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Original Research

Influence of Chromium on the Natural Antioxidant Barrier

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Abstract

Hexavalent chromium (VI) compounds are widely used in industry, while trivalent chromium (III) is used as a nutritional supplement. CrVI is generally considered more toxic and carcinogenic than its trivalent form and the toxicity of this metal is associated with free radical processes generating reactive oxygen species (ROS). The detailed mechanism of ROS formation by chromium and its compounds remains largely unknown.

This paper investigates the effects of both chromium III and VI on the activity of antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx), the level of reduced glutathione (GSH), and the ferric-reducing ability of plasma (FRAP) in human blood. Our study shows that chromium III does not affect the levels of GSH, but it decreases FRAP. It also affects SOD in a dose-dependent manner while having no effect on the activity of GPx. CrVI, on the other hand, causes a decrease of SOD activity, increases the activity of GPx, reduces the level of GSH and, similarly to CrIII, shows a negative effect on FRAP.

Keywords: chromium, superoxide dismutase, glutathione peroxidase, reduced glutathione, ferric-reducing ability of plasma

Introduction

Chromium compounds are extensively used and released to the environment, in some countries hundreds of tons a year [1]. Chromium III is a microelement essential in the glucose metabolism, while chromium VI is carcinogenic (IARC list). Occupational exposure to CrVI is associated with pulmonary carcinomas, allergic dermatitis, and tissue damage, especially to the central nervous and reproductive systems. Recently the ability of chromium to interact with estrogen receptor was discovered. This metal can replace zinc in zinc fingers area of estrogen receptor and is classified as metalloestrogen [2, 3]. Our previous studies on the influence of chromium on the erythrocyte membrane lipid peroxidation have identified both CrIII and Cr VI as stimulants for this process [4]. To further investigate the mechanism of chromium-induced oxidative stress we have tested its capacity to generate the hydroxyl radical [5]. Other reports show that during intracellular reduction of Cr(VI), a number of reactive oxygen species (ROS) is probably produced by the Fenton-like and Haber-Weiss reaction [6]. These products can injure cellular proteins, lipids, and DNA, leading to pathological changes.

Our present analyses focused on testing the tri- and hexavalent chromium influence on the natural antioxidant barrier. The effect of chromium on human erythrocyte superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, the level of reduced glutathione (GSH), and the ferric-reducing ability of plasma (FRAP) was eval-

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Table 1. The influence of chromium on SOD activity.

Chromium μg/mL	CrIII 0.05	CrIII 0.5	CrIII 1.0	CrVI 0.05	CrVI 0.5	CrVI 1.0	Control
SOD U/gHb	1100	636	645	597	526	504	707
SD	296.31	149.86	41.36	47.62	41.98	32.07	47.62
p value *vs*. control	0.02*	0.40	0.03*	0.06	0.005*	0.005*	

*Differences considered significant ($p<0.05$).

uated. The natural antioxidants (enzymatic or non-enzymatic) are important defensive systems against oxidative stress. They preserve homeostasis for normal cell function. SOD and GPx are very useful enzymatic ROS scavengers. SOD converts highly toxic superoxide anion radical to hydrogen peroxide. This hydrogen peroxide is then reduced to water by GPx in the presence of reduced glutathione. Both SOD and GPx belong to the metalloenzyme class and are sensitive to metal balance. The data on influence of chromium on antioxidative barrier are not uniquivocal. Some studies show that SOD and GPx were significantly decreased by exposing human T-lymphocytes to chromate [7]; some studies report no changes [8]. The oral administration of CrVI to Swiss mice caused a significant increase of ROS level in the liver, after both 1 and 5 days of exposure accompanied by dose- and time-dependent changes in SOD activity [9]. In our study, the human erythrocytes were used as material for research. It seemed a proper model since chromium exhibits a high affinity toward red blood cells. Recently the toxic effect of chromium is widely considered in the free radical processes [10-12], but the mechanism has not yet been explained. It is not clear at least, how chromium influences the antioxidative barrier in the body and whether there are differences between CrVI and CrIII.

Materials and Methods

Fresh human blood obtained from healthy donors of Academic Hospital taken on EDTA (for SOD, GSH, FRAP measurements) or heparin (GPx) was used directly or after fractionation to erythrocytes or plasma. Erythrocytes were used for SOD and GSH measurements; whole blood for GPx and plasma for FRAP determination. Erythrocytes were obtained from whole blood by centrifuging for 10 min at 3000 rpm, plasma and white blood cells were rejected, erythrocyte pellets were washed 3 times with 0.9% sodium chloride. The suspension of 10% (SOD) or 50% (GSH) erythrocytes in PBS was prepared. Plasma samples for FRAP measurements were obtained from the whole blood centrifugation at 3,000 rpm at room temperature by careful aspiration without disturbing the erythrocyte/white blood cell pellet.

SOD activity was determined with a RANSOD kit from RANDOX Laboratories. The results were expressed in U/gHb [13]. GPx activity was determined in whole blood with a RANSEL kit from RANDOX Laboratories according to manufacturers' instructions; the results were expressed in U/l [14]. Levels of reduced glutathione were determined with Ellman's modified method, 1959 [15]. FRAP was determined as the ability of plasma to reduce Fe^{3+} to Fe^{2+} ions with TPTZ (2,4,6-tripirydyl-s-triazine) as described [16].

$CrCl_3$ (Ubichem, Hampshire) and $K_2Cr_2O_4$ (POCh) were used as chromium sources in experiments at the concentrations of 0.01 to 40.0 μg of chromium per 1 ml. The exact chromium concentrations for every parameter tested are specified in the text.

Absorbances were determined with a U-2900 Spectrophotometer (Hitachi) at wavelengths of 340, 505, 412, and 593 nm for GPx, SOD, GSH, and FRAP measurements, respectively. Every measurement was conducted in eight replicates, including the control sample without chromium.

All the data were evaluated with a T-Student's test, differences between the groups were considered statistically significant at $p<0.05$. The data were expressed as means of eight independent measurements ±SD.

Results

A differential, concentration-dependent influence of CrIII on SOD activity was found. At lower concentrations (0.05 μg/mL) chromium III statistically significantly ($p<0.02$) increased SOD activity to 1100 U/gHb compared to the control (707 U/gHb). In the concentration of 0.5 μg/mL, chromium did not influence the enzyme, while the chromium concentration of 1 μg/mL resulted in a statistically significant ($p<0.03$) decrease of SOD activity to 645 U/gHb (Table 1). But a low statistical significance obtained (p values ranging between 0.02-0.03) suggests minor influence of CrIII on SOD activity. CrVI, on the other hand, significantly ($p<0.005$) inhibited SOD activity at the two highest concentrations used (0.5 and 1.0 μg/mL) (Table 1).

CrIII in concentrations of 0.05, 0.5, and 1 μg/mL had an inhibiting effect on GPx activity. This inhibitory effect was statistically significant in the two highest chromium III concentrations used (0.5 and 1.0 μg/mL; $p<0.001$ and $p<0.05$, respectively) (Fig. 1). Cr VI, on the other hand, increased the GPx activity at the concentration of 1.0 μg/mL with statistical significance of $p<0.05$.

A different effect of Cr III and CrVI was observed for reduced glutathione (GSH) levels in human erythrocytes. CrIII in the whole concentration range tested (0.025-10.0 μg/mL) did not exhibit any effect on GSH levels. CrVI, on

Table 2. GSH levels upon exposure of human erythrocytes to chromium.

CrIII µg/mL	0.025	0.05	0.1	1.0	5.0	10	Control
GSH µmol/gHb	3.13	3.09	3.17	3.23	3.30	3.30	3.23
SD	0.06	0.10	0.08	0.09	0.08	0.11	0.09
p value *vs*. control	0.09	0.05	0.23	1.00	0.22	0.31	
CrVI µg/mL	0.025	0.05	0.1	1.0	5.0	10	Control
GSH µmol/gHb	2.94	2.90	2.84	2.91	2.65	2.50	2.90
SD	0.29	0.04	0.10	0.18	0.17	0.04	0.04
p value *vs*. control	0.69	0.15	0.34	0.87	0.00*	0.00*	

*Differences considered significant ($p<0.05$).

the other hand, showed no effect only at the concentrations of 0.025 to 1 µg/mL, while higher doses (5 and 10 µg/mL) statistically significantly (p=0.0000) decreased the level of GSH to 2.65 and 2.50 µmol/g Hb, respectively, compared to the control (2.90 µmol/g Hb) (Table 2).

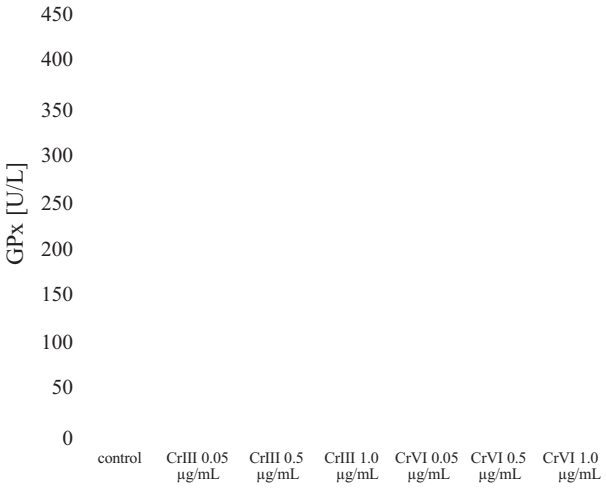

Fig. 1. The influence of chromium on GPx activity. *Differences considered significant ($p<0.05$).

The influence of both chromium forms on FRAP was manifested by a high, statistically significant (p=0.0000) decrease of ferric-reducing ability of plasma (expressed as plasma Fe^{2+} concentration) in all chromium III and VI concentrations used ranging from 0.01 to 40 µg/mL. As an example: chromium III in the dose of 0.5 µg/mL decreased the plasma Fe^{2+} concentration to 0.083 mmol/L compared to the control of 0.129 mmol Fe^{2+} per liter (Fig. 2).

The data obtained so far suggest that Cr III and CrVI have varying effects on all the parameters tested. Trivalent chromium at higher concentrations inhibits the activity of SOD and GPx, while hexavalent chromium increases GPx activity. Moreover, high concentrations of CrVI decrease the levels of GSH in human erythrocytes. Both CrIII and CrVI have a deleterious effect on FRAP, decreasing the antioxidant potential of plasma.

Discussion of Results

Recent studies have reported the free radical formation capability of chromium [2-6]; the molecular mechanisms of this process are, however, largely unknown. Our studies have shown no significant influence of chromium on the

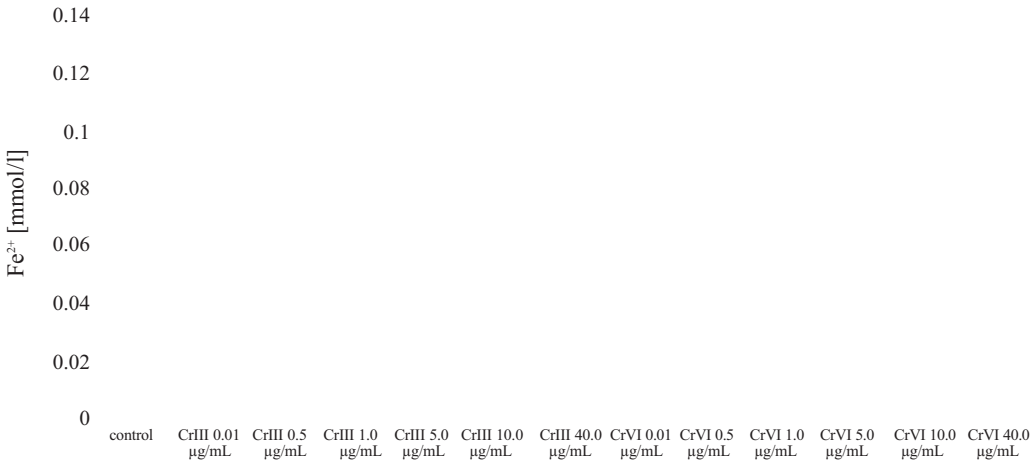

Fig. 2. Ferric-reducing ability of plasma (FRAP) is decreased upon plasma treatment with chromium. *Differences considered significant ($p<0.05$).

hydroxyl radical formation in erythrocytes. In order to look more deeply into mechanisms we explored the effect of chromium on antioxidant barrier factors such as SOD, GPx, GSH, and FRAP.

Chromium content in erythrocytes is used as a biomarker of hexavalent chromium exposure, because of easy transport into erythrocytes via the sulfate ion channel and subsequent reduction with the binding to hemoglobin. Chromium VI is carcinogenic. The role of oxidative damage in metal-induced carcinogenesis is described [17]. Heavy metals disturb the homeostasis and metabolic cascades. The formation of ROS is considered as a molecular mechanism of chromium-induced toxicity. Chromium could catalyze the Fenton/Haber-Weiss reaction, leading to hydroxyl radical formation. Antioxidant enzymes SOD and GPx preserve homeostasis during oxidative stress; SOD by dismutation of toxic superoxide ions to hydrogen peroxide while GPx by their reduction. It was proved that SOD inhibited chromium-induced DNA damage, playing the protective role. GPx also protects the membrane lipids from oxidative damage in Cr-induced toxicity. Both SOD and GPx belong to the metaloenzymes class. Superoxide dismutase exists as Cu, Zn-, or Mn-SOD, GPx contains selenium. Glutathione is an efficient antioxidant and cofactor of glutathione-dependent peroxidases and transferases [18].

The toxicity of chromium III and VI is different also in free radical processes. Chromium VI passes through cell membranes more easily than CrIII and it is reported as more toxic and carcinogenic. It is also known that CrVI is reduced to CrV, IV, and III. These subsequent reduction reactions generate many reactive oxygen species [19]. Other authors report an increase in lipid peroxidation (malondialdehyde level – MDA) in workers exposed to chromium compounds [8] and in laboratory animals [17]. The data concerning the activity of SOD, GPx, and GSH levels, however, are largely ambiguous. The chrome-plating factory workers who had elevated blood chromium levels (5.98-3.17 μg/L; versus control levels of 0.89-0.46 μg/L) were found to exhibit higher peroxidation levels measured with MDA in blood and urine, while the activities of SOD and GPx remained unchanged [8]. Rats intoxicated with CrVI ($K_2Cr_2O_4$) at a dose of 15 mg/kg body weight showed reduced GPx activity in kidneys, on the third day of the experiment. However, the SOD activity in this organ was unchanged [20]. There was a correlation between the route of intake and the stress intensity response shown as well, along with organ response differences, e.g. a stronger oxidative stress response in the liver than kidney [9]. Other studies report a dose-dependent SOD activity increase in the liver and kidney in rats after intraperitoneal intoxication with $K_2Cr_2O_4$ at doses 2.5-10 mg/kg body weight [17]. The slight induction in SOD activity can be a result of an adaptive response to oxidative stress, but higher doses of chromium can make the enzymes exhausted. The studies of chronic chromium intoxication in the epithelial L-41 cell line have shown that while chromium doses of 2 mM activate the antioxidant barrier, the prolonged exposure to 20 mM of CrVI increases apoptosis rate, destroys the glutathione antioxidant system, and reduces the activity of Cu, Zn-SOD [11].

Our findings demonstrate that CrIII- and CrVI-induced oxidative stress is not significantly manifested with SOD and GPx activity changes; rather, it is more pronounced by FRAP decrease (this study) or the lipid peroxidation increase [4], which has also been documented [8]. The studies indicate a varying influence of CrIII and CrVI on SOD and GPx activity. Hexavalent chromium seems to exhibit a strong inhibiting effect on SOD and stimulative effect on GPx. CrIII, on the other hand, shows differing effects on SOD and inhibition of GPx activity in blood. It is reported [21] that chromium VI reduction intermediates are believed to react with hydrogen peroxide to form hydroxyl radical. This pathway could be responsible for different influences of CrIII and VI on GPx activity. The induction of enzymes could be an adaptive response to oxidative stress caused by chromium. The induction of GPx by CrVI confirms the large participation of hydrogen peroxide in CrVI-induced toxicity.

The *in vitro* experiment [7] with human leukemic T-lymphocytes exposed to chromate show a significant decrease in SOD and GPx activities. The results of Huang investigation with people exposed to chromium [8] point that antioxidant enzymes are not suitable biomarkers for occupational chromium exposure. The subjects had a higher LPO level but no changes in SOD and GPx activities. The authors conclude that the activity of these enzymes is due to several factors such as age, sex, smoking habit, etc. Other reports show that the activity is also time-dependent and tissue specific. That seems to be the reason of variable results. The authors [20] who described the time-dependent changes of GPx activity in rats exposed to chromium confirm that peroxynitrite impairs GPx activity and suggest that factors other than oxidative/nitrosative stress is involved in these processes.

It was reported that CrIII exposure decreases the level of glutathione in brain, liver, and kidney [22], while some studies showed actually no changes in glutathione levels [23]. The toxic effect of chromium is certainly tissue-specific, time-dependent, and different for hexavalent and trivalent chromium.

Conclusions

1. CrIII and CrVI differently influence SOD and GPx activities.
2. CrIII decreases, while CrVI increases GPx activity.
3. Hexavalent chromium decreases the levels of GSH.
4. Both CrIII and CrVI decrease the antioxidant potential of serum (FRAP).

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