

Effect of Chromium on Glutathione-S-Transferase and Catalase Activity and their Respective Gene Expressions in the Brain Tissue of F1 Generation Mice Following Prenatal Exposure: Modulation by Quercetin

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Abstract

Background: The increased use of Cr in several anthropogenic activities and subsequent contamination of water and soil is an increasing concern worldwide. It has also recently drawn attention for its widespread use in dietary supplements.

Objective: In this study we proposed to study whether chromium (Cr) could alter the antioxidant enzymes in brain and their gene expression in the in F1 mice when administered to their dams during gestation and whether quercetin could modulate this effect.

Methods: Animals were administered chromium alone and in combination with quercetin for seven days during their gestation period. Their new born pups (F1 mice) were reared till adulthood. The brain tissue of F1 mice was collected and GST (glutathione-S-transferase) and CAT (catalase) activity was measured and fold increase in their respective gene expression was observed using RT-PCR method.

Result: Cr significantly reduced both activity and expression of GST in the brain tissue of F1 generation mice. There was a slight increase in catalase activity and expression on exposure to Cr. Quercetin was observed to significantly enhance the GST and catalase activity as well as increase the gene expression of these enzymes when administered along with Cr.

Conclusion: Our study shows that Cr altered enzyme activity in brain tissue. Quercetin can strengthen the antioxidant system of the cell by affecting expression of antioxidant enzymes at the transcription level and may attenuate the ill effects of exposure to heavy metals.

Keywords: Chromium; Glutathione-S-transferase; Catalase; RT-PCR; Quercetin

Introduction

In the last few decades the increased use of chromium (Cr) in several anthropogenic activities and subsequent contamination of water and soil is an increasing concern worldwide [1]. Chromium is widely used in industry as plating, alloying, tanning of animal hides, textile dyes, pigments, ceramic glazes and refractory bricks. Chromium also has been shown to have deleterious effects due to its ability to form DNA adducts and generate free radicals during cellular reduction of hexavalent form to intermediate species. Any damage to the DNA carries the possibility to be carried over to the next generation [2,3].

Contrarily, Cr(III) is considered a micronutrient in humans essential for carbohydrate and lipid metabolism. It has recently drawn attention for its widespread use in dietary supplements, especially in patients with diabetes. Clinical studies have demonstrated that

chromium supplementation improved memory in Alzheimer's disease. Very often, these effects have been associated with alteration in antioxidant system in the brain which includes glutathione-S-transferase (GST) and catalase (CAT) activity [4-6].

It is established that glutathione-S-transferase (GST) isoenzymes mediate conjugation of compounds with the intracellular tripeptides glutathione. GST acts to protect against oxidative damage and peroxidation of lipids and DNA. Similarly catalase (CAT) is an important member of the cellular defence system against oxidative stress. Reports show that CAT activity decreases in oxidative stress. Gene expression for CAT in *Schizosaccharomyces pombe* was enhanced in oxidative stress. Thus the enzyme activity for GST and CAT and their respective gene expression are valuable indicators of oxidative damage occurring in brain [6-9].

Quercetin, a flavonoid is naturally present in fruits, vegetables and red wine and is a known antioxidant. It has been shown to offer protection against Cd-induced nephrotoxicity and hepatotoxicity. Quercetin has also been reported to improve spatial learning in

galactose treated aging mice model. Chromium-induced enhancement in memory was observed to be modulated by quercetin when administered to mice during lactation period to their dams. Thus in this study we proposed to study whether Cr could alter the antioxidant enzymes in brain and their gene expression in the in F1 mice when administered to their dams during gestation and whether quercetin could modulate this effect [9,10].

Materials and Methods

Animals

Young (8-10 weeks old) male and female Swiss albino mice weighing 20–25 g were used in the study. The animals were procured from the Central Animal House, University College of Medical Sciences, Delhi. Animals were housed in groups of 4 per cage with free access to pellet diet and water in a temperature controlled facility. All the experiments were performed at daytime between 09:30 and 15:30 h. Care of animals was as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India, New Delhi. The study was duly approved by the Institutional Animal Ethics Committee, University College of Medical Sciences, Delhi (Approval No. IAEC/2011/65 dated December 2, 2011).

Preparation of animals

Nulliparous female mice determined to be estrous by vaginal cytology were randomly divided into pairs and were placed overnight with one male mouse for mating. The onset of pregnancy was confirmed by presence of spermatozoa in vaginal smears on the following morning. The pregnant female animals were housed individually in breeding cage. Following delivery, the next day was regarded as post natal Day 1 (PND1). On PND 1, animals were randomly distributed to treatment and control groups for different vehicle/drugs. The F1 generation produced mice were reared till 100 days age, and the experiments were performed on animals of either sex.

Plant material

Quercetin was obtained from M/s Sigma Aldrich (Product no. Q4952 CAS no.117-39-5, dated March 8, 2010) in the form of yellow amorphous powder. As per the literature provided by the manufacturer, the purity was 98% as assessed by HPLC. For the purpose of the study, the quercetin was suspended in double distilled water to prepare solutions of the required doses (25, 50, and 100 mg/kg).

Drugs and dosing schedule

Chromium chloride (M/s Sigma Aldrich, product no. 230723) was dissolved in distilled water (or vehicle), and was administered daily in dosages of 200 µg/kg/day intraperitoneally (i.p.). Quercetin was administered in doses of 25, 50 and 100 mg/kg/day, i.p. Control groups received equal volume of vehicle used for different treatment groups. Piracetam (Nootropil–M/s UCB, Maharashtra, India) of 98% purity was administered at 400 mg/kg as the standard drug. The administration of drugs/vehicles was started on the day following delivery (PND 1) and continued for 7 days. On postnatal day 21 (PND21), the litter mates were weaned, separated, housed together by sex, and were grouped in same laboratory conditions as their parents.

For performing the experiments, animals from preferably different litters were selected. Six to eight animals were selected in each experimental group.

Estimation of glutathione-S-transferase (GST) activity

GST estimation was done by a kinetic method as described by Mannervik and Danielson (1988) with slight modification. Briefly, 10 µL of samples were transferred to wells of a 96 well plate in duplicates. 190 µL of substrate solution, consisting of 300 mM phosphate buffered saline at pH 6.5, 200 mM of reduced glutathione solution and 100 mM of CDNB in the ratio of 98:1:1, was added to each well. The plate was read in a multimode reader at 340 nm for 5 min. $\Delta A_{340}/\text{min}$ was calculated by finding the difference between the initial read and final read and dividing it by 5 min. GST activity was calculated by the following formula:

$$\text{GST Activity} = (\Delta A_{340}/\text{min} \times \text{reaction volume}(200 \mu\text{L}) \times 1000) / (\text{extinction Coefficient}(9.6) \times \text{sample volume}(10 \mu\text{L}) \times \text{protein (mg)})$$

Protein concentration was measured using nanodrop spectrophotometer by taking absorbance of 2 µL sample at 260 nm.

Estimation of catalase activity

Catalase activity was assayed following the method of Luck (1974). H₂O₂-phosphate buffer (3 ml) was taken in an experimental cuvette, 40 µL of lysate was added and mixed thoroughly. The decrease in absorbance was recorded at 240 nm in a nanodrop spectrophotometer for 5 min (Thermo Scientific, USA). The enzyme solution containing H₂O₂-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

Protein estimation

Protein concentration was measured using Nanodrop spectrophotometer by taking absorbance of 2 µL sample at 260 nm. Catalase and GST activity was expressed per mg total protein.

RT-PCR for glutathione-S-transferase and catalase

RT-PCR for analyzing the expression of above mentioned anti-oxidant genes will be done following the method described by Sharma et al. Total RNA will be isolated using Tri-reagent from Sigma Chemicals, USA. The bands obtained by RT-PCR will be analyzed and quantified on an AlphaImager densitometer (Alpha Innotech, USA) and normalized with the bands of constitutively expressed G3PDH.

Disposal of animals: The dead animals shall be disposed by incineration.

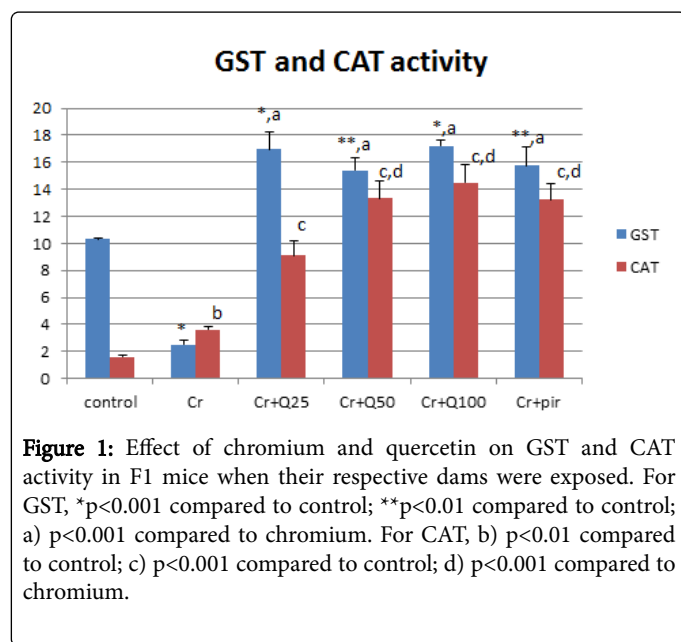
Statistical analysis

Results of the above experiments were expressed as mean \pm S.E.M. (Standard Error of Mean), and the difference between means was analysed by analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test.

Results

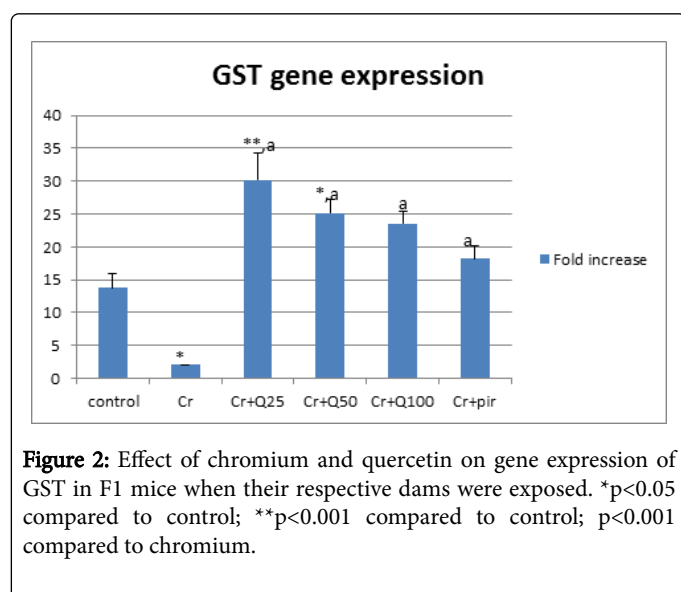
Glutathione-S-transferase (GST) activity in brain

Cr reduced the activity of GST compared to control ($p < 0.001$). Coadministration of quercetin (dose 25, 50, 100 mg/kg) reversed this effect ($p < 0.001$). Piracetam coadministration also had the same effect ($p < 0.001$). Figure 1.



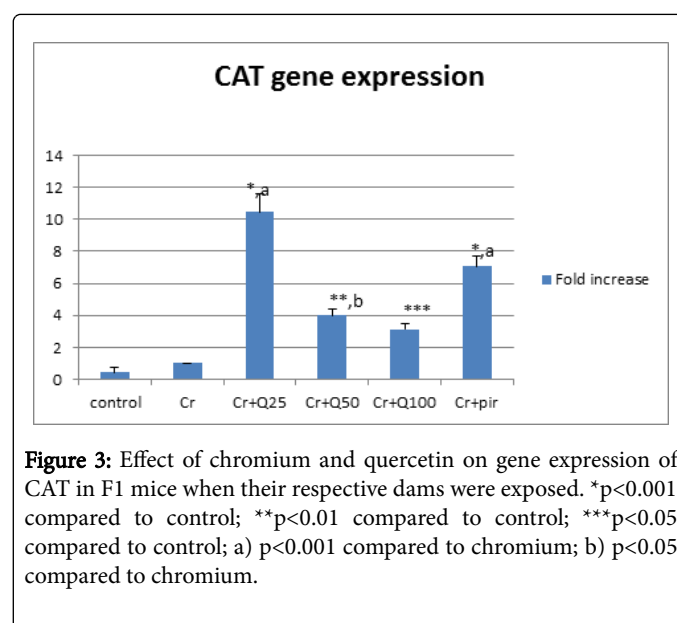
Catalase activity in brain

Catalase activity was increased significantly ($p < 0.01$) compared to control and when quercetin was coadministered, catalase activity was further enhanced ($p < 0.001$). Piracetam coadministration also had the same effect ($p < 0.001$) Figure 2.



Relative gene expression for glutathione-S-transferase and catalase

The relative fold increase in GST gene expression is significantly decreased in Cr treated animals ($p < 0.05$) compared to control group. Quercetin as well as piracetam cotreatment reversed this effect significantly compared to Cr treated alone ($p < 0.001$). The relative fold increase in gene expression of CAT is increased in Cr treated animals however this effect was not statistically significant. The groups treated with both Cr and quercetin (all doses) as well as piracetam increased CAT gene expression significantly ($p < 0.001$, $p < 0.01$, $p < 0.05$, $p < 0.001$) compared to control. Quercetin (dose 25, 50 mg/kg when cotreated with Cr reversed the effect of Cr significantly ($p < 0.001$, $p < 0.05$). Piracetam also reversed the effect of Cr on CAT gene expression significantly ($p < 0.05$). Figure 3.



Discussion

Chromium in various forms finds its way into the environment due to its increasing use in plating, alloying, tanning of animal hides, textile dyes, and pigment industry. Serum chromium level normally is less than or equal to 1.4 $\mu\text{g/mL}$. Lower levels occur in rural areas and higher levels occur in large urban centres. Majority of specimens submitted for analysis from unexposed individual contain 0.3 $\mu\text{g/mL}$ to 0.9 $\mu\text{g/mL}$ [11-14]. Moreover studies have shown that Cr compounds are essential micronutrients having important physiological functions and are also considered to improve glucose tolerance in patients suffering from diabetes [15,16]. This has led to an increased use of chromium compounds in food supplements. All these factors have resulted in high levels of chromium exposure in humans. Despite having beneficial effects, exposure to high level of chromium has raised concerns about its toxic effects. Like other heavy metals numerous studies have shown chromium to increase oxidative stress in cells by generating reactive oxygen species (ROS) [17-20]. However, the mechanism by which chromium increases ROS is still debatable. It may directly generate ROS, or interact with the antioxidant enzymes and inhibit their activity or act at the genetic level and decrease transcription of antioxidant enzymes. Acting at the genetic level is more alarming as it may affect the next generation. In this study, we

evaluated the effect of chromium exposure on F1 generation when their dams were exposed to the metal during pregnancy.

The effect of chromium on two important antioxidant enzymes, GST and Catalase was studied in terms of activity and gene expression. Cr significantly reduced both activity and expression of GST in the brain tissue of F1 generation mice. The decrease in GST activity may be due to decrease in expression of the GST gene. Gunaratnam et al has shown decreased GST activity in hepatocytes exposed to Cr. Another reason for decreased activity may be due to involvement of GST proteins in the detoxification of Cr. There was a slight increase in catalase activity on exposure to Cr but no significant change in its gene expression. Probably all antioxidant enzymes are affected to different extent by Cr. Our findings are to some extent in line with earlier studies which have shown that Cr exposure does not much affect catalase activity significantly in other organisms [21-24].

Quercetin is a known antioxidant and has been shown to decrease ROS in various animal models. The average quercetin intake through onions, tomatoes, asparagus, green tea, green pepper and fruits is on an average 16.2 mg/day. Several studies have shown quercetin to independently increase activity of antioxidant enzymes, like GST, catalase, SOD, and glutathione peroxide [25-28]. These studies have however not reported the effect of quercetin on expression of these antioxidant enzymes. In the present study, we administered quercetin at 25, 50 and 100 mg/kg/day and it was observed to enhance the GST and catalase activity as well as increase the gene expression of these enzymes when administered along with Cr. Our study shows that quercetin can strengthen the antioxidant system of the cell by affecting expression of antioxidant enzymes at the transcription level and may attenuate the ill effects of exposure to heavy metals [29-32].

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