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Protective effects of Selenium (Se) on Chromium (VI) induced nephrotoxicity in adult rats

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ABSTRACT

Chromium is a toxic metal implicated in human diseases. This study was focused on investigating the possible protective effect of Se against $K_2Cr_2O_7$. Female Wistar rats, used in this study, were divided into four groups of six animals each: group I served as control which received standard diet; group II received orally only $K_2Cr_2O_7$ (700 ppm equivalent to 67 mg/kg bw); group III received both $K_2Cr_2O_7$ and Se (0.5 mg/kg of diet); group IV received Se (0.5 mg Na_2SeO_3/kg of diet). The exposure of rats to $K_2Cr_2O_7$ for 21 days provoked renal damages with a significant increase in kidney malondialdehyde, superoxide dismutase, plasma creatinine, and uric acid levels, while catalase, glutathione peroxidase, non-protein thiol, Metallothionein and plasma urea levels decreased. Coadministration of Se in the diet of chromium-treated group improved malondialdehyde, renal biomarkers levels and antioxidant enzyme activities. Kidney histological studies confirmed biochemical parameters and the beneficial role of selenium.

1. Introduction

Chromium (Cr) is one of the most toxic chemical compounds because of its increased level in the environment as a result of industrial and agricultural practices. It has become one of the most abundant pollutants in aquatic and terrestrial ecosystems (Costa et al., 2003). In nature, Cr exists mostly in two valence states: hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)]. The oxidation state and solubility of Cr compound determine its toxicity. Potassium dichromate ($K_2Cr_2O_7$), a hexavalent form of Cr(VI), is widely used in metallurgy, chrome plating, textile manufacture, wood preservation, photography and photoengraving, refractory and stainless steel industry and cooling systems (Barceloux, 1999). Exposure to Cr (VI) has several adverse human health effects including neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity and immunotoxicity (Norseth, 1981; Stohs and Bagchi, 1995; Barceloux, 1999; Kawanishi et al., 2002; O'Brien et al., 2003). Cr(VI) compounds are the most toxic since they can be easily absorbed and transported across membranes via non-specific anion carriers (Buttner and Beyersmann, 1985; Stohs et al., 2001). Once inside the cell, Cr(VI) is reduced to Cr(III). This reduction process generates reactive oxygen species (ROS) and induces soft tissues' damage such as liver, pancreas, cerebellum and kidney (Solis-Heredia et al., 2000; Bagchi et al., 2002; Fatima et al., 2005). The latter is the main target organ for chromium accumulation, more sensitive to the toxic effects of chromium than other tissues. Related studies have demonstrated that exposure to chromium induces acute renal failure (ARF) in humans (Sharma et al., 1978; Verschoor et al., 1988; Picaud et al., 1991) and in animals (Schmidt et al., 1960; Michael et al., 1991; Pedraza-Chaverri et al., 1995). Chronic exposure to chromium can damage the renal proximal tubular epithelial cells and causes proximal tubular dysfunction (Pedraza-Chaverri et al., 2005). Thus, it is believed that antioxidants should be considered as important compounds for effective treatment from chromium poisoning. Indeed, $K_2Cr_2O_7$-induced injury can be inhibited by non-enzymatic antioxidants such as ascorbic acid, vitamin E, N-acetyl cysteine, garlic powder and GSH (Hojo and Satomi, 1991; Sugiyama, 1992; Arreola-Mendoza et al., 2006 Pedraza-Chaverri et al., 2008). Subsequent studies have shown the beneficial effect of selenium (Se), an essential trace element, on the nephrotoxicity induced by heavy metals (Xiao et al., 2002; Ognjanovic et al., 2008).

Selenium is a structural component of several enzymes including glutathione peroxidase (GPx) and thioredoxine (Perottoni et al., 2004), which play a key role in the cellular oxidative defense and have been shown to be induced by oxidative stress (Lechner et al., 2002). In recent years, there has been a great deal of studies carried out on selenium metabolism

Abbreviations: $K_2Cr_2O_7$, potassium dichromate; Se, selenium; MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; ROS, reactive oxygen species; MT, metallothionein; NPSH, non-protein thiol

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(Shi et al., 2004). In most of these studies selenium was administered to experimental animals in sodium selenite form (Ates et al., 2008). Many efforts have been undertaken in evaluating the relative antioxidant potential of Se against environmental pollutants which cause health problems (El-Demerdash, 2001; El-Demerdash, 2004; Ersteniuk, 2004).

To our knowledge, the potential protective effect of Se on $K_2Cr_2O_7$-induced nephrotoxicity has not been explored. Based on the above information, we hypothesize that Se can reduce $K_2Cr_2O_7$-induced renal injury. The aim of this study is to examine the effects of Se on $K_2Cr_2O_7$-induced nephrotoxicity and oxidative stress.

2. Materials and methods

2.1. Chemicals

Potassium dichromate ($K_2Cr_2O_7$), was obtained from Merck (Darmstadt, Germany). Sodium selenite (Na_2SeO_3) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

2.2. Animals and treatments

Female Wistar rats of initial body weight of 130 ± 10 g, obtained from the Central Pharmacy (SIPHAT. Tunis. Tunisia), were used in this study. They were maintained under standard laboratory conditions (temperature 22 ± 2 °C; 12 h light–dark cycle). The animals had free access to water and commercial standard pellet diet (SICO. Sfax. Tunisia). The content of Se in standard diet (0.17 mg Na_2SeO_3/kg) was determined after mineralization, by the Electrothermic Atomic Absorption Spectrometry technique (ETAAS). Measurements were performed on a Perkin-Elmer 5100/Zeeman Atomic Absorption Spectrometer with a 196-nm wavelength.

The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethical Committee of our Institution.

One week after acclimatization, rats were randomly divided into four groups of six animals each: group I served as control which received standard diet; group II received via drinking water only $K_2Cr_2O_7$ (700 ppm equivalent 67 mg/kg bw, orally); group III received both 700 ppm of $K_2Cr_2O_7$ and Se (0.5 mg/kg of diet); group IV served as a positive control and received Se (0.5 mg/kg of diet) as sodium selenite (Na_2SeO_3). The major route of exposure to chromium for the general population is the oral way. The present study was designed to investigate the toxicity of $K_2Cr_2O_7$ given to rats via the oral route. Se is added in the diet (0.5 mg/kg of diet). $K_2Cr_2O_7$ and Se were administrated during 21 days. The dose of $K_2Cr_2O_7$ and the period of treatment were selected on the basis of previous studies (Trivedi et al., 1989; Junaid et al., 1995, 1996a, b; Kanojia et al., 1996, 1998). The levels of $K_2Cr_2O_7$ used in the present study are not usually found in the environment but may be encountered in the workplace or in the vicinity of industrial establishments (Junaid et al., 1996a). The selenium dose (0.5 mg/kg of diet) used in our experiment and in other findings gave high protection against stress conditions in several tissues (Ognjanovic et al., 2008; Ben Amara et al., 2009). Lower doses of selenium gave less protection while higher doses were not much effective Hotz et al. (1997). During this period, food and water intake, body weight of the animals were monitored daily. The amount of ingested diet was calculated as the difference between the weight of feed that remained in the food bin (D_a) and the amount placed one day before (D_b). These data were then used to calculate the daily average feed intake, according to the formula:

$$\text{Average feed intake} = (D_b - D_a)$$

Quantities of $K_2Cr_2O_7$ and Se ingested by each rat were calculated from water and diet intake, respectively (Table 2).

At the end of the experimental period, animals of different groups were killed by cervical decapitation to avoid animal stress. Blood was collected into EDTA tubes and centrifuged at 2200*g* for 10 min. Plasma samples were drawn and stored at −20 °C until analysis. Kidneys were dissected out, cleaned and weighed. Some samples were rinsed and homogenized (10% w/v) in an appropriate buffer (pH=7.4) and centrifuged. The resulting supernatants were used for biochemical assays. Other samples were immediately removed, cleaned and fixed in 10% formalin solution and embedded in paraffin for histological studies.

Urinary samples were obtained from each animal housed in a specially designed metabolic cage, where faecal contamination was avoided. Urine samples were collected into bottles within 24-h cycles. The volume of each sample was recorded and centrifuged at 3000*g* for 5 min.

2.3. Biochemical determinations

2.3.1. Estimation of urea, uric acid, creatinine and creatinine clearance

The levels of urea, uric acid and creatinine in plasma and urine were estimated spectrophotometrically using commercial diagnostic kits, respectively, (ref 20151, 20143, 20091) purchased from Biomagreb (Ariana, Tunisia). Creatinine clearance, an index of glomerular filtration rate was calculated by UV/P equation (Charrel, 1991), where U is the urinary creatinine level, V the volume of urine sample collected within 24 h and P the plasma creatinine concentration.

2.3.2. Lipid peroxidation

Concentration of MDA in tissues, an index of lipid peroxidation, was determined spectrophotometrically according to Draper and Hadley method (1990). A 0.5 ml aliquot of kidney extract supernatant was mixed with 1 ml of trichloroacetic acid solution and centrifuged at 2500*g* for 10 min. One millilitre of a solution containing 0.67% thiobarbituric acid (TBA) and 0.5 ml of supernatant were incubated for 15 min at 90 °C and cooled. Absorbance of TBA–MDA complex was determined at 532 nm using a spectrophotometer. Lipid peroxidation was expressed as nmol of thiobarbituric acid reactive substances (TBARS), using 1,1,3,3-tetra-ethoxypropane as standard.

2.3.3. Non-protein thiols (NPSH) content in kidney

Kidney NPSH levels were determined by the method of Ellman (1959). A 500 µl aliquot of supernatant was mixed with 10% trichloroacetic acid (1 V/1V). After centrifugation, the protein pellet was discarded and free-SH groups were determined in a clear supernatant. A 100 µl aliquot of supernatant was added to 850 µl of 1 M potassium phosphate buffer (pH=7.4) and 50 µl of DTNB (10 mM) 5,5-dithio-bis (2-nitrobenzoic acid). The colorimetric reaction was measured at 412 nm.

2.3.4. Metallothionein (MT) content in kidney

Metallothionein in kidney was assayed according to the method of Viarengo et al. (1997) modified by Petrovic et al. (2001). 1 ml of supernatant was added to 1.05 ml of cold absolute ethanol and 80 µl of chloroform, which were then centrifuged at 6000*g* for 10 min. The collected supernatant was combined with three volumes of cold ethanol, maintained at −20 °C for 1 h and centrifuged at 6000*g* for 10 min. The metallothionein-containing pellets were rinsed with 87% ethanol and 1% chloroform and centrifuged at 6000*g* for 10 min. The metallothionein content in the pellet was evaluated using the colorimetric method with Ellman's reagent. The pellet was resuspended in 150 µl NaCl (0.25 M). Then an aliquot of 150 µl HCl (1 N) containing EDTA (4 mM) was added to the sample. A volume of 4.2 ml NaCl (2 M) containing DTNB (0.43 Mm) buffered with Na-phosphate (0.2 M; pH=8) was then added to the sample at room temperature. The sample was finally centrifuged at 3000*g* for 5 min and the supernatant absorbance was evaluated at 412 nm.

2.3.5. Antioxidant defense system assays

The catalase (CAT) activity was determined according to the method of Aebi (1984). The H_2O_2 decomposition rate was followed by monitoring absorption at 240 nm. One unit of CAT activity is defined as the amount of enzymes required to decompose 1 µmol of hydrogen peroxide in 1 min. The enzyme activity was expressed as µmol H_2O_2 consumed/min/mg protein.

Glutathione peroxidase (GPx) activity was measured according to Flohe and Gunzler, (1984). The enzyme activity was expressed as nmol of GSH oxidized/min/mg protein.

Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM of tissue homogenates in potassium phosphate buffer (pH=7.4), 0.1 mM L-methionine, 2 µM riboflavine and 75 µM Nitro Bleu Tetrazolium (NBT). The developed blue color reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as U/mg protein.

Reduced glutathione levels (GSH) were determined by the method of Ellman (1959) modified by Jollow et al. (1974) based on the development of a yellow color when DTNB (5,5-dithiobis-2 nitro benzoic acid) was added to compounds containing sulfhydryl groups. Five hundred milliliters of tissue homogenate were added to 3 ml of 4% sulfosalicylic acid. The mixture was centrifuged at 1600*g* for 15 min. Five hundred milliliters of supernatant were taken and added to Ellman's reagent. The absorbance was measured at 412 nm after 10 min. Total GSH content was expressed as µg/g tissue.

2.3.6. Protein analysis in kidney

Protein content in kidney was determined according to Lowry et al. (1951) using Bovine serum albumin as standard.