranes via sulfate/phosphate channels [71]. It may be hypothesized that if extracellular reduction is overwhelmed in the respiratory tract, toxic intermediates may lead to severe effects. The consequence (either

detoxification or toxicity) basically depends on the delicate balance between several factors such as the site of reduction, its proximity to biomolecules, and characteristics of reducers [1].

Another point regarding lung NPSH content was the difference between pre- and post-treatments of antioxidants. The alterations were effectively prevented in pre-treatment groups; however, both for NAC and taurine, the effect remained only partial with post-treatment, which may be explained by the critical importance of pre-treatment in the lung. As described by Afolaranmi and Grant, the membrane permeability for Cr(VI) is the rate-limiting step that determines organ accumulation, and a higher or faster transfer into the cell compared with extracellular reduction may lead to higher Cr levels [72], which may apply to our results especially in the lung. Our findings are consistent with the reports highlighting the more efficient role of pre-treatment of NAC or taurine [9, 10, 22]. The importance and benefit provided by application of antioxidant before toxic-insult have also been underlined in other studies [27, 73, 74]. Specifically, Cr(VI)-induced apoptosis and oxidative DNA damage produced by reactive oxygen species in lung cells of rats have been shown to be mitigated by NAC pre-treatment [75]. Dose-dependent beneficial effect of NAC pre-treatment against Cr(VI) cytotoxicity and reactive oxygen species in osteoblasts [76] has also been demonstrated.

Based on the current data on the testis, NAC and taurine appear equipotent in compensation of lipid peroxidation (Fig. 1); however, NPSH assay (Fig. 2) reveals that pre- and post-treatments of taurine were able to significantly ameliorate thiol depletion in line with a previous report [77]. The lack of improvement on GSH level by NAC in the testis (Fig. 2) might be due to insufficient NAC delivered to the tissue, thus limiting the direct effect of NAC. Although convincing data showed us the potential of NAC in other issues, it may be possible that a higher dose would be necessary to provide protection in the testis. In this context, the need of higher NAC dose to obtain an elevation of GSH level in patients, despite an evident increase of cysteine in plasma, has been previously described [78]. Moreover, the critical role of treatment duration for NAC has been reported in a study on male germ cells: It has been suggested that total glutathione could be replenished following NAC twice/week intervention, while once/week had no positive effect [79]. Therefore, despite lack of an effect on thiols, the fact that treatment with NAC curtailed the lipid peroxidation suggests that other possible mechanisms other than GSH synthesis may have contributed, at least in part, to the present outcome; therefore, it is essential to elucidate the underpinning mechanism responsible for this effect.

In conclusion, our results indicate that NAC and taurine may offer protection to or amelioration of Cr(VI)-induced oxidative damage in mice heart, lung, spleen, and testis. Given that Cr(VI) compounds are commonly used in industrial processes, and can cause serious health effects, further studies are required to investigate whether NAC or taurine could afford protection to chronically exposed individuals.

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Compliance with Ethical Standards

For care and use of animals, all applicable international and national guidelines were followed, and experimental design was approved by Ankara University Local Committee for Ethics.

Conflict of Interest The authors declare that they have no conflict of interest.

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