BIOMEDICAL AND ENVIRONMENTAL SCIENCES **19**, 197-204 (2006)

DNA Damage, Apoptosis and C-myc, C-fos, and C-jun Overexpression Induced by Selenium in Rat Hepatocytes[1]

RI-AN YU, CHENG-FENG YANG, AND XUE-MIN CHEN

Department of Occupational and Environmental Health, MOE Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China

Objective To study the effects of selenium on DNA damage, apoptosis and c-myc, c-fos, and c-jun expression in rat hepatocytes. **Methods** Sodium selenite at the doses of 5, 10, and 20 μmol/kg was given to rats by i.p. and there were 5 male SD rats in each group. Hepatocellular DNA damage was detected by single cell gel electrophoresis (or comet assay). Hepatocellular apoptosis was determined by TUNEL (TdT-mediated dUTP nick end labelling) and flow cytometry. C-myc, c-fos, and c-jun expression in rat hepatocytes were assayed by Northern dot hybridization. C-myc, c-fos, and c-jun protein were detected by immunohistochemical method. **Results** At the doses of 5, 10, and 20 μmol/kg, DNA damage was induced by sodium selenite in rat hepatocytes and the rates of comet cells were 34.40%, 74.80%, and 91.40% respectively. Results also showed an obvious dose-response relationship between the rates of comet cells and the doses of sodium selenite (r=0.9501, P<0.01). Sodium selenite at the doses of 5, 10, and 20 μmol/kg caused c-myc, c-fos, and c-jun overexpression obviously. The positive brown-yellow signal for proteins of c-myc, c-fos, and c-jun was mainly located in the cytoplasm of hepatocytes with immunohistochemical method. TUNEL-positive cells were detected in selenium-treated rat livers. Apoptotic rates (%) of selenium-treated liver cells at the doses of 5, 10, and 20 μmol/kg were (3.72 ± 1.76), (5.82 ± 1.42), and (11.76 ± 1.87) respectively, being much higher than those in the control. Besides an obvious dose-response relationship between apoptotic rates and the doses of sodium selenite (r=0.9897, P<0.01), these results displayed a close relationship between DNA damage rates and apoptotic rates, and the relative coefficient was 0.9021, P<0.01. **Conclusion** Selenium at 5-20 μmol/kg can induce DNA damage, apoptosis, and overexpression of c-myc, c-fos, and c-jun in rat hepatocytes.

Key words: Selenium; DNA damage; Apoptosis; C-myc; C-fos; C-jun

INTRODUCTION

Selenium, an important nutritional trace element, is an essential component of glutathione peroxidases and thioredoxin reductases. Selenium-dependent glutathione peroxidases and thioredoxin reductases protect the body from cellular metabolism of the endogenous products that has been implicated in DNA damage, mutagenesis, and carcinogenesis[1]. Numerous studies in experimental animal models indicate that high levels of selenium compounds prevent cancer. Most importantly, clinical intervention studies using different selenium compounds (for example, se-enriched yeast or selenite) in China and USA have established chemopreventive activity of selenium compounds against cancers in humans. Epidemiologic evidence, laboratory bioassays, and human clinical intervention trials support a protective role of selenium against cancer development in intestines, prostate, lung, and liver[2-4].

Several hypotheses have been proposed to account for the selenium-mediated inhibition of tumorigenesis. As a constituent of selenoproteins, selenium plays a role as an antioxidant. Studies have also demonstrated that selenium stimulates apoptosis and enhances carcinogen detoxification at pharmacologic doses[5]. Despite decades of research into the mode of action of selenium, detailed mechanisms underlying its cancer chemopreventive activity remain largely unclear. Selenium deficiency has been implicated in the development of many

[1]This work was supported by National Natural Science Foundation of China (No. 30271110, 30471500).

Correspondence should be addressed to Dr. Ri-An YU and Prof. Xue-Min CHEN, Department of Occupational and Environmental Health, MOE Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China. E-mail: yurian.tj@163.com

Biographical note of the first author: Ri-An YU, male, born in, 1964, Ph. D., professor, majoring in environmental and molecular toxicology. Tel: 86-27-83692715. Fax: 86-27-83692701.

0895-3988/2006
CN 11-2816/Q

diseases, including cancer, cardiovascular, and immune disorders[6]. Low selenium status results in a differential gene expression pattern indicative of activation of genes involved in DNA damage, oxidative stress, and cell cycle control, and a decrease in expression of genes involved in detoxification[7]. While selenium compounds, for example selenite, are supplemented at lower concentrations, the major effects of selenite appear to be related to its role as a micronutrient[2-4]. At intermediate concentrations, selenite appears to exert its chemopreventive activity[2-4]. At higher concentrations, selenite induces oxidative stress and may become toxic[8]. Excess intake of selenium may result in oxidative damage leading to genomic instability[9]. In this study we investigated the effects of sodium selenite at the doses of 5, 10, and 20 μmol/kg on DNA damage, apoptosis, and overexpression of c-myc, c-fos, and c-jun in rat hepatocytes.

MATERIALS AND METHODS

Animal Handling

Male Sprague-Dawley rats weighing approximately 200±50 g were used in all experiments. Rats were housed in polycarbonated cages with compressed fiber bedding with free access to commercial pelleted diet and water. Four groups were used and each group had five animals. The first (control) group received no selenite treatment, the second received Na_2SeO_3 at the dose of 5 μmol/kg, the third received Na_2SeO_3 at the dose of 10 μmol/kg, and the last received Na_2SeO_3 at the dose of 20 μmol/kg. Na_2SeO_3 was prepared in 0.9% NaCl and administered intraperitoneally (i.p.) and the animals in the control group received equal volume of 0.9% NaCl. The animals were sacrificed two days after the initial injections and their livers were removed immediately for use.

SCGE Assay

Selenium-induced DNA damage in the liver was analyzed by single cell gel electrophoresis or comet assay. Comet assay was carried out as previously described[10]. Briefly, fully frosted slides were covered with 80 μL of 0.65% normal melting point agarose (NMA) in Ca^{2+} and Mg^{2+} free PBS (pH 7.4) as first layer. After solidification, 80 μL of mixture of freshly prepared cell suspension (10 μL,1-5×10^6/mL) with 70 μL of 0.65% low melting point agarose (LMA) in Ca^{2+} and Mg^{2+} free PBS (pH 7.4) was rapidly pipetted onto the first layer as the second layer, and then covered with coverslip and kept at 4℃ for 5 minutes. After removal of the coverslip, 80 μL of 0.65% LMA (without cells) was added onto cell layer as the third layer. After solidification at 4℃ and removal of coverslip, the slides were lysed in freshly prepared ice-cold lysing solution for 1 hour, removed and placed in a horizontal electrophoresis tank side by side, avoiding space and with the agarose end facing the anode. The tanks were filled with freshly prepared electrophoresis solution, and the slides were left in the solution for 20 minutes to allow the unwinding of DNA and expression of alkali labile damage before electrophoresis. Electrophoresis was conducted at 4℃ for 20 minutes using 25 V and the current was adjusted to 0.3 A by raising or lowering the buffer level. After electrophoresis, the slides were neutralized 3 times in 0.4 mol/L Tris-HCl buffer (pH 7.5), and then 45 μL of 20 μg/mL ethidium bromide was added to each slide and covered with a coverslip. The slides were placed in a humidified air-tight container at 4℃ and examined within 2 days. Slides were examined at 400× magnification under a fluorescence microscope (Nikon, Japan). Images of 100 randomly selected cells were analyzed. The degree of DNA damage was graded by naked eye into 5 categories according to the amounts of DNA in the tail: Grade 0: no damage, <5%; grade 1: low level damage, 5%-20%; grade 2: medium level damage, 20%-40%; grade 3: high level damage, 40%-95%; grade 4: total damage, >95%.

Flow Cytometry

It is well established that DNA fragmentation during apoptosis may lead to extensive loss of DNA content and distinct sub-G1 peak when analyzed by flow cytometry. Selenium-induced apoptosis was evaluated by the determination of sub-G1 cells. At the end of various designated treatments, cells were washed, fixed and permeated with 70% ice-cold ethanol at 4℃ for 2 hours. Cells were then incubated with freshly prepared propidium iodide (PI) staining buffer (0.1% Triton X-100, 200 μg/mL RNase A, and 20 μg/mL PI in PBS) for 15 min at 37℃, followed by flow cytometry analysis of 20 000 cells in each group. The histogram was abstracted and percentage of cells in the sub-G1 phase was then calculated to reflect the apoptotic cells.

TUNEL

Apoptosis was demonstrated *in situ* by TUNEL assay. The 3′-hydroxy end of DNA fragments was labelled with deoxy-UTP-digoxigenin and the digoxigenin was detected using specific antibodies and the peroxidase-diaminobenzidine system. TUNEL assay was performed using a test kit from Boehringer Mannheim according to the manufacturer's

instructions. Briefly, sections were deparaffinized in xylene and hydrated in graded alcohol. Endogenous peroxidase was blocked with 2% H_2O_2 in PBS. Terminal deoxynucleotidyl transferase (TdT) and digoxigenin-labelled dUTP were applied to the sections for 1 h at 37℃. Sections were washed in wash buffer, treated with peroxidase-conjugated anti-digoxigenin antibody for 1 h at room temperature, stained lightly with diaminobenzidine (DAB), dehydrated in alcohol series, cleared in xylene, and mounted in Permount.

RNA Extraction

Total RNA was isolated using TRIzol reageat. Briefly, 10-100 mg of fresh liver tissue was homogenized in a homogenizer. Following homogenization, the homogenate was stored for 5 min at 4℃ to permit the complete dissociation of nucleoprotein complexes. Then 0.2 mL of chloroform was added, samples were covered tightly, vigorously shaken for 15 s and placed on ice at 4℃ for 5 min. The mixture was centrifuged at 12 000 g (4℃) for 15 min. After centrifugation, volume of the aqueous phase was 40%-50% of the total volume of the homogenate plus chloroform. The aqueous phase was carefully transferred to a fresh tube and an equal volume of isopropanol was added, and stored for 10 min at 4℃. The samples were centrifuged at 12 000 g for 10 min. RNA precipitation formed a white pellet at the bottom of the tube. After the supernatant was removed, the RNA pellet was washed twice with 1 mL of 75% ethanol by swirling and subsequent centrifugation for 5 min at 7500 g (4℃). At the end of the procedure, the pellet was briefly dried under a vacuum for 5-10 min. It is important to prevent the RNA pellet from drying completely, as it greatly decreases its solubility. At last, the RNA pellet was dissolved in 50-100 μL DEPC-treated water by swirling for 1 min. Purity of the RNA was determined by the ratio of the absorbance at 280 and 260 nm. RNA yield was calculated based on the absorption at 260 nm.

Northern Dot Hybridization

Aliquots of total RNA (60 μg) were denatured for 15 min at 68℃ in 50% formamide, 17% formaldehyde, and 1×saline sodium citrate (SSC; 0.015 mol/L sodium citrate, 0.15 mol/L NaCl, pH 7). Then, denaturated RNA was blotted on nylon membranes with successive decreasing dilutions. RNA was fixed by irradiating the membranes with uv light. The cDNA probes of c-myc, c-fos, and c-jun were labelled with digoxigenin by random priming labelling kit. RNA blots were prehybridized for 2 h at 42℃ in 20% formamide with 3×saline sodium phosphate and ethylene diaminetetraacetic acid solution, 1% SDS, 5×denhardt's in the presence of 0.1 mg/mL salmon sperm DNA. RNA blots were then hybridized for 18 h at 42℃ in the same solution containing 9% of dextran sulfate and the digoxigenin-labelled probe. Membranes were then washed four times at 20℃ for 5 min in 2×SSC, 0.1% of SDS, and then, two times at 55℃ for 20 min in 1×SSC with 0.1% of SDS. For color reaction, diaminobenzidine (DAB) was used. Levels of mRNA were quantified by densitometry.

Immunochemistry

The sections were deparaffinized in xylene, hydrated in grated ethanol continuously, and then covered with 3% hydrogen peroxide in PBS to block the endogenous peroxidase activity for 10 min. The sections were pretreated in citrate buffer (0.01 mol/L, pH 6.0) under microwave heating for 20 min to retrieve the antigen. Normal goat serum was added to the slides for 30 min at room temperature. After that, the sections were incubated overnight at 4℃ with monoclonal primary antibody, anti-c-myc, anti-c-fos, and anti-c-jun (Santa Cruez). SP kit was purchased from Beijing Zhongshan Biotechnology Ltd. (China). After the slides were washed three times in PBS for 5 min each, the biotinylated goat secondary antibody was added for 30 min followed by avidin-biotinylated peroxidase complex for another 30 min at room temperature. After washed with PBS, the slides were stained with DAB, and then counterstained with haematoxylin.

Statistical Analysis

The data were tested with statistical programs. Student's *t*-test or Chi-square test was used and *P* value <0.05 was defined as statistically significance.

RESULTS

DNA Damage Induced by Selenium in Rat Hepatocytes

Table 1 shows the effects of sodium selenite at the doses of 5, 10, and 20 μmol/kg on DNA damage. Statistically significant differences were seen between three dose levels and the control, $P<0.01$. There was an obvious dose-response relationship and statistical analysis yielded a relative coefficient value of 0.9501 ($P<0.01$) for the dose range examined.

Hepatocyte Apoptosis Induced by Selenium in Rats

Table 2 shows the effects of sodium selenite at the doses of 5, 10, and 20 μmol/kg on hepatocyte

TABLE 1

Effects of Selenium on DNA Damage in Rat Hepatocytes

Groups	Cell Number	Grades of DNA Damage					Rates of Comet Cells
		0	1	2	3	4	
Na_2SeO_3 (5 μmol/kg)	500	328	95	54	9	14	34.40**
Na_2SeO_3 (10 μmol/kg)	500	126	165	92	54	63	74.80**
Na_2SeO_3 (20 μmol/kg)	500	43	68	106	131	152	91.40**
Control (0 μmol/kg)	500	455	35	7	3	0	9.00

Note. ** $P<0.01$, *vs* control.

TABLE 2

Apoptosis Induced by Selenium in Rat Hepatocytes ($\bar{x} \pm s$)

Groups	Animal Number	Apoptotic Rates
Na_2SeO_3 (5 μmol/kg)	5	3.72 ± 1.76*
Na_2SeO_3 (10 μmol/kg)	5	5.82 ± 1.42**
Na_2SeO_3 (20 μmol/kg)	5	11.76 ± 1.87**
Control (0 μmol/kg)	5	2.22 ± 0.43

Note. * $P<0.05$, ** $P<0.01$, *vs* control.

apoptosis. There was a statistically significant difference between the selenium-treated groups and the controls in apoptotic rates and the dose-response relationship was obvious. Statistical analysis yielded a relative coefficient value of 0.9897, $P<0.01$. The relative coefficient between DNA damage rates and apoptotic rates was 0.9021, $P<0.01$.

TUNEL method was utilized to immunologically detect individual cells in the three selenium-treatment groups. Representative results are depicted in Fig. 1. The black, brown, and dark-brown immunoreaction products, indicative of TUNEL positivity, were observed in the groups treated with sodium selenite at the doses of 5, 10, and 20 μmol/kg.

Overexpression of c-myc, c-fos, and c-jun mRNA Induced by Selenium in Rat Hepatocytes

Table 3 shows the effects of sodium selenite at the doses of 5, 10, and 20 μmol/kg on c-myc, c-fos, and c-jun mRNA expression in rat hepatocytes. Value of mRNA was expressed as integrated optical density (IOD). IODs of c-myc mRNA, c-fos mRNA, and c-jun mRNA induced by sodium selenite at the doses of 5, 10, and 20 μmol/kg were higher than those in control. Representative results of Northern dot hybridization are depicted in Fig. 2. The positive brown-yellow signal for c-myc, c-fos, and c-jun mRNA expression products. Proteins of c-myc, c-fos, and c-jun were mainly located in the cytoplasm of hepatocytes. Representative results are depicted in Fig. 3.

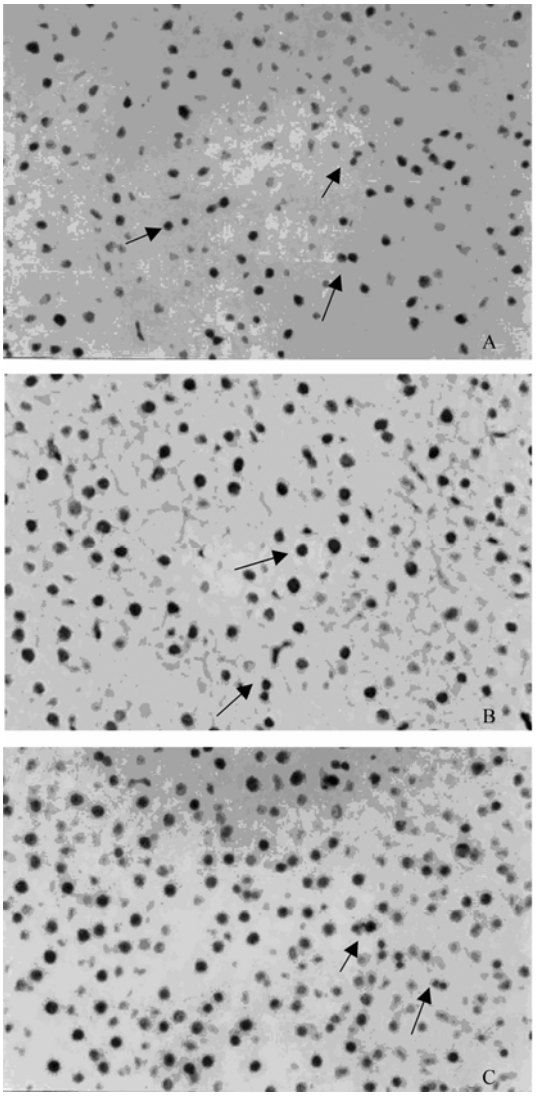

FIG. 1. Apoptosis induced by sodium selenite at the doses of 5 (A), 10 (B), and 20 μmol/kg (C) with TUNEL method.

TABLE 3

Effects of Selenium on C-myc, C-fos, and C-jun mRNA Expression in Rat Hepatocytes ($\bar{x} \pm s$)

Groups	Value of Integrated Optical Density (IOD)		
	C-myc mRNA	C-fos mRNA	C-jun mRNA
Na_2SeO_3 (5 μmol/kg)	$20.35 \pm 7.01^{*}$	$13.37 \pm 4.93^{*}$	$15.67 \pm 6.46^{\triangle}$
Na_2SeO_3 (10 μmol/kg)	$21.82 \pm 5.28^{*}$	$15.15 \pm 4.02^{**}$	$17.81 \pm 4.37^{*}$
Na_2SeO_3 (20 μmol/kg)	$19.98 \pm 5.84^{*}$	$19.88 \pm 5.01^{**}$	$19.23 \pm 6.53^{*}$
Control (0 μmol/kg)	8.89 ± 3.19	6.01 ± 1.41	9.16 ± 2.56

Note. $^{\triangle}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$, *vs* control.

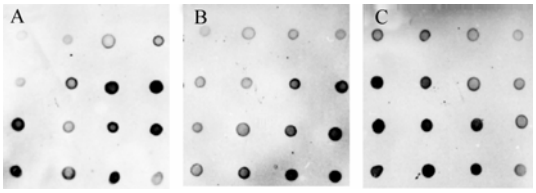

FIG. 2. C-myc (A), c-fos (B), and c-jun (C) mRNA overexpressions induced by sodium selenite. In each picture, from upper to bottom, the first line represents control, the second line represents 5 μmol/kg sodium selenite, the third line represents 10 μmol/kg sodium selenite, the last one represents 20 μmol/kg sodium selenite.

DISCUSSION

Several research reports have described DNA damage as measured by the demonstration of single strand DNA breaks and DNA ladders[11-12]. Our results showed that sodium selenite at the doses of 5, 10, and 20 μmol/kg could cause DNA single strand breaks in hepatocytes of rats and there was an obvious dose-response relationship. Selenium is an essential dietary nutrient for all mammalian species, but is also toxic *in vivo* and to cells in culture[9,13]. Wycherly *et al*.[14] showed that high dietary intake of inorganic selenium as sodium selenite may promote *in vivo* DNA oxidation. Our results are consistent with their finding and support the hypothesis that high selenium intake may be toxic to rat hepatocytes. Toxicity of selenium occurs due to its prooxidant ability to catalyze the oxidation of thiols and simultaneously generates，superoxide (O_2^-). Cytotoxicity of selenium, being dose-dependent, is limited to those compounds that can generate the selenide anion by metabolism or by reduction of diselenides[9]. Inorganic forms of selenium undergo reductive metabolism through a number of intermediate steps leading to the generation of hydrogen selenide. Depending on cellular requirements for selenium, hydrogen selenide is either used for selenoprotein

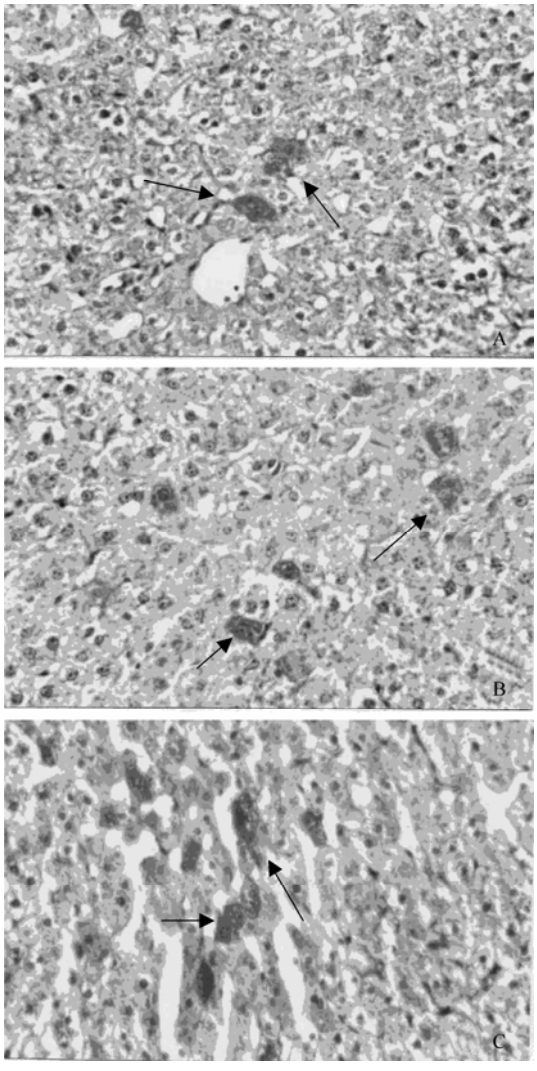

FIG. 3. C-myc (A), c-fos (B), and c-jun (C) mRNA expression products, proteins of c-myc, c-fos, and c-jun induced by sodium selenite.

synthesis or is further metabolized via methylation in the process of its elimination from the cells. The complete methylation of selenium occurs in three steps in which methylselenol, dimethylselenide, and

trimethylselenonium are formed. This methylation pathway is considered detoxifying[15]. Spallholz and Hoffman[16] have summarized the toxicological mechanism of selenium. Hydrogen selenide is a key intermediate in the selenium methylation of inorganic and organic selenium compounds in animals. Hydrogen selenide accumulates in animals receiving excess selenocysteine as a consequence of inhibition of selenium methylation. Excess hydrogen selenide contributes to hepatotoxicity and contributes to other selenium-related injuries. Another important mechanism appears to involve the formation of CH_3Se^- which either enters a redox cycle and generates superoxide and oxidative stress, or forms free radicals that bind to and inhibit important enzymes and proteins. We studied the effects of sodium selenite on production of superoxide anion ($\cdot O_2^-$) and hydroxyl free radical ($\cdot OH$) in rat hepatocytes *in vivo* and *in vitro*. The results showed that selenium at certain doses could inhibit the production of free radicals and improve the state of oxidative stress, but selenium at a relatively high dose could increase the formation of free radicals and result in oxidative stress significantly in rat hepatocytes *in vivo* and *in vitro*[17]. One notable effect of oxidative stress is to cause DNA damages, such as DNA strand breaks and base modifications[18]. The level of DNA damage detected at any given time point is the result of a balance between the induction of DNA damage and its repair. Cells are capable of repairing most lesions induced by physical or chemical agents[19]. Abul-Hassan *et al*.[20] have demonstrated direct evidence of the inhibitory effect of inorganic Se on cellular DNA repair capacity. We infer that selenium induces DNA damage by inducting oxidative stress and inhibiting DNA repair.

Apoptosis is an active mode of cell death in physiological and toxicological processes characterized by chromatin condensation, membrane blebbing, oligonucleosomal DNA fragmentation, and apoptotic body formation. The integrity of cellular organelles and plasma membrane is maintained until late in the process. DNA is often the target of toxicants, and DNA damage is the leading candidate for initiating the process of apoptosis[21]. Indeed, the key molecular event in apoptosis is the internucleosomal fragmentation of DNA, caused by a specific endogenous endonuclease that is either induced or activated from a pre-existing inactive state[22]. Selenium compounds induce Ca^{2+} release from the isolated sarcoplasmic reticulum vesicles[23], and endonuclease can be activated by increasing calcium[24]. DNA damages such as single strand breaks induced by selenite also can activate endonuclease. Selenite treatment induces DNA single strand breaks. Accumulation of DNA single strand breaks triggers endonuclease activation. Endonuclease cleaves DNA as double strand breaks and the final stage of this cleavage can be detected as oligonucleosomal size fragments. Cells that incur irreparable DNA double strand breaks die due to apoptosis[25]. Our results showed that the relative coefficient between DNA damage rates and apoptotic rates was 0.9021 ($P<0.01$). It is suggested that DNA damage is an important inducing factor for apoptosis.

Caspases are involved in the apoptosis induced by various stimuli and are regarded as important mediators of apoptosis. Among more than 10 kinds of caspases, caspase-3 is known as the most important one involved in most kinds of apoptosis. Jung *et al*.[26] showed that the enzymatic activity of caspase-3 increases after Se-methylselenocysteine treatment, and a significant activation of caspase-3 is evident 9 hours after the treatment[26], suggesting that methylseleninic acid-induced DU-145 cell detachment is a prerequisite for caspase activation and poly (ADP-ribose) polymerase cleavage is an apoptosis execution pathway that is principally initiated by caspase-8 → caspase-9, caspase-7 → caspase-3 and is likely amplified by feedback loops from caspase-3[27].

Our results showed that selenium could induce TUNEL positive cells in rat hepatocytes and display an obvious dose-response relationship between apoptotic rates and the doses of sodium selenite, and also a close relationship between DNA damage rates and apoptotic rates. Although caspases are important mediators of apoptosis, in our study DNA damage was the mechanism of apoptosis induced by selenium, and another mechanism may be c-myc, c-fos, and c-jun overexpression.

C-myc, c-fos, and c-jun belong to immediate early response genes (IEGs) that undergo early transcriptional activation when quiescent cells are exposed to mitogenic substances. We have reported that selenium at certain doses can inhibit hepatocellular c-myc, c-fos, and c-jun overexpression induced by cadmium in rats[28]. In this study, sodium selenite at the doses of 5, 10, and 20 μmol/kg caused c-myc, c-fos, and c-jun overexpression obviously. The positive brown-yellow signal for proteins of c-myc, c-fos, and c-jun was mainly located in the cytoplasm of hepatocytes. Tian *et al*.[29] reported that selenium could enhance expressions of c-fos and c-jun mRNA and their proteins, and may affect the differentiation and development of hippocampus neurons. Gene array analysis has suggested that c-myc, cyclin C, proliferating cell nuclear antigen, cyclin-dependent kinase (cdk)1, cdk2, cdk4, cyclin B, and cyclin D2 mRNA levels are lower in selenium-deficient HL-60 cells than in HL-60 cells

supplemented with 0.25 μmol/L selenomethionine. Decreased c-myc mRNA level in selenium-deficient HL-60 cells has been confirmed by reverse transcription-polymerase chain reaction analysis. In other words, supplementation with selenomethionine (0.25 μmol/L) up-regulates c-myc and cyclin C mRNA levels in HL-60 cells[30]. To our knowledge, we first report that sodium selenite at the doses of 5, 10, and 20 μmol/kg can induce overexpression of c-myc, c-fos, and c-jun in rat hepatocytes.

C-myc encodes an oncogenic helix-loop-helix leucine zipper transcription factor that acts as a heterodimer with its partner protein, Max, to activate genes regulating the cell cycle machinery as well as critical metabolic enzymes. The additional ability of c-myc to repress transcription of differentiation-related genes suggests that c-myc is a central and key molecular integrator of cell proliferation, apoptosis, and differentiation[31]. Desbiens *et al*.[32] showed that c-myc potentiation of the mitochondrial pathway of apoptosis, at least in part results from a sensitization of Ask1 activation, allowing DNA-damaging agents to induce cascade Ask1, p38alpha and Bax. C-myc promotes apoptosis by destabilizing mitochondrial integrity, leading to release of the proapoptotic effectors including holocytochrome c. Juin *et al*. indicated that c-myc triggers a proapoptotic mitochondrial destabilizing activity that cooperates with proapoptotic members of the Bcl-2 family[33]. SeO_2 at 30 μmol/L could markedly inhibit cell proliferation and viability, and prompt apoptosis of both normal hepatocytes and hepatoma cells. The apoptotic rate induced by SeO_2 is closely correlated with down-regulation of Bcl-2 and up-regulation of P53 proteins. The Bcl-2/P53 value is closely correlated with the apoptotic rate as well as SeO_2 concentrations[34].

The nuclear transcription factor AP-1, composed of dimers of Fos and Jun proteins may function to modulate stress-induced apoptosis either positively or negatively, depending on the microenvironment and the cell type in which the stress stimulus is induced[35]. AP-1 has been linked to a startling breadth of cellular events including cell transformation, proliferation, differentiation, and apoptosis. AP-1 is often portrayed as a general nuclear decision-maker that determines life or death of cells in response to extracellular stimuli. However, it is increasingly clear that the cellular context is critical for determining the contribution of AP-1 to cellular fates, and the role of AP-1 in apoptosis should be considered within the context of a complex network of nuclear factors that respond simultaneously to a wide range of signal transduction pathways[36]. Dhar *et al*.[37] have summarized that generation of reactive oxygen species (ROS) stimulates transcription by activating transcription factors such as activator protein 1 (AP-1) and nuclear factor kappaB (NF-κB). ROS and other free radicals can activate AP-1 and NF-κB transcription coordinately. Selenium can cause ROS[9,17,38] and induce overexpression of c-jun and c-fos[29], suggesting that under our experimental conditions, AP-1 plays an important role in the induction of rat hepatocyte apoptosis.

In conclusion, selenium at the doses of 5-20 μmol/kg can induce DNA damage, apoptosis, and overexpression of c-myc, c-fos, and c-jun in rat hepatocytes. Further studies are needed to display the exact mechanisms of selenium-induced apoptosis and overexpression of c-myc, c-fos, and c-jun.

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(*Received April* 2, 2005 *Accepted October* 11, 2005)