GLUTATHIONE DYNAMICS IN THE SECOND GENERATION YOUNG RATS BLOOD AS A CONSEQUENCE OF FEMALE EXPOSURE TO Cr(VI) INTOXICATION DURING GESTATION

DINAMICA GSH-ULUI CELEI DE A DOUA GENERAŢII DE ŞOBOLANI MATURI DIN PUNCT DE VEDERE SEXUAL CONSECUTIV INTOXICĂRII CU Cr(VI) A FEMELELOR MAME ÎN TIMPUL GESTAŢIEI

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Chromium compounds are found in the environment, due to erosion of chromium containing rocks and can be distributed by volcanic eruptions in food, water. Metals being non-biodegradable persist in the environment for a long period and cause serious ecotoxicological problems. Chromium, which exists in nature mostly in the trivalent form (Cr^{+3}), is essential for activating certain enzymes and for stabilizing proteins and nucleic acids. We have studied the influence of the glutathione dynamics in the second generation rats blood, as a consequence of females chromium (VI) intoxication during the gestation. This study was carried out on 7 Wistar adult female rats, control group (C), 21 adult Wistar female rats, devided in three experimental groups (E) and theire young rats. The rats were feet, durind the gestation, with 25ppm (LOAEL), 50ppm and 75ppm potassium dichromate, ad libitum, in drinking water. The control batch received tap water. Reduced glutathione (GSH) was measured quantitatively after the wean using a Perkin-Elmer spectrophotometer, through Beutler et al. method, at 412nm. The study reports also the depletion of young rats blood GSH.
Keywords: GSH, potassium dichromate, oxidative stress, young rats.

Introduction

Chromium is the 21st most abundant element in Earth's crust with an average concentration of 100 ppm (Cronin, 2004).

The results of several experiments with humans, rats, mice, and other species, are many physiological and biochemical symptoms of chromium deficiencies (Anderson, 1994).

Research with animals has confirmed that chromium from dietary organic complexes, such as chromium picolinate (CrPic), chromium nicotinate (CrNic), and high-chromium yeasts, is absorbed more efficiently than is chromium from chromium chloride ($CrCl_3$). Some research data indicate that chromium is an essential nutrient for food-producing and laboratory animals.

The trivalent state of chromium (Cr^{3+}) is responsible for its nutritional activity. The principal route by which trivalent chromium enters the body is the digestive system. Chromium in foods is present both in the inorganic form and as organic complexes. Intestinal absorption of chromium is low (0.5–2%), and the mechanism has not yet been fully elucidated. Absorbed chromium circulates as free Cr^{3+}, as Cr^{3+} bound to transferrin or other plasma proteins, or as complexes, such as glucose tolerance factor (GTF)-Cr. Circulating trivalent chromium can be taken up by tissues, and its distribution in the body depends on the species, age, and chemical form. It is excreted primarily in the urine by glomerular filtration or bound to a low-mol-wt organic transporter. Chromium metabolism is still imperfectly understood. (Ducros, 1992).

Materials and Methods

This study was carried out on 28 Wistar adult female rats and theire 40 young rats, divided in one control batch (C) and 3experimental batches (E_1, E_2, E_3).

The experimental baches are:

- E_1 = young rats – from females exposed during the gestation, to 25ppm Cr^{+6} (LOAEL) form ($K_2Cr_2O_7$) in drinking water, ad libitum
- E_2 = young rats – from females exposed during the gestation, to 50ppm Cr^{+6} (LOAEL) form ($K_2Cr_2O_7$) in drinking water, ad libitum
- E_3 = young rats – from females exposed during the gestation, to 75ppm Cr^{+6} (LOAEL) form ($K_2Cr_2O_7$) in drinking water, ad libitum
- Control batch (C) – young rats – maintained tape water without Cr^{+6}

Potassium dichromate was administered to the adult female rats in drinking water, ad libitum, only during the gestation.

The young rats were euthanatized after the wean, the blood samples being used for determinations.

GSH was measured quantitatively at a Perkin-Elmer spectrophotometer through Beutler et al. method (Beutler, 1963), 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 (Ghergariu, 2000) at the automatic analyzer MS-9 VET.

Results and Discussion

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

Table 1.

Young rats blood GSH values in the control group (C) and experimental groups (E_1, E_2, E_3) after the wean.

Group	GSH (μmol/gHb)		
	X±Sx	DS	Confidence level 95%
C	0.65±0.01	0.01	0.01
E_1(25ppm)	0.64±0.01	0.01	0.01
E_2(50ppm)	0.60±0.01	0.01	0.01
E_3(75ppm)	0.57±0.01	0.01	0.01

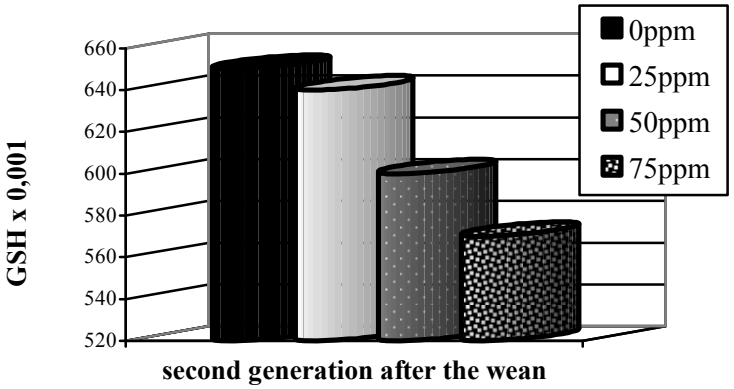

Figure.1. GSH value in the experimental and control groups

Metal induced toxicity and carcinogenicity, with an emphasis on the generation and role of reactive oxygen and nitrogen species (Valko, 2005).

In vitro and in vivo studies demonstrate that chromium cations are involved in Fenton reaction, the production of oxidative stress that results in oxidative deterioration of biological macromolecules. Chromium undergoes redox cycling, resulting in enhanced production of reactive oxygen species such as superoxide ion, hydroxyl radicals, and hydrogen peroxide. These reactive oxygen species result in increased lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA damage, membrane damage, altered gene expression, and apoptosis. (Stohs, 2001).

Hexavalent chromium (Cr^{+6}) is reduced intracellularly to Cr^{+5}, Cr^{+4} and Cr^{+3} by ascorbate (Asc), cysteine and glutathione (GSH). These metabolites induce a spectrum of genomic DNA damage resulting in the inhibition of DNA replication.

GSH not acts only as a reductant of Cr (VI) but also becomes crosslinked to DNA by Cr. The cellular and molecular mechanisms of Cr(VI)-carcinogenesis is still unclear.

Many Cr^{+6} compounds exist as chromate oxyanions (CrO_4^{2-}) in solution which readily enter cells through relatively non-selective anion channels for SO_4^{2-}. Once inside cells, Cr^{+6} is reduced to Cr^{+3} and other reductive intermediates which react with nucleic acids, resulting in a spectrum of DNA lesions and mutations. (Travis, 2001).

Conclusions

Hexavalent chromium is an established carcinogenic agent, which is not directly reactive with DNA. Its genotoxicity involves a reduction step, producing reactive oxygen species and radicals, and also lower valence forms which form stable complexes with intracellular macromolecules.

Cr induced ROS formation, may cause protein damage.

Excess chromium may replace other metals in metalloprotein or may interact directly with SH- groups of proteins or GSH, the important component of the body's intracellular antioxidant defenses, protecting cytosolic organelles.

The consequences of ad libitum $K_2Cr_2O_7$ intoxication of females during the gestation was the induced oxidative stress, the GSH values decrease in the second generation rats blood.

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