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

XU Zhao[a,b], WANG Zhou[c], LI Jian-jun[b], CHEN Chen[d], ZHANG Ping-chuan[a], DONG Lu[a], CHEN Jing-hong[a], CHEN Qun[a], ZHANG Xiao-tian[a], WANG Zhi-lun[a,*]

a.X'ian Jiaotong Univ, Sch Med, MinistEduc, Key Lab Environm & Genes Related Dis, Xi'an 710061, Shaanxi, Peoples R. China

b. Xi'an Jiaotong Univ, SchSci, Dept Chem, Xi'an 710049, Shaanxi, Peoples R. China

c. Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, Peoples R. China

d. Univ Queensland, Sch Biomed Sci, Brisbane, Qld 4072, Australia

* Corresponding author. Address: Xi'an Jiaotong University School of Medicine, No. 76 Yanta West Road, Xi'an, Shaanxi 710061, Peoples R China. Tel.: +862982655193.

E-mail address: leeb_01@163.com; wzlun@mail.xjtu.edu.cn (Z. Wang).

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.

***Key words*:** Na_2SeO_3; $NaAsO_2$; Liver; Oxidative stress; HSPs

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 (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 showed 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 (El-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 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℃) 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 (17.0mg /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 125~185 ml/ (kg b.w)/d and $NaAsO_2$ intakes between 1.62~2.42 mg/ (kg b.w)/d. The third group consumed water between 113~169 ml/ (kg b.w)/d and Na_2SeO_3 intakes between 1.92~2.90 mg/ (kg b.w)/d, whereas the last group consumed water between 95~145 ml/ (kg b.w)/d, the $NaAsO_2$ intakes 1.24~1.90mg/ (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℃ for molecular analysis.

2.3 Assessment of liver damage

Serum was separated from blood by centrifugation at 3000 rpm for 15 min at 4℃ 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 532nm in accordance with the manufacturer's instructions. About 0.5g of liver sample was homogenized in 4.5ml 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℃ until assay. Protein concentration of homogenate was determined by the Bradford method (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 (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℃ for 5 min to denature the reverse transcriptase, and stored at -20℃ for PCR.

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

Table 1

Primers sequences for qRT-PCR

Gene	Forward primer sequence	Reverse primer sequence	size (bp)
SOD1	5′−ACACAAGGCTGTACCACTGC−3′	5′−CCACATTGCCCAGGTCTCC−3′	103
CAT	5′−TGCCGTCCGATTCTCCACAG−3′	5′−TCCCACGAGGTCCCAGTTAC−3′	115
GSH-Px	5′−GTCCACCGTGTATGCCTTCTCC−3′	5′−TCTCCTGATGTCCGAACTGATTGC−3′	218
Txnrd1	5′−ACACAAAGCTTCAGCATGTCA−−3″	5′−CAATTCCGAGAGCGTTCC−3′	236
HSP70	5′−ATCTCCTGGCTGGACTCTAACA−3′	5′−CACCCATCTGTCTCCTAGATCA−3′	241
β-actin	5′−ACTATCGGCAATGAGCGGTTCC−3′	5′−CTGTGTTGGCATAGAGGTCTTTACG−3′	148

The reactions were conducted in a final volume of 50μl containing 5μl of cDNA (200ng/l), 0.5μl of each primer (30 μmol/l), SYBR Green Master 25.0μl (Roche, Basel, Switzerland) and RNAase free water 19.0μl. Amplifications were preformed for all samples under the following conditions: 95℃ for 15 s, and 58.5℃ for 60 s, 72℃ for 2 min for 40 cycles. The results were normalized on *β*-actin gene expression (*β*-actin expression in samples are 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℃ 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 15V for 30min. 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℃for 1 hr, and washed three times with TBS/0.05% Tween-20 (TBST) for 5min (each time). Membranes were incubated overnight with primary antibodies at 4℃, washed in TBST and re-probed with secondary antibody at room temperature for 1 hr. 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 activitties 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 activitties 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).

Table 2

Effect of sodium selenite on serum biomarker enzymes in experimental chronic arsenic poisoning rat liver

Treatment groups	Numbers of rats	ALT(U/l)	AST(U/l)	GLB(g/l)
Control	10	38.6 ± 6.8	116.9 ± 10.1	28.4 ± 1.2
$NaAsO_2$	10	62.7 ± 7.4[a]	145.1 ± 12.3[a]	29.1 ± 1.5
Na_2SeO_3	10	40.6 ± 3.7[b]	112.7 ± 7.8[b]	28.7 ± 1.1
$NaAsO_2 + Na_2SeO_3$	10	44.7 ± 3.2[b]	126.1 ± 23.5	27.8 ± 1.8

Data are mean ± S.E.

a $P < 0.05$ vs. control group.

b $P < 0.05$ vs. $NaAsO_2$ group.

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 arsenic-treated group compared to the other groups. Combined treatment with arsenic and selenium caused a significant ($p < 0.05$) decrease in the MDA level of liver tissue compared to

that in liver tissues from rats treated with only arsenic (Table 3).

3.3. Effect of treatments on hepatic antioxidant enzyme activity

Three antioxidant enzymes (SOD, CAT and Gpx) of rat liver were determined, and the results are shown in Table 3. All the antioxidant enzyme activities were reduced significantly ($p < 0.05$) in the arsenic-treated group compared to the control, but only Gpx shows a significant ($p < 0.05$) recovery in response to the presence of selenium and arsenic. The hepatic SOD and CAT activities of the combined treatment group are similar to arsenic-treated levels, and these changes did not reach statistical significance.

Table 3

Effect of sodium selenite on liver oxidative stress and antioxidant enzyme activity in experimental chronic arsenic poisoning rat

Treatment groups	Numbers of rats	SOD(U/mg protein)	CAT(U/mgprotein)	GPx(U/mg protein)	MDA(nmol/mg protein)
Control	10	507.6 ± 18.1	24.5±1.9	313.1 ± 32.1	4.1 ± 1.0
$NaAsO_2$	10	462.7 ± 26.7[a]	18.3±1.5[a]	106.8±21.7[a]	6.3 ±0.9[a]
Na_2SeO_3	10	484.2 ± 38.9	19.2 ± 3.7[a]	293.2 ± 19.1[b]	4.7 ±0.8[b]
$NaAsO_2 + Na_2SeO_3$	10	478.5 ±31.3	20.3 ± 2.7[a]	282.7 ± 36.6[ab]	4.6 ± 1.3[b]

Data are mean ± S.E.

a $P < 0.05$ vs. control group.

b $P < 0.05$ vs. $NaAsO_2$ .group.

3.4. Effect on expression of oxidative stress –related genes in liver

The mRNA expression of SOD1, CAT, GPx, Txnrd1 and HSP70 were measured by

Real-time quantitative PCR. The results showed that the expression of all of the oxidative stress related genes in $NaAsO_2$ treated rats were reduced significantly compared with control group, except the expression of HSP70, which increased significantly ($P < 0.05$). Compare to the $NaAsO_2$ group, the expressions of SOD1, CAT, GPx, Txnrd1 mRNA were increased significantly in the group with combined treatment of $NaAsO_2$ and Na_2SeO_3, whereas mRNA expression of HSP70 decreased significantly ($P < 0.05$) (Fig. 1) .

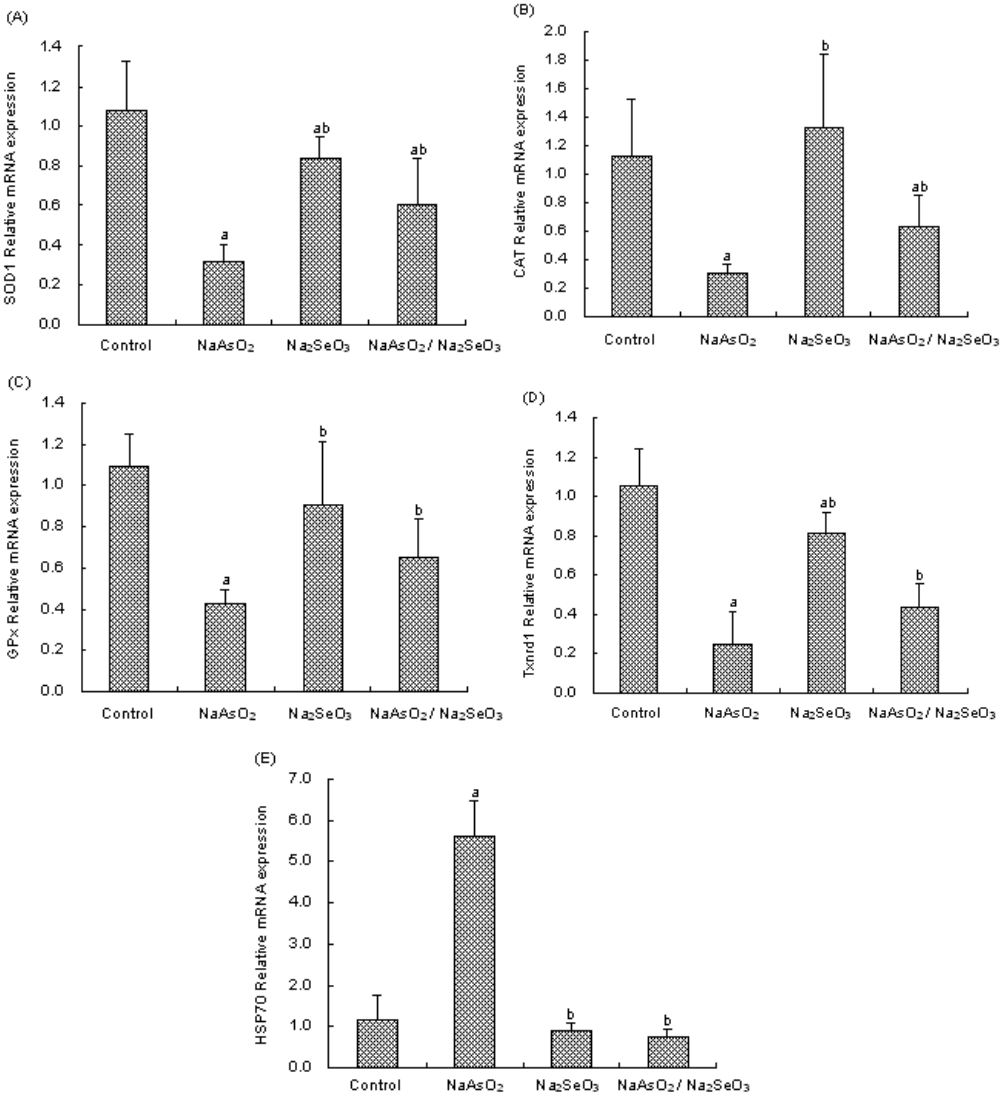
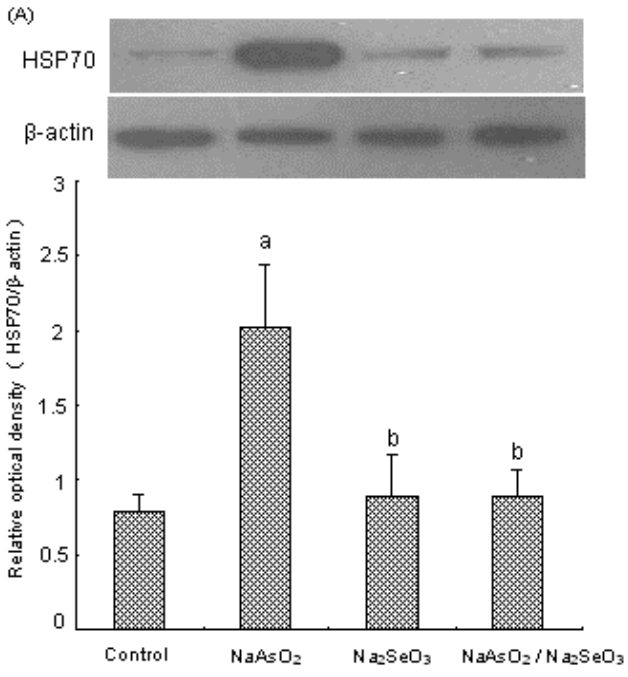


Fig. 1. Effects of sodium selenite on oxidative stress-related genes expression of experimental rat livers.(A) Real-time RT-PCR analysis of SOD1 mRNA expression in different treated groups. (B) Real-time RT-PCR

analysis of CAT mRNA expression in different groups. (C) Real-time RT-PCR analysis of GPx mRNA expression in different groups. (D) Real-time RT-PCR analysis of Txnrd1 mRNA expression in different groups. (E) Real-time RT-PCR analysis of HSP70 mRNA expression in different groups. All data are expressed as means ± S.E., a $P < 0.05$ vs. control group, b $P < 0.05$ vs. $NaAsO_2$ group.

3.5. Effect on expression of TrxR and Heat Shock Proteins

Comparing with the control group, HSP70 and HO-1 protein levels in the $NaAsO_2$ group were increased significantly ($P < 0.05$), but the expression of TrxR protein was decreased. Comparing with the $NaAsO_2$ group, the heat shock proteins levels in the combined treatment group with $NaAsO_2$ and Na_2SeO_3 were decreased significantly ($P < 0.05$), whereas TrxR protein expression was increased (Fig. 2).



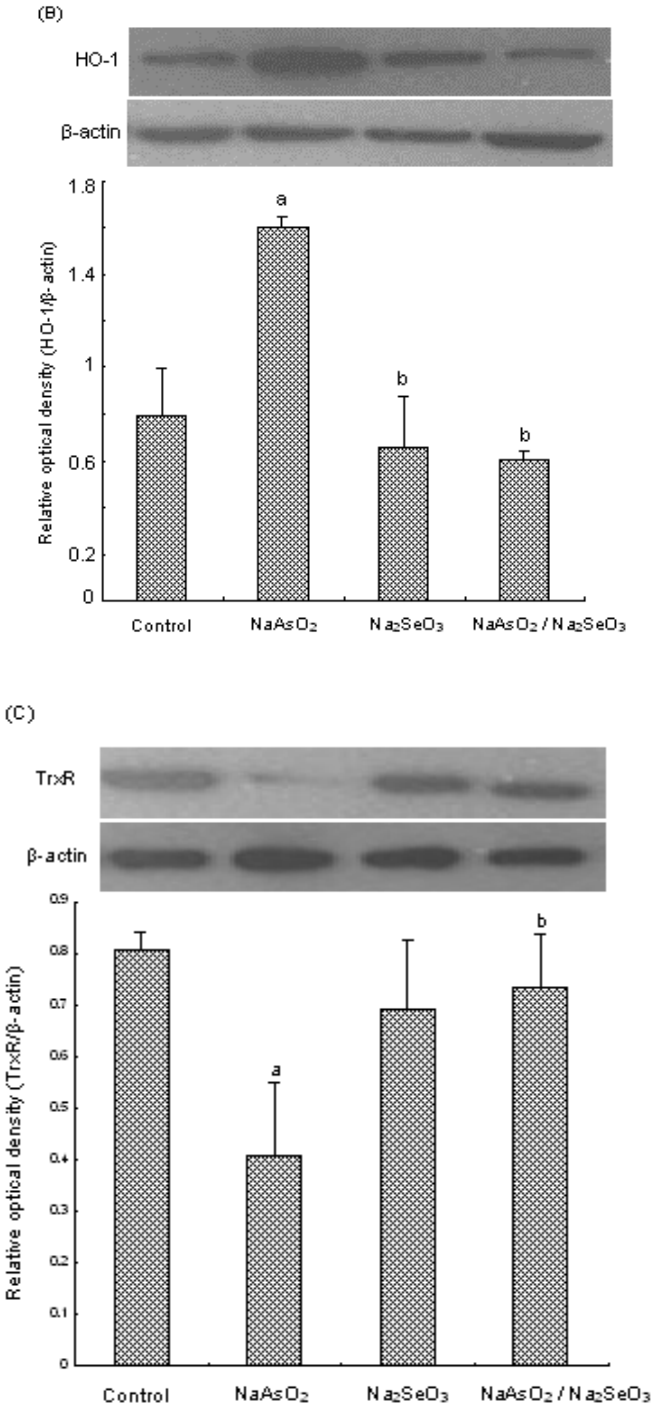


Fig. 2. Effect of sodium selenite on expression of Heat Shock Proteins and TrxR of experimental rat livers.(A) Western blot analysis and semiquantitative analysis of HSP70 in rat livers of control and treated groups.(B) Western blot analysis and semiquantitative analysis of HO-1 in rat livers of control and treated groups. (C) Western blot analysis and semiquantitative analysis of TrxR in rat livers of control and treated groups. The optical density of protein bands were normalized to that of β-actin. All data are expressed as means ± S.E.,

n⩾3 relative optical density value, a P < 0.05 vs. control group, b P < 0.05 vs. $NaAsO_2$ group.

4. Discussion

In the present study, rats treated for 20 weeks with $NaAsO_2$ containing water *ad libitum* established a situation similar to human chronic arsenic poisoning(Santra et al., 2000; Luo et al., 2009). An investigation by Chen (2005) indicated that although the consumption of arsenic-contaminated water is different among different individuals who live in high-arsenic areas, the gene-gene and gene-environment interactions are involved in arsenic-induced health hazards through toxicological mechanisms including genomic instability and oxidative stress. Believing these gene-environmental interrelationships, we provided *ad libitum* water in this study and measured liver function, hepatic lipid peroxidation, hepatic antioxidant enzyme activity and some oxidative stress -related genes at molecular levels. The results suggested that long-term intake of $NaAsO_2$ causes oxidative damage in rat liver, which can be alleviated by treating with Na_2SeO_3.

The amount of ALT and AST in blood is used to evaluate the liver function. The results showed that ALT and AST in serum increased in $NaAsO_2$ group and decreased in combined treatment of Na_2SeO_3 and $NaAsO_2$. It is therefore suggested that long-term intake of $NaAsO_2$ causes oxidative damage in rat liver which can be alleviated by treating with Na_2SeO_3.

Increasing evidence has showed that oxidative stress is one of the important mechanisms of chronic arsenic poisoning. Oxidative stress is the term generally used to describe an imbalanced state between the oxidation system and antioxidation system, in which cellular antioxidant defenses are inadequate to inactivate the reactive oxygen species (ROS). Therefore, ROS overproduction or antioxidant reductions are the direct causes of oxidative stress. Many factors may cause the production of ROS, including arsenic poisoning (Liu et al., 2001; Li et al, 2002;

Flora et al, 2008). The literature (Nandi et al, 2006) indicates that arsenic exerts its toxicity by generating ROS such as superoxide, hydroxyl and peroxyl radicals during the metabolism of arsenic, which results in the oxidative damage of DNA and different tissues. In the present study, rats were exposed to arsenic through drinking water for 20weeks. The increase of MDA and the decrease in the enzymatic activities of GPx, SOD and CAT in the liver confirmed that the rats suffered oxidation damage after long-term exposure to inorganic arsenic. These results are in line with the findings of Santra (Santra et al., 1999; 2000) and suggest that long-term excessive arsenic intake leads to the lipid peroxidation enhancement and the oxidative damage in the liver.

MDA is the production of lipid peroxidation. Increased MDA level is an index of enhanced lipid peroxidation (Kamalakkanan and Prince, 2004). In our findings, $NaAsO_2$ treatment in the experiment resulted in a significant increase in the level of MDA in serum. Meanwhile, Na_2SeO_3 suppressed lipid peroxidation. It means that selenium evoked an antioxidant effect and eliminated oxide such as hydrogen peroxide and lipid peroxide (Mates et al., 2000). This detoxification occurs likely because of the formation of seleno-bis(S-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$ in the mammalian bloodstream(Xue et al., 2010).

Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. SOD, CAT and GPx are the important antioxidant enzymes in the liver. Among them, SOD reduces superoxide radicals to water and molecular oxygen. SOD presents in three isoforms, copper-zinc-containing superoxide dismutase (Cu-Zn-SOD, SOD1), manganese containing superoxide dismutase (Mn-SOD, SOD2) and extracellular superoxide dismutase (EC SOD SOD3) The SOD1 isoenzyme is the most abundant one in the cytoplasm（Bera et al., 2011）. Reduction of SOD activity in arsenic-exposed animals in this experiment might be due to the enhanced

production of superoxide radical anions. While CAT reduces hydrogen peroxide, the activity of CAT has been decreased during arsenic poisoning due to deficiency of NADH. GPx is an antioxidant enzyme, which modifies the poisonous peroxide to a non-toxic hydroxyl compound in order to protect the membrane structure and function. The decrease in the activity of GPx during the period of arsenic treatment in this experiment indicated an increase in the levels of peroxide. If the antioxidant ability of cells is insufficient, then supplements are necessary to sustain the balance of oxidative stress.

Selenium is an essential trace element of human and many animals and is an antioxidant, which removes hydrogen peroxide and lipid peroxide. Selenium is also an important component of GPx, located in the active center of the enzyme. Therefore, the action of antioxidant enzyme on GPx depends on the content of selenium. Our epidemiological survey (Xue et al., 2010) on chronic arsenic toxicity in human revealed that inorganic arsenic exposure was associated with oxidative stress and the antioxidant enzyme activity of people exposed to arsenic was enhanced in high selenium state. Other reports (Biswaset al., 1999; Messarahet al., 2012) on animal models also showed selenium played an important role in protecting the hepatic cellular injury induced by arsenic. In China, some clinical investigations (Hou et al., 2000; Wang et al., 2009) showed that selenium had a clear curative effect on liver injury caused by endemic arsenic. In the present study, the results showed that selenium reduced oxidative damage in the rat liver caused by arsenic poisoning through improving the activity of antioxidant enzymes and reducing the expression of HSPs and other oxidative stress related genes in rat liver. However, selenium may be toxic if taken in excess (Wycherly et al., 2004; Letavayova et al., 2008; Valdiglesias et al., 2010). Different chemical forms of selenium have been reported (Biswas et al., 2000; Kim et al., 2007; Machado et

al., 2009) to produce variety of genotoxic effects and there is no sufficient data to determine the optimal dose of selenium. Thus, potential hazard of selenium should be considered carefully and further study is necessary to estimate the right dose range of selenium in the treatment of arsenic toxicity.

In present study, some proteins and genes related to oxidative stress were also investigated. Thioredoxin (Trx) is an important index, which represents the body oxidative stress levels (Nakamura, 2005). Thioredoxin systems involves redox active of thioredoxins and thioredoxin reductases. Mammalian thioredoxin reductases are selenium-containing flavoproteinoxido reductases, dependent on a selenocysteine residue for reduction of the active site disulfide in thioredoxins (Arner, 2009). Masatoshi (Masatoshi et al., 2003) examined alterations in enzyme activities of hepatic thioredoxin reductase from prolonged exposure of male rabbits to inorganic arsenate and the results showed a significant suppression of hepatic thioredoxin reductase activities. Our results showed that the Txnrd1 mRNA level was sharply reduced in the $NaAsO_2$ group as compared with the control group, whereas selenium attenuated this reduction. The Thioredoxin Reductases(TrxR) expression was significantly decreased in the $NaAsO_2$ group and reversed in selenium intervention group. It is therefore suggested that the oxidative damage induced by arsenic should be at least partly attributable to the lack of Txnrd, and selenium may have an ability to antagonize arsenic induced oxidative stress through increase in the TrxR levels.

Heat Shock Proteins (HSPs) were synthesized from stress genes under stress response conditions. In general, the major HSPs are expressed at low levels and functional as molecular chaperones (Hendrick and Hartl, 1993; Gupta et al., 2010). Under stress conditions, the newly synthesized stress proteins play an essential role in maintaining cellular homeostasis by assisting

in the correct folding of nascent and stress-accumulated misfolded proteins (Schlesinger, 1990; Morimoto, 1993; Sikora and Grzesiuk, 2007; Salujaand Dudeja, 2008). It is clear that heavy metals cause expression of heat shock proteins (Mutwakil et al., 1997). Both HSP32 and HSP70 are activated in the cellular defense mechanisms against stress injury caused by arsinc (Del Razo et al., 2001). The HSP70 is one of the most abundantly induced proteins under a variety of stress conditions and the most extensive oxidative stress marker at present (Lachapelle et al., 2007; Webb and Gagnon, 2009), and HO-1 is recognized as the major isoform of Hsp32. The effective capacity of arsinc to induce HO-1 synthesis has been fully demonstrated both in vitro and in vivo (Conner et al., 1993; Menzel et al., 1998; Kitchin et al., 1999; Liu et al., 2001). In the present study, the expression of HSPs mRNA and protein were increased by 5 and 3 folds in the arsenic group compared with the control group, respectively. The expression difference of HSPs mRNA and protein between the arsenic-treated group and the combined treatment group was analyzed to estimate the effect of selenium. It showed that selenium prevented the occuring of oxidative damage from arsenic by significantly reducing the expression of HSPs mRNA and protein. However, the expression of every HSP was not decreased by high selenium status. The epidemiological survey (Chen et al., 2009) showed that selenium was an inducer of HSP70 and the expression of HSP70 in human peripheral blood mononuclear cells was increased too. Further studies will have to be performed to reveal the mechanism of selenium on different heat shock proteins.

5. Conclution

The results obtained in the present study provide further evidence that long-term intake of

inorganic arsenic causes oxidative damage in rat liver cells and Na_2SeO_3 protects the cells by adjusting the expression of oxidative stress related genes and improving the activities of antioxidant enzymes.

Acknowledgments

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