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Study on the blood glutathione protective effects induced during three rats generations by $K_2Cr_2O_7$ intake

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Abstract

Chromium exists in a series of oxidation states with a valence from -2 to +6. Trivalent (Cr[III]) and hexavalent (Cr[VI]) compounds are the most biologically significant. After entering the body from an exogenous source, Cr(VI) is rapidly taken up by erythrocytes after absorption and reduced to Cr(III) inside the cell. We have studied the influence of potassium dichromate ($K_2Cr_2O_7$) on blood GSH values in female rats. This study was carried out on 49 adult white Wistar adult female rats divided in 6 experimental groups and one control group. The rats were feed with 25ppm (LOAEL), 50ppm and 75ppm $K_2Cr_2O_7$, ad libitum, in drinking water. The control group received tap water. Reduced glutathione (GSH) was measured quantitatively using a spectrophotometer method. This study reports that potassium dichromate exposure induces depletion of blood GSH because Cr(VI) can generate reactive oxygen species (ROS). It can provoke oxidative stress and toxicity.

Keywords: GSH, potassium dichromate, oxidative stress, female rats

1. Introduction

The work with reduced glutathione (-glutamyl-cysteinyl-glycine; GSH) [1] has greatly advanced biochemical and nutritional sciences over the past 125 years [2]. Glutathione is the predominant low-molecular-weight thiol (0.5–10 mM) in animal cells. Most of the cellular GSH (85–90%) is present in the cytosol, with the remainder in many organelles (including the mitochondria, nuclear matrix, and peroxisomes) [3]. With the exception of bile acid, which may contain up to 10 mM GSH, extracellular concentrations of GSH are relatively low (e.g., 2–20 µM in plasma) [4].

Chromium exists in a series of oxidation states with a valence from -2 to +6. Trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) compounds are the most biologically significant.

Cr^{+3} is an essential dietary mineral in low doses. It is required to potentiate insulin and for the normal glucose metabolism. Chromium carcinogenicity was first identified over a century ago and Cr+6 compounds were amongst the earliest chemicals to be classified as carcinogens [5]. Chromium is used in three basic industries: metallurgical, chemical, and refractory (heat-resistant applications). Occupational exposure to Cr^{+6}-containing compounds is known to induce lung toxicity and increased incidence of respiratory-system cancers [6-8]. After entering the body from an exogenous source, Cr^{+3} binds directly to transferrin, an iron-transporting protein in the plasma. In contrast, after entering the body from an exogenous source, Cr^{+6} is rapidly taken up by erythrocytes after absorption and reduced to Cr^{+3} inside the cell [9].

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Membrane damage is one of the important consequences of Cr, an environmental toxicant, induced cytotoxicity. Cr^{+6} can actively enter the cells through channels for the transfer of the isoelectric and isostructural anions, such as SO_4^{2-} and HPO_4^{2-} channels [9]. Transport of Cr through the cell membrane concerned exclusively the Cr^{+6} species. Recent models considered the uptake of reduced Cr species generated by extracellular redox mechanisms [10]. Extracellulary generated Cr^{+5} and Cr^{+3} complexes also have high permeabilities through the cell membrane [11].

GSH, a membrane protectant may, be used to reduce the Cr-induced membrane damage [12]. Intracellular metabolism of Cr^{+6} may lead to oxidative stress.

2. Materials and methods

This study was carried out on 49 adult white Wistar adult female rats, divided in 9 experimental batches (F_{01}, F_{02}, F_{03}, F_{11}, F_{12}, F_{13}, F_{21}, F_{22}, F_{23}) and one control batch (C).

The experimental batches are divided in 3 groups, 3 generations:

- $F_0 = F_{01}, F_{02}, F_{03}$ – first generation - female rats – exposed for 3 months to 25ppm (LOAEL), 50ppm and 75ppm $K_2Cr_2O_7$ in drinking water, ad libitum;
- $F_1 = F_{11}, F_{12}, F_{13}$ – second generation - female rats, obtained from the mating of female rats belonging to the groups F_0 with male rats that were exposed for 3 months to 25ppm, 50ppm and 75ppm $K_2Cr_2O_7$ in drinking water, ad libitum, before mating;
- $F_2 = F_{21}, F_{22}, F_{23}$ – third generation - female rats, obtained from the mating of female rats belonging to the groups F_1 with male rats that were exposed for 3 months to 25ppm, 50ppm and 75ppm $K_2Cr_2O_7$ in drinking water, ad libitum, before mating;
- Control batch (C) – female rats – maintained tape water without $K_2Cr_2O_7$;

The female rats were euthanized at sexual maturity, blood samples being used for determinations. GSH was measured quantitatively at a Perkin-Elmer spectrophotometer through Beutler et al. method [13], at 412nm of yellow color developed by adding 5,5'-dithiobis(2-nitrobenzoic acid) to sulphahidryl compounds.

The results were expressed as µmol GSH/gHb. Hemoglobin (Hb) was determined through Drabkin method [14] at the automatic analyzer MS-9 VET.

3. Results and Discussion

The blood test results are presented in Tables 1, 2, 3 and Figure 1.

Table 1. GSH mean values in the control batch (C) and first generation experimental batches F_0 (F_{01}, F_{02}, F_{03}).

Group	GSH (µmol/gHb)		
	X±Sx	DS	Confidence level
C	0.65±0.01	0.01	0.01
F_{01}(25ppm)	0.64±0.01	0.01	0.01
F_{02}(50ppm)	0.53±0.01	0.01	0.01
F_{03}(75ppm)	0.50±0.01	0.01	0.01

Table 2. GSH mean values in the control batch (C) and second generation experimental batches F_1 (F_{11}, F_{12}, F_{13})

Group	GSH (µmol/gHb)		
	X±Sx	D.S.	Confidence level
C	0.65±0.01	0.01	0.01
F_{11}(25ppm)	0.64±0.01	0.01	0.01
F_{12}(50ppm)	0.52±0.01	0.01	0.01
F_{13}(75ppm)	0.46±0.01	0.01	0.01

Table 3. GSH mean values in the control batch (C) and third generation experimental batches F_2 (F_{21}, F_{22}, F_{23})

Group	GSH (µmol/gHb)		
	X±Sx	D.S.	Confidence level
C	0.65±0.01	0.01	0.01
F_{21}(25ppm)	0.64±0.01	0.01	0.01
F_{22}(50ppm)	0.52±0.01	0.01	0.01
F_{23}(75ppm)	0.46±0.01	0.01	0.01

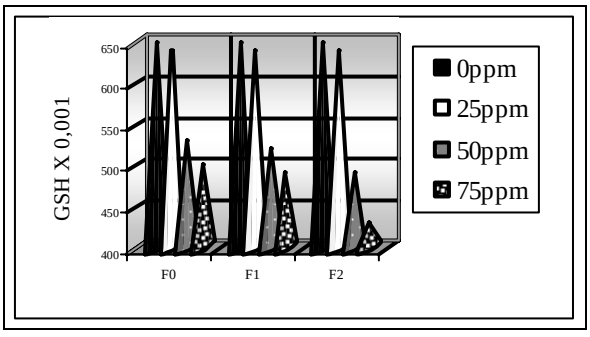

Figure 1. GSH value in the experimental and control groups

Glutathione participates in many cellular reactions. He effectively scavenges free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxyl radical, peroxynitrite, and H_2O_2) directly, and indirectly through enzymatic reactions [15].

In such reactions, GSH is oxidized to form GSSG, which is then reduced to GSH by the NADPH-dependent glutathione reductase. In addition, glutathione peroxidase (a selenium-containing enzyme) catalyzes the GSH-dependent reduction of H_2O_2 and other peroxides [16].

The cysteine residue induce a nonenzymatically oxidation of GSH to glutathione disulfide (GSSG) by electrophilic substances (e.g., free radicals and reactive oxygen/nitrogen species). The GSSG efflux from cells contributes to a net loss of intracellular GSH. Cellular GSH concentrations are reduced markedly in response to protein malnutrition, oxidative stress, and many pathological conditions [17]. Cr^{+6}, inside the cell, in the presence of cellular reductants, causes a variety of DNA lesions including Cr–DNA adducts, DNA–protein crosslinks, DNA–DNA crosslinks, and oxidative damage [18]. Within the cell GSH rapidly forms a complex with Cr(VI), followed by a slow reduction of Cr^{+6} to yield Cr^{+5} [11]. GSH can act as an intramolecular stabiliser of Cr^{+6} via the formation of a thiolate ester. Once formed, Cr^{+5} species alter the DNA conformation.

Several in vitro studies of the reaction of Cr^{+6} with GSH were conducted, with an spin trapping technique, to demonstrate the formation of Cr Cr^{+5} species (most probably the Cr^{+5} -glutathione complex) and the glutathione-derived thiyl radical (GS•) [19]. Once formed Cr^{+5} can react via Fenton reaction with H_2O_2 forming the hydroxyl radical capable of causing DNA damage [20]. In addition to the cellular damaging effect of the GS• radical, it can further react with other thiol molecules in oxygenated tissues to give the superoxide radical. Superoxide can further reduce Cr(VI) to Cr^{+5} which can then catalyze the decomposition of H_2O_2 thus creating the DNA damaging hydroxyl radical.

Cr^{+5} can also be reduced by cellular reductants (e.g. ascorbate, GSH) to Cr^{+4}, again participating in Fenton chemistry generating hydroxyl radical. GSH/GSSG is the major redox couple that determines the antioxidative capacity of cells, but its value can be affected by other redox couples, including $NADPH/NADP^+$ and $thioredoxin_{red}$/ $thioredoxin_{ox}$ [4].

4. Conclusion

Hexavalent chromium, a potent teratogen, is significantly reduced by GSH in all tissues.

GSH is the principal reducing agent in erythrocytes, the principal scavenger of reactive oxygen species in mitochondria. The consequences of ad libitum $K_2Cr_2O_7$ administration at 25, 50 and 75ppm during three generation female rats, was the induced ROS production, the inhibition of the regeneration of GSH from the oxidized form, a limited decrease of GSH values in F_0, F_1, F_3, groups.

References

1. Badaloo, A., Reid, M., Forrester, T., Heird, W. C. & Jahoor, F., Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition, *Am. J. Clin. Nutr.*, **2002**, 76(3), 646-652
2. Sies, H., Glutathione and its cellular functions. *Free Radic. Biol. Med.,* **1999**, *27*(9-10), 916-921, doi:10.1016/S0891-5849(99)00177-X
3. Lu, S. C., Regulation of glutathione synthesis. *Curr. Top. Cell Regul*., **2000**, *36*, 95-116
4. Jones, D. P., Redox potential of GSH/GSSG couple: assay and biological significance. *Methods Enzymol*., **2000**, *348*, 93-112
5. Stern, R.M., *Biological and Environmental Aspects of Chromium*, Elsevier, S. Langård, Amsterdam, **1982**, 5–47
6. Langård S., One hundred years of chromium and cancer: a review of epidemiological evidence and selected case reports, *Am. J. Ind. Med.*, **1990**, *17*(2), 189–215
7. Hayes, R.B., Review of occupational epidemiology of chromium chemicals and respiratory cancer, *Sci. Total Environ.,* **1988**, *71*(3), 331–339, doi:10.1016/0048-9697(88)90205-7
8. De Flora, S., Bagnasco, M., Serra, D., Zanacchi, P., Genotoxicity of chromium compounds. A review, *Mutat. Res.* **1990**, *238*(2), 99–172, doi:10.1016/0165-1110(90)90007-X
9. Codd R, Dillon CT, Levina A, Lay PA: Studies on the Genotoxicity of Chromium: From the Test Tube to the Cell., *Coord Chem Rev,* **2001**, *216-217*, 537-582.
10. Gunaratnam, M., Pohlscheidt, M., Grant, M.H., Pretreatment of rats with the inducing agents phenobarbitone and 3-methylcholanthrene ameliorates the toxicity of chromium (VI) in hepatocytes, *Toxicol. In Vitro,* **2002**, *16*(5), 509-516, doi:10.1016/S0887-2333(02)00040-1
11. O'Brien, T., Xu, J., Patierno, S.R., Effects of glutathione on chromium-induced DNA. Crosslinking and DNA polymerase arrest, *Mol.Cell.Biochem.* **2001**, *222*(1-2), 173-182, doi: 10.1023/A:1017918330073
12. Sankar Kumar Dey, Somenath Roy, Role of GSH in the amelioration of chromium-induced membrane damage, *Toxicological & Environmental Chemistry*, *92*(2) 2010, 261 – 269, doi: 10.1080/02772240902955669

13. E. Beutler, O. Duron, B.M. Kelly, Improved method for the determination of blood glutathione, *J.Lab.Clin Med*., **1963**, *61*, 882-890
14. Ghergariu, S., Pop, A., Kadar, L., Sînu, M., *Manual de laborator clinic veterinar*, Ed ALL Educational, Bucuresti, **2000**, 65-66
15. Fang, Y. Z., Yang, S., Wu, G., Free radicals, antioxidants, and nutrition, *Nutrition,* **2002**, *18*, 872-879
16. Lei, X. G., In vivo antioxidant role of glutathione peroxidase: evidence from knockout mice. *Methods Enzymol*., **2002**, 347, 213-225, doi:10.1016/S0076-6879(02)47021-8
17. Griffith, O. W., Biologic and pharmacologic regulation of mammalian glutathione synthesis, *Free Radic. Biol. Med*., **1999**, *27*(9-10), 922-935, doi:10.1016/S0891-5849(99)00176-8
18. Kasprzak, K.S., Possible role of oxidative damage in metal-induced carcinogenesis, *Cancer Investig.* **1995**, *13*(4), 411-430.
19. Aiyar J, Berkovits HJ, Floyd RA, Wetterhahn KE., Reaction of chromium(VI) with glutathione or with hydrogen peroxide: identification of reactive intermediates and their role in chromium(VI)-induced DNA damage, *Environ Health Perspect*. **1991**, *92*, 53–62
20. Liu, K.J.; Shi, X.L., In vivo reduction of chromium (VI) and its related free radical generation, *Mol. Cell. Biochem.* **2001**, *222*(1-2), 41-47, doi: 10.1023/A:1017994720562