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RESEARCH ARTICLE

ROLE OF ANTIOXIDANTS IN DETOXICATION OF HEXAVALENT CHROMIUM TOXICITY IN LABORATORY RATS

***Dr. Dharmendra Kumar**

Department of Zoology, Govt. P. G. College Lohaghat -262524, Champawat, Uttarakhand, India

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ABSTRACT

Antioxidative protection offered by three nutrients viz: α-tocopherol (vitamin E), reduced glutathione (GSH) and selenium (Se) against hexavalent chromium {Cr(VI)} toxicity has been studied in laboratory rats. Chromium like other transition metal ions induced oxidative stress in liver and kidney. However, treatments with antioxidants inhibited lipid peroxidation and activated serum transminases and glutathione -S-transferases activity in both the organs. Selective preferences were shown by these antioxidants for these parameters in each organs. Our results confirm that antioxidants offer protection by different mechanisms. Disturbances in cellular equilibrium of anti and pro-oxidants caused by chromium can be overcome by antioxidants with certain preferences.

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INTRODUCTION

Chromium is a toxic heavy metal, which primarily exists in two inorganic forms, Cr(VI) and Cr(III). Highly soluble Cr(VI) is carcinogenic due to its oxidizing nature. Current concept about Cr(VI) toxicity implicate the formation of reduced oxygen metabolites such as the superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radicals ($OH^{\bullet}$) as mechanisms producing cell damage the so called "oxidative stress". Antioxidants are molecules which can safely interact with Free Radicals (FR) and the terminate the chain reaction before vital molecules are damaged. Although, there are several enzyme system within the body that scavenge FR, The principal micronutrient antioxidants which have been used in this study are α-tocopherol, selenium and GSH. The body cannot manufacture these micronutrients so they must be supplied in the diet. Like other xenobiotics, heavy metals are potent inducers of oxidative stress (Rana, 1997). Several reports (Rana and Kumar 1984, Stohs *et al*., 1984, Sunderman Jr. 1986) suggest that elements such as iron, copper, cadmium, lead, mercury, nickel and vanadium have the ability to generate Reactive Oxygen Species (ROS) that induce lipid peroxidation (LP), causes DNA damage, deplete sulphydryls and alter calcium homeostasis. Further, a large body of experimental evidence suggests that oxidative tissue damage does depend on the antioxidative status of the cell and tissue. The involvement of oxidative mechanisms in chromate induced mutagenic and carcinogenic processes has also been suggested (Standeven and Wetterhahn, 1991 and Sugiyama, 1992). However, the protective influence of antioxidants on chromate induced oxidative disturbances is not well known. Therefore, a study on the effects of three antioxidants viz: glutathione (GSH), α-tocopherol and selenium in chromium treated was undertaken.

Corresponding author*: ***Dr. Dharmendra Kumar,*
Department of Zoology, Govt. P. G. College Lohaghat -262524, Champawat, Uttarakhand, India.

MATERIALS AND METHODS

Chemicals

Glutathione (GSH), α-tocopherol, NADP, NADH, thiobarbituric acid, 5-5 dithiobis (2-nitrobenzoic acid), 1,1,3 tetramethoxypropane and 1 chloro, 2-4 dinitrobenzene were purchased from Sigma Chemical Co. (St. Louis Mo, USA). Potassium dichromate and sodium selenite were procured from Glaxo (India). P-dimethyl-aminobenzaldehyde was supplied by SISCO Research Laboratory (Mumbai, India). Kits for the estimation of serum transminases (AST and ALT) were procured from Span Diagnostics Private Limited (Surat, India). All other reagents and chemicals used in this study were either of analytical grade or highest purity.

Animal procedure

Four months old Wistar strain rats (150 ± 10 gm) were obtained from Central Animal Facility of All India Institute of Medical Sciences (AIIMS), New Delhi. Each rat was housed in a polypropylene cage and maintained under standard laboratory conditions (Room temperature 25 ± 5 ° C and relative humidity 50 ± 10%). They were fed commercial pellets (Lipton, India) and tap water *ad libitum.* After acclimatization to standard laboratory conditions for two week, the rats were divided into five groups. Rats of group A were administered predetermined (Rana and Kumar, 1984) sublethal dose of Cr (5 mg / 100 gm body weight) by gavage on each alternate day for 30 days. Rats of group B were fed on Cr as rats of group A but treated with selenium (1 mg /100 gm body weight) also. The rats of group C were treated with Cr and α-tocopherol (0.5 IU /100 gm body weight). Similarly, the rats of group D were treated with Cr and supplemented with GSH (0.25 mg /100 gm body weight) as described earlier (Rana and Verma, 1996).

Biochemical assay

On 31st day all the rats were starved overnight and sacrificed next morning by light anesthesia.

Serum transminases

Blood was collected from each rat through cardiac puncture. Serum was separated and processed for the estimation of Alanine Amino Transferases (ALT) and Aspartate Amino Transferases (AST) using commercial kits and following the method of Reitman and Frankel (1957). Small pieces of liver and kidney were quickly removed, homogenized and processed for the estimation of malondialdehyde, reduced glutathione and oxidized glutathione and glutathione-S-transferase as prescribed.

Malondialdehyde

Microsomes were separated by differential centrifugation. They were washed twice in 0.25M sucrose and used for the estimation of malondialdehyde by thiobarbituric acid (Jordan and Schenkman, 1982) was used as the standard.

Glutathione-s-transferase

The enzyme was assayed according to the method of Habig *et al*. (1974) using 1-chloro, 2, 4 dinitrobenzene (CDNB) as the substrate. The production of CDNB glutathione adduct was monitored at 340 nm.

Statistical analyses

All data are represented as mean ± standard error (SEM). Analyses of variance (ANOVA) with post hoc Scheffe test was used for multiple comparison.

RESULTS

Results on serum transminases show that administration of Cr(VI) causes significant damage to liver. An improvement in liver function was recorded in rats treated with antioxidants. Maximum protection was offered by selenium followed by GSH and α-tocopherol. These observations suggest that treatments with antioxidants improve liver functions of Cr(VI) treated rats (Table 1).

Table 1. Influence of antioxidants on serum transminases in Cr(VI) fed rats

Group	Treatments	AST	ALT
A	Cr(VI)	70 ± 1.83*	43 ± 1.21*
B	Cr(VI) + GSH	54 ± 3.23**	32 ± 0.56**
C	Cr(VI) + α-tocopherol	53 ± 1.66**	27 ± 1.84**
D	Cr(VI) + Selenium	45 ± 2.01**	23 ± 1.66**
E	Control	39 ± 2.51	19 ± 1.60

Results are expressed as mean ± SE (n = 5).
*Significantly different ($p < 0.05$) when compared with saline treated control rats.
**Significantly different ($p < 0.05$) when compared with Cr(VI) fed rats.
Analyses of variance F (ANOVA) was significant among all these groups for AST (19.53, $P < 0.05$) and ALT (2.97, $P < 0.05$).

Cr(VI) induced lipid peroxidation in liver as well as kidney. However, it was inhibited in both the organs by the co-treatments with antioxidants. In liver, α-tocopherol offered highest protection but in kidney selenium was highly protective (Table 2).

Table 2. Influence of antioxidants on microsomal lipid peroxidation (n moles malondialdehyde / mg protein) in liver and kidney of Cr(VI) fed rats

Group	Treatments	Liver (nmoles MDA /mg protein)	Kidney (nmoles MDA / mg protein)
A	Cr(VI)	0.260 ± 0.017*	0.267 ± 0.015*
B	Cr(VI) + GSH	0.233 ± 0.011**	0.234 ± 0.013**
C	Cr(VI) + α-tocopherol	0.198 ± 0.005**	0.221 ± 0.021**
D	Cr(VI) + Selenium	0.238 ± 0.015**	0.214 ± 0.014**
E	Control	0.199 ± 0.005	0.204 ± 0.020

Results are expressed as mean ± SE (n = 5).
*Significantly different ($p < 0.05$) when compared with saline treated control rats.
**Significantly different ($p < 0.05$) when compared with Cr(VI) fed rats.
Analyses of variance F (ANOVA) was significant among all these groups for Liver (4.44, $P < 0.05$) and Kidney (4.06, $P < 0.05$).

Glutathione-S-transferase activity was significantly inhibited by Cr(VI) in liver and kidney of rats. The enzyme activity was restored by treatments with these antioxidants. GSH was highly effective in restoring the enzyme activity in comparison to selenium and α-tocopherol (Table 3).

Table 3. Influence of antioxidants on glutathione-S-transferase (nmoles/NADPH/min. / mg protein) in liver and kidney of Cr(VI) fed rats

Group	Treatments	Liver (μmoles / min / mg protein)	Kidney (μmoles/ min / mg protein)
A	Cr(VI)	0.329 ± 0.075*	0.299 ± 0.033*
B	Cr(VI) + GSH	0.576 ± 0.038**	0.558 ± 0.094**
C	Cr(VI) + α-tocopherol	0.462 ± 0.017**	0.473 ± 0.195**
D	Cr(VI) + Selenium	0.495 ± 0.161**	0.363 ± 0.047**
E	Control	0.881 ± 0.069	0.817 ± 0.169

Results are expressed as mean ± SE (n = 5).
*Significantly different ($p < 0.05$) when compared with saline treated control rats.
**Significantly different ($p < 0.05$) when compared with Cr(VI) fed rats.
Analyses of variance F (ANOVA) was significant among all these groups for Liver (13.43, $P < 0.05$) and Kidney (8.81, $P < 0.05$).

DISCUSSION

Chromium is one of the most toxic chemical compound because of its increased level in the environment as a result of metallurgies refractory, chemical and tannery industries as well as by agricultural practices. It has become one of the most abundant pollutant in aquatic and terrestrial ecosystems (Costa *et al*., 2003). Hexavalent Cr(VI) and trivalent Cr(III) are two stable chromium oxidation states found in nature. As an analogue of sulphate, chromate can enter mammalian cells readily via sulphate transport system (Ackerley *et al*., 2004). Cr(III), which is less toxic and less soluble than Cr(VI), is readily being converted into Cr(VI) under natural conditions through various oxidation processes and this oxidized Cr(VI) reacts with nucleic acid and other cell components to produce mutagenic and carcinogenic effects on biological systems (McLean and Beveridge, 2001). However, Cr(III) is considered to be a trace element essential for the proper functioning of living organisms (Zayed and Terry 2003, Wang, *et al*., 2009). Our results show that Cr(VI) readily and selectively accumulate in soft tissues like liver and kidney. The retention was higher in liver than kidney. Cr(VI) which is taken up by mammalian cell is ultimately converted to Cr(III) species by sulphydryl groups. Reduction of Cr(VI) involves membrane bound chromate reduce during anaerobic respiration or a soluble cytosolic chromate reductase under aerobic conditions and activity of which is enhanced by NADPH or glutathione as enzyme co-factors (Elangovan *et al*., 2006). This intracellular reduction effectively traps Cr inside cell. Cr(III) species thus formed can not easily cross cell membrane. The end result is the massive build up of Cr(III) inside cell with intracellular and intranuclear levels reaching several nmoles/l.

Antioxidants protection was found to mobilize Cr these organs. Antioxidants stimulate metallothionein against metal toxicity (Kumar, 2012). GSH has a high affinity for several metals and the complexes formed are excreted in bile. Formation of a transportable Cr-GSH complex seems to be an attractive hypothesis. GSH can chelate Cr as suggested by Chaberel and Martell (1959). Selenium has been found to protect rats against cadmium (Rana and Verma, 1996) and copper (Rana and Verma,1997). Furthermore, it is suggested that Se-Cr interaction is brought by endogenous glutathine which reduces selenite to selenide compounds (Iwata *et al*., 1981). The high lipo-affinity of this compound might alter or reduce its distribution and toxicity in critical tissues. Nevertheless, α-tocopherol seems to have no direct effect. It might indirectly promote metal ions sequestration by proteins. The relative importance of an antioxidant is determined by physical factors such as effective concentration and mobility at the specific environment. α-tocopherol binding proteins found in hepatic cytosol may mediate intermembrane transport in cells (Murphy and Moris, 1981). Reddy and colleague (1988) suggested that α-tocopherol is required for expression of antioxidant activity by GSH-dependent factor. The involvement of oxidative mechanism in chromate induce mutagenic and carcinogenic processes has been proposed by a few workers (Standeven and Watterhahn, 1991 and Sugiyama, 1992).

Present results also suggest that Cr(VI) induce lipid peroxidation in liver and kidney of rats which is manifested by ROS particularly H_2O_2 producing $OH^{\bullet}$ ions in a Fenton-type mechanism (Shi and Dalal,1990 a, b). α-tocopherol could act as $OH^{\bullet}$ scavengers and O_2 quenchers at sites where lipid peroxidation is likely to occur. Mattagaja-Singh and Mishra (1997) suggested that Cr(VI) induce the generation of oxidants in the cell, probably through the direct interaction of a reduced form of Cr with molecular oxygen. The administration of these antioxidants together with Cr inhibited lipid peroxidation. α-tocopherol was more protective in liver whereas selenium was more protective in kidney. GSH acts as a reductant in the metabolism of hydroperoxides and FR. Selenium protection can be offered by the induction of glutathione dependent enzymes as reported earlier (Rana and Verma, 1996). In biological system, α-tocopherol scavenges peroxy radicals. Peroxy radicals endogenously oxidize α-tocopherol to the tocopheryl radical that does not allow further propagation of the radical chain (Burton and Ingold, 1981).

The accumulation of Cr in soft tissues and resultant oxidative stress disturbed liver function. The range is wide in terms of threshold concentration that result in inhibition by the various heavy metals, but they are in the order of100 mg/litre (Chen *et al*., 2008). Heavy metals are recognized as a strong biotoxicants, because of their persistent nature and cumulative action to the living life (Sharma and Agrawal 2005). However, an improvement was indicated by diminished activities of AST and ALT recorded after antioxidant protection. Inhaled Cr(VI) dust might not affect the enzyme markers of liver functions (Lee *et al*., 1988) but ingested Cr did injure the liver parenchyma. Antioxidants have been found to improve liver function in Cd and Hg fed rats (Rana and Verma, 1996).

The glutathione-S-transferase appear to perform several detoxication functions. They catalyze all reactions where GSH acts as a nucleophilic agent. The mechanism envisages glutathione transferase as a binding protein with a second site for GSH close by. It has also been shown that a single protein known as ligandin is responsible for such activities. Our results show that transferases are stimulated by antioxidants in liver and kidney. As a matter of fact, antioxidants might offer protection by stimulating glutathione-S-transferases. Heavy metals are known to inhibit the activity of glutathione-S-transferases.

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