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Protective effects of selenium on oxidative damage and oxidative stress related gene expression in rat liver under chronic poisoning of arsenic

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ABSTRACT

Arsenic (As) is a toxic metalloid existing widely in the environment, and chronic exposure to it through contaminated drinking water has become a global problem of public health. The present study focused on the protective effects of selenium on oxidative damage of chronic arsenic poisoning in rat liver. Rats were divided into four groups at random and given designed treatments for 20 weeks. The oxidative damage of liver tissue was evaluated by lipid peroxidation and antioxidant enzymes. Oxidative stress related genes were detected to reflect the liver stress state at the molecular level. Compared to the control and Na_2SeO_3 groups, the MDA content in liver tissue was decreased and the activities of antioxidant enzymes were increased in the Na_2SeO_3 intervention group. The mRNA levels of SOD1, CAT, GPx and Txnrd1 were increased significantly ($P < 0.05$) in the combined Na_2SeO_3 + $NaAsO_2$ treatment group. The expressions of HSP70 and HO-1 were significantly ($P < 0.05$) increased in the $NaAsO_2$ group and reduced in the combined treatment group. The results indicate that long-term intake of $NaAsO_2$ causes oxidative damage in the rat liver, and Na_2SeO_3 protects liver cells by adjusting the expression of oxidative stress related genes to improve the activities of antioxidant enzymes.

1. Introduction

Arsenic is widely distributed in the environment in both organic and inorganic forms, and it was found in water, air and soil. Inorganic arsenic facilitates cancer of skin, bladder, lung, kidney and liver (Guha Mazumder et al., 2000; Morales et al., 2000; Yoshida et al., 2004; Chen et al., 2005; Rahman et al., 2009), and increases the risk of diabetes and cardiovascular disease (Tseng et al., 2002; Liao et al., 2009; Islam et al., 2012). The major sources of human exposure to inorganic arsenic compounds are occupational or environmental contacts, drinking water contamination, and industrial pollution. Investigations have shown that millions of people worldwide are at risk of chronic arsenic poisoning (Harvey, 2008; Fendorf et al., 2010), and arsenic toxicity has become a global health problem.

Arsenic is toxic because it affects many biological processes. Various studies (Kitchin and Ahmad, 2003; Izquierdo-Vega et al., 2006; Ei-Demerdash et al., 2009) reported that arsenic toxicity involved oxidative damage. Formation of excess reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals was proven to be the main cause of oxidative damage (Liu et al., 2001; Kitchin and Ahmad, 2003). The liver is one of the target organs of arsenic toxicity and carcinogenesis (Liu and Waalkes, 2008). Epidemiology studies have indicated that chronic arsenic exposure causes abnormal liver function, hepatomegaly, liver fibrosis and cirrhosis (Santra et al., 1999; Guha Mazumder, 2005). Studies (Santra et al., 2000; Ghatak et al., 2011) on chronic arsenic toxicity have revealed that oxidative stress and hepatic stellate cell activation are key events in arsenic induced liver fibrosis. Therefore, increasing the antioxidant capacity of cells has become a therapeutic strategy to antagonize arsenic poisoning (Ei-Demerdash et al., 2009; Das et al., 2010; Rana et al., 2010).

Selenium is an essential dietary trace element, which acts as an antioxidant. The most important evidence of this function is the close localization of selenium to the active site of many antioxidant enzymes, e.g., thioredoxin reductase and glutathione peroxidase (GPx) in the cell. GPx is known to prevent the harmful effects of free radicals and reduce the formation of the reactive metabolites induced by arsenic. Selenium may therefore play a protective role on toxicity caused by arsenic. Several studies (Biswas et al., 1999; Ozardali et al., 2004; Messarah et al., 2012) have shown that

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selenium plays an important role in preventing hepatic cellular injury inducted by hepatotoxic agent including arsenic. There is however no report yet of the effect of selenium on oxidative stress related gene expression (such as HSPs and Txnrd1) under chronic arsenic poisoning.

In this study, the protective capacity of selenium on arsenic-induced liver injury in rats was evaluated by measuring the activity levels of AST, ALT and MDA. In addition, hepatic antioxidant enzymes (SOD, CAT and GPx) activities and oxidative stress related gene (Txnrd1 and HSPs) expression in rat liver were determined to provide the protective mechanism of selenium in liver cells.

2. Materials and methods

2.1. Chemicals and antibodies

Sodium arsenite ($NaAsO_2$) was purchased from Sigma (St. Louis, MO, USA) and sodium selenite (Na_2SeO_3) from Shanghai sinpeuo fine chemical co., Ltd. (Shanghai China). All the chemicals used in the experiment were of analytical grade from Sigma.

Rabbit polyclonal antibodies against HO-1 (heme oxygenase-1; diluted 1:300) or HSP70 (diluted 1:500), and horseradish peroxidase conjugated secondary antibodies against rabbit IgG (diluted 1:5000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibodies against TRx (diluted 1:500) and β-actin (diluted 1:2000) were obtained from Bioworld (Bioworld Technology Co. Ltd., Minnesota, USA).

2.2. Animals and experimental design

Forty weaning Sprague–Dawley rats (60–80 g) were obtained from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an China). All rats were housed in an animal holding room with controlled temperature (25 ± 1 °C) and relative humidity (55 ± 5%). The rats received humane care and all experimental procedures with animals in present study were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Xi'an Jiaotong University and approved by the Animal Ethics Committee of the University.

The rats were divided into four groups (control, $NaAsO_2$, Na_2SeO_3 and Na_2SeO_3 + $NaAsO_2$ groups; $n = 10$, equal number of male and female) randomly and fed with water containing designed chemicals. The control group was given drinking water, and the remaining groups were treated with $NaAsO_2$ (13.0 mg/l), Na_2SeO_3 (17.0 mg/l) or a combination of $NaAsO_2$ and Na_2SeO_3 in drinking water respectively. All the rats were given standard food and the water was available ad libitum. The dose of $NaAsO_2$ and the period of treatment were selected on the basis of previous studies (Santra et al., 2000; Pal and Chatterjee, 2004; Yousef et al., 2008; Luo et al., 2009), whereas Na_2SeO_3 dose was selected based on the clinical application and on results from previous studies on human and experimental animals (Biswas et al., 1999; Chattopadhyay et al., 2003).

During the experiment, the $NaAsO_2$ and Na_2SeO_3 containing water were changed and the volumes of consumed water were measured every day. The daily intake was calculated. The second group consumed water between 125 and 185 ml/(kg b.w)/d and $NaAsO_2$ intakes between 1.62 and 2.42 mg/(kg b.w)/d. The third group consumed water between 113 and 169 ml/(kg b.w)/d and Na_2SeO_3 intakes between 1.92 and 2.90 mg/(kg b.w)/d, whereas the last group consumed water between 95 and 145 ml/(kg b.w)/d, the $NaAsO_2$ intakes 1.24–1.90 mg/(kg b.w)/d and Na_2SeO_3 1.61–2.49 mg/(kg b.w)/d.

After 20 weeks, the rats were anesthetized with diethyl ether. Blood was collected by heart puncture for serum biochemical assays. The livers were removed. Liver fragments were immediately frozen in liquid nitrogen and stored at −80 °C for molecular analysis.

2.3. Assessment of liver damage

Serum was separated from blood by centrifugation at 3000 rpm for 15 min at 4 °C and kept in a freezer. To analyze hepatic function, activities of serum Aspartate transaminase (AST), alanine transaminase (ALT) and globulin (GLB) were measured using assay kits (Nanjing Jiancheng Bioengineering Company, Nanjing, China), according to the manufacturers' instructions.

2.4. Assessment of hepatic lipid peroxidation

Lipid peroxidation in liver was determined by measuring the amount of malondialdehyde (MDA) by the method of Bloom and Westerfe (1971) using a commercial MDA kit (Nanjing Jiancheng Bioengineering Company, Nanjing, China). The spectrophotometric absorbance was assessed at 532 nm in accordance with the manufacturer's instructions. About 0.5 g of liver sample was homogenized in 4.5 ml of ice-cold phosphate buffer saline for preparing liver homogenate, then the homogenates were centrifuged for 10 min at 3000 rpm and the supernatant was stored at −20 °C until assay. Protein concentration of homogenate was determined by the Bradford, 1976, and bovine serum albumin was used as the standard. The results were expressed as nmol MDA per mg protein.

2.5. Evaluation of hepatic antioxidant enzyme activity

The status of antioxidative defense systems in liver homogenate was evaluated by measuring following antioxidants. The activity of superoxide dismutase (SOD) was measured according to the method of Minami and Yoshikawa (1979). The activities of glutathione peroxidase (GPx) and catalase (CAT) were assayed by the methods of Paglia and Valentine (1967) and Cohen et al., 1970, respectively.

2.6. Real-time quantitative PCR

Total RNA was isolated from approximately 100 mg of frozen liver tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and extracted according to the manufacturer's instructions. RNA purity and concentration were determined by trace ultraviolet spectrophotometer. The cDNA was generated from total RNA using cDNA reverse transcription kit (Roche, Basel, Switzerland). After reverse transcription, the sample was heated to 95 °C for 5 min to denature the reverse transcriptase, and stored at −20 °C for PCR.

The primers for qRT-PCR are listed in Table 1. All primers were synthesized by Sangon Biological Engineering Co. Ltd. (Shanghai China).

The reactions were conducted in a final volume of 50 μl containing 5 μl of cDNA (200 ng/l), 0.5 μl of each primer (30 μmol/l), SYBR Green Master 25.0 μl (Roche, Basel, Switzerland) and RNAase free water 19.0 μl. Amplifications were preformed for all samples under the following conditions: 95 °C for 15 s, and 58.5 °C for 60 s, 72 °C for 2 min for 40 cycles. The results were normalized on β-actin gene expression (β-actin expression in samples is consistent). Relative mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.7. Western blotting analysis of TRx, HO-1 and hsp70 in liver

Total protein was extracted from the liver following Hummon's method (Hummon et al. 2007). The concentration of protein was measured by BCA reagent (Pierce Chemical Co., Rockford, IL, USA). For each sample, 20 μg of total protein extracts were diluted in SDS-sample buffer, denatured at 95 °C for 5 min, and separated with a 10% SDS–polyacrylamide gel at 110 V. After electrophoresis of the gel, the proteins were transferred to a PVDF (Polyvinylidene Fluoride) membrane (Millipore, Billerica, MA, USA) using a Semi dry trans blot apparatus (Bio-Rad) at 15 V for 30 min. Membrane was blocked in 5% non-fat milk in Tris-buffered saline(TBS: 25 mmol/l Tris, 150 mmol/l NaCl, 2 mmol/l KCl, pH 7.4) (Sigma, St. Louis, MO, USA) with 0.05% Tween-20 at 25 °C for 1 h, and washed three times with TBS/0.05% Tween-20 (TBST) for 5 min (each time). Membranes were incubated overnight with primary antibodies at 4 °C, washed in TBST and re-probed with secondary antibody at room temperature for 1 h. The proteins were visualized by the ECL system (Pierce Chemical Co., Rockford, IL, USA). The images were captured and the bands were quantified using Work-Lab software (UVP, Upland, CA, USA).

2.8. Statistical analysis

Data have been expressed as mean ± S.E. and n refers to the number of animals used. Statistical analysis of data was performed using SPSS 13.0 software. The significance of differences between means was analyzed by one-way analysis of variance (ANOVA) and means were compared with Duncan's multiple comparison post hoc test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of treatments on liver function

The activities of ALT and AST in blood are commonly used to evaluate the liver function. Table 2 shows that the activities of AST ($P < 0.05$) and ALT ($P < 0.05$) were increased in the arsenic-treated group compared to control, indicating liver damage in this group. The activities of ALT ($P < 0.05$) and AST were decreased in the combined arsenic and selenium group compared to that of the arsenic-treated group. This suggests that selenium eases the liver injury induced by arsenic (Table 2).

3.2. Effect of treatments on hepatic lipid peroxidation

Malondialdehyde (MDA) assay was routinely used to measure the extent of lipid peroxidation. Results in Table 3 shows that the MDA level was significantly ($P < 0.05$) increased in the