BIOMEDICAL AND ENVIRONMENTAL SCIENCES **19**, 302-308 (2006)

Effect of Selenium Supplementation on Activity and mRNA Expression of Type 1 Deiodinase in Mice With Excessive Iodine Intake[1]

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Objective To investigate the effect of selenium supplementation on the selenium status and selenoenzyme, especially the activity and mRNA expression of type 1 deiodinase (D1) in mice with excessive iodine (EI) intake and to explore the mechanism of selenium intervention on iodine-induced abnormities. **Methods** Weanling female BALB/c mice were given tap water or 3 mg/L of iodine or supplemented with 0.5 mg/L or 1.0 mg/L of selenium in the presence of excessive iodine for 5 months. Selenium status, thyroid hormone level, hepatic and renal D1 activity and mRNA expression were examined. **Results** Excessive iodine intake significantly decreased the selenium concentration in urine and liver, and the activity of glutathione peroxidase (GSH-Px) in liver. Meanwhile, serum total T_4 (TT_4) increased while serum total T_3 (TT_3) decreased. Hepatic D1 enzyme activity and mRNA expression were reduced by 33% and 86%, respectively. Renal D1 enzyme activity and mRNA were reduced by 30% and 55%, respectively. Selenium supplementation obviously increased selenium concentration, activity of GSH-Px and D1 as well as mRNA expression of D1. However, increasing the supplementation of Se from 0.5 to 1.0 mg/L did not further increase selenoenzyme activity and expression. **Conclusion** Relative selenium deficiency caused by excessive iodine plays an essential role in the mechanism of iodine-induced abnormalities. An appropriate dose of selenium supplementation exercises a beneficial intervention.

Key words: Excessive iodine; Selenium; Glutathione peroxidase; Type 1 deiodinase

INTRODUCTION

Previous studies[1] revealed that the relationship between the iodine intake level of a population and the occurrence of thyroid diseases is U-shaped. When insufficient dietary iodine is consumed, goiter usually develops, with occasional induction of hypothyroidism. On the other hand, when excessive iodine is ingested, hypothyroidism[2] or hyperthyroidism[3] associated with goiter may also develop. Iodine-induced goiter has been reported[4] in many nations and areas, resulting from ingestion of excessive iodine in foods[5] such as seaweed, in drinking water[6-7] as a natural pollutant, and in medications such as amiodarone[8] or from excessive iodine supplement in iodated salt[9]. It has been reported that nearly 16 million people are threatened by excessive iodine intake in 10 provinces of China[10] indicating that iodine-induced goiter has become a new public health problem.

However, the mechanism of iodine-induced thyroid dysfunction has not yet been clarified. The studies of Vitel *et al*.[11] and Denef *et al*.[12] indicated that excessive iodide-induced thyroid inhibition, cell necrosis or apoptosis are consequences of oxidative stress mechanism. According to Wolff and Chaikoff[13], organic binding of iodine within the thyroid glands can be almost completely blocked by excessive inorganic iodine, thus causing damage. But these theories could not fully explain the changes in morphology and thyroid hormone level induced by excessive iodine intake. A new hypothesis-deiodinase activity decrease theory[14] has been proposed, which indicates that the mechanism of iodine-induced goiter is due to inactivation of iodothyronine deiodinase, a selenium-containing enzyme, as the result of relative or absolute selenium deficiency. The result of antioxidants (Vit C, Vit E, and selenium) in the treatment of Graves' disease (an iodine-induced autoimmune disease) gives some clue to the hypothesis, and great attention has paid to the

[1]This work was supported by the National Science Foundation of China (NSFC) (No. 30230330).
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0895-3988/2006
CN 11-2816/Q

relationship between the selenium and iodine-induced thyroid dysfunction.

Microelement selenium is essential to thyroid hormone metabolism as it is an important part of both glutathione peroxidase (GSH-Px), a selenocysteine-containing enzyme which protects cellular structures against oxidative damages, and deiodinase, another selenocysteine-containing enzyme which regulates inter-conversion of active and inactive forms of iodothyronines[16-17]. There are three kinds of deiodinases responsible for the major part of thyroid hormone metabolism. Type 1 deiodinase (D1) is predominantly found in the liver, kidney, and thyroid, and is the only selenodeiodinase that can function as either an outer (5') or an inner (5) ring iodothyronine deiodinase. The conversion of T_4 to T_3 catalyzed by D1 is responsible for generating most of the circulating T_3[18]. The results of animal studies[19] suggested that iodine-induced thyroid hormone abnormalities, namely increased T_4 and decreased or unchanged T_3, are related to the decreased activity of D1. Other studies[20] found that selenium deficiency decreases D1 in the liver and kidney at transcriptional or translational level. Hotz *et al*.[21] reported that high iodine intake when selenium is deficient, may inflict damage to thyroid tissue as a result of low thyroidal GSH-Px activity during thyroid stimulation. These results suggest that selenium status is related to thyroid dysfunction induced by excessive iodine intake.

However, previous studies have not provided data on the nutritional status of selenium when people are exposed to excessive iodine so that whether selenium supplementation can normalize the thyroid hormone abnormalities induced by excessive iodine intake remains unclear. Our study may provide novel evidence for the important role of relative selenium deficiency induced by excessive iodine intake in iodine-induced disorders. Supplementation of selenium may protect against the damage caused by excessive iodine intake by influencing type 1 activity and mRNA expression.

MATERIALS AND METHODS

Animals and Treatment

Forty weanling female BALB/c mice purchased from Laboratory Animal Center of Hubei Provincial Center for Disease Control and Prevention were maintained in plastic cages with wood chip bedding at a constant temperature (20℃±2℃) and humidity (60%-80%) in a 12 h/12 h light/dark cycle. The animals had free access to food and water for 5 months.

The mice were divided into 4 groups. Control group was given tap water. Excessive iodine (EI) intake group was given 3.0 mg/L iodine in the form of potassium iodate. The other two groups, were supplemented with 0.5 mg/L (EI+0.5 mg/L Se) and 1.0 mg/L (EI+1.0 mg/L Se) selenium in the form of sodium selenite in drinking water, in addition to 3.0 mg/L iodine. The drinking water was prepared weekly. The diet was the BALB/c mouse chow in all groups. The contents of iodine and selenium were 365 μg/kg and 140 μg/kg in the diet and 8 μg/L and 16 μg/L in tap water.

The mice were weighed weekly. At the end of the experiment, they were placed into the metabolic cages, and urine samples were collected in the morning for urinary iodine and selenium analysis. Then, the mice were killed by cervical dislocation. Serum was separated and stored at -20℃ for use in the thyroid hormone analysis. Liver and kidney were removed and stored at -80℃ till analysis.

Selenium and Iodine Concentration, and Thyroid Hormone Analysis

Iodine concentrations in diet, water, and urine were measured by Cer-Arsenite color metric method modified by Fischer *et al*.[22]. Urinary creatinine concentrations were determined by alkaline picrate method. Fluorimetric assay with 2, 3-diaminonaphthalene was used to measure selenium contents in diet, water, urine, and liver. The urinary iodine or selenium to creatinine ratio (μg/g Cr) was used to estimate iodine or selenium concentration in urine. Serum total thyroxine (T_4) and serum total triiodothyronine (T_3) were measured by RIA kits obtained from the Chinese Academy of Atomic Energy in Beijing.

Enzyme Activity Assays

Glutathione peroxidase (GSH-Px) activity in the liver was assayed according to the method of L'Abbe *et al*.[23]. The assay was based on the coupled reaction with glutathione reductase. The unit definition is the amount of enzyme which causes the oxidation of one micromole of GSH per minute at 37℃. Protein concentration was determined according to the method of Lowry.

To determine D1 activity, tissues were homogenized in cold D1 homogenization solution (1 mmol/L DTT, 0.32 mmol/L sucrose, and 10 mmol/L Hepes buffer, pH 7.0) at 1:39 and 1:24 ratios (w/v) for livers and kidneys, respectively. Homogenates were centrifuged (1500×g) for 10 min at 4℃. Floating debris were removed, and aliquots of the supernatant were pipetted into tubes. Homogenates were recentrifuged (20 000×g) for 5 min at 4℃,

floating debris were removed, and the supernatant was used for D1 assay.

D1 activity was assayed by using ^{125}I-rT_3 (0.005 μmol/L ^{125}I-rT_3, 1000 μCi, obtained from the Chinese Academy of Atomic Energy in Beijing, 0.49 5 μmol/L 5'-L rT_3) as substrate and in the presence of 2 mmol/L DTT, 1 mmol/L EDTA, and 100 mmol/L potassium phosphate buffer, pH 7.0, based on the methods previously described[24]. Enzyme activity was expressed as pmol of I^- released per mg protein per minute of reaction. Protein content in homogenates was measured by the modified Lowry's method with reagent obtained from Biorad.

RT-PCR Analysis

Liver and kidney tissues of mice were homogenized in TriZol solution (Life Technologies, Rockville, MD). Two μg of total RNA was reverse-transcribed with random hexamers, and then PCR was carried out using the following primers published previously[25-26]: D1 sense primer, 5′-CTTGTGATATTCCTGCAGGTAGC-3′; and antisense primer, 5′-ACAGCTCGGACTTCCTCAG 3′, product 692 bp; glyceraldehydes-3-phosphate dehydrogenase (GAPDH) sense primer, 5′-TCACTCAAGATTGTCAGCAA-3′; and antisense primer 5′-AGATCCACGACGGACACAT T-3′, product 308 bp. Thermal cycling was performed under the following conditions: D1:1 cycle at 95℃ for 2 min, 34 cycles at 94℃ for 1 min, at 55℃ for 45 s, at 72℃ for 1 min, and a final extension at 72℃ for 10 min; GAPDH: 1 cycle at 95℃ for 12 min, 30 cycles at 94℃ for 45 s, at 58℃ for 45 s, at 72℃ for 80 s, and a final extension at 72℃ for 7 min. The PCR products were analyzed by 1.5% agarose gel electrophoresis with the running gel containing ethidium bromide. Quantification of the D1 and GAPDH mRNA was performed by scanning the intensities of ethidium bromide and analyzed by BioDocAnalyze system. D1 mRNA levels relative to GAPDH mRNA were standardized.

Statistical Analysis

Most quantitative data were analyzed by ANOVA. Because of its skewed distribution, the medians were used to describe the central tendency of urinary iodine concentration. The Kruskal-Wallis method was used to test the differences in ranking the iodine concentration in urine. The other data are presented as $\bar{x} \pm s$.

RESULTS

Urinary Iodine and Selenium Concentration, GSH-Px Activity and Selenium Content in Liver

The concentration of iodine in urine is currently the most widely used biochemical marker of iodine intake. Figure 1a shows that the median urinary iodine concentration in mice of EI group was significantly higher than that in the control group (P<0.01). However, there was no significant difference between the EI group and the two selenium supplementation groups (P>0.05).

There are many potential indicators of Se status. Mice treated with excessive iodine significantly decreased their urinary selenium excretion (0.83±0.19 mg/g Cr) compared with normal mice (0.58±0.13 mg/g Cr) (P<0.05) (Fig. 1b). Meanwhile, the urinary selenium concentration in mice treated with excessive iodine plus 0.5 mg/L and 1.0 mg/L selenium was 0.89±0.17 mg/g Cr and 1.02±0.25 mg/g Cr, respectively, significantly higher than that in EI group (P<0.05).

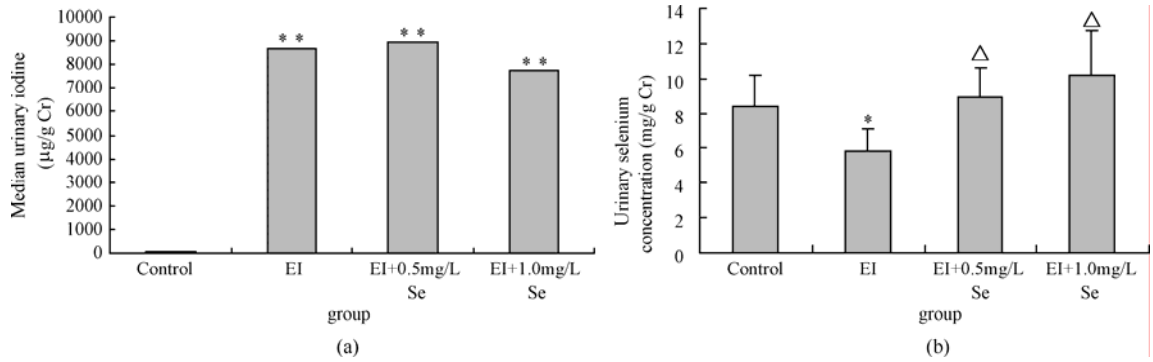

FIG. 1. Urinary iodine (a) and selenium (b) concentrations in different groups. Urinary iodine to creatinine ratio (μg/g Cr) was used to estimate iodine concentration in urine. Data were expressed as median, and each bar represents the median in a group of six samples. Kruskal-Wallis method was used to test the differences in ranking of iodine concentrations in urine, $^{**}P$<0.01 *vs* control group. Urinary selenium to creatinine ratio (mg/g Cr) was used to estimate selenium concentration in urine, and each bar represents the mean and SE in a group of six samples. $^{*}P$<0.05 *vs* control group, $^{\triangle}P$<0.05 *vs* EI group. EI: excessive iodine intake group. EI+0.5 mg/L Se: 0.5 mg/L selenium supplementation group. EI+1.0 mg/L Se: 1.0 mg/L selenium supplementation group.

Selenium content in liver indicated the same tendency as the urinary selenium concentration (Fig. 2). Determination of GSH peroxidase activities in blood or tissue could give a useful indication to functional Se status. Compared with normal group, the activity of GSH-Px in liver was reduced by 47% in excessive iodine intake group (P<0.05) while significantly increased that in the group supplied with 0.5 mg/L selenium (P<0.05, compared with EI group). No further increase in the GSH-Px activity was observed when they were supplemented with 1.0 mg/L selenium (Fig. 3).

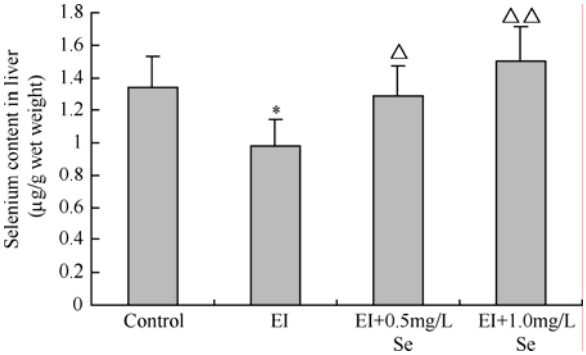

FIG 2. Selenium contents in liver of different groups. Each bar represents the mean and SE in a group of eight animals. $^{*}P$<0.05 *vs* control group. $^{\triangle}P$<0.05 *vs* EI group. $^{\triangle\triangle}P$<0.01 *vs* EI group. EI: excessive iodine intake group. EI+0.5 mg/L Se: 0.5 mg/L selenium supplementation group. EI+1.0 mg/L Se: 1.0 mg/L selenium supplementation group.

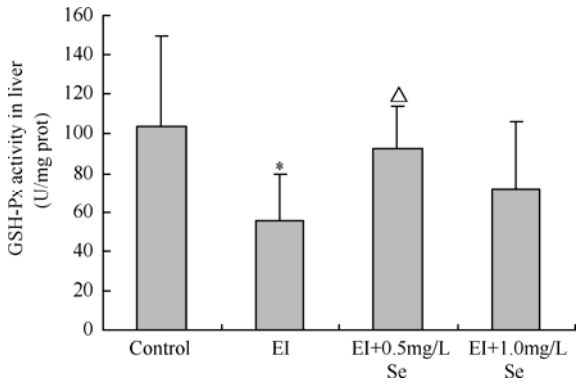

FIG. 3. GSH-Px activities in liver of different groups. The unit definition was the amount of enzyme which caused the oxidation of one micromole of GSH per minute at 37℃. Each bar represents the mean and SE in a group of ten animals. $^{*}P$<0.05 *vs* control group. $^{\triangle}P$<0.05 *vs* EI group. EI: excessive iodine intake group. EI+0.5 mg/L Se: 0.5 mg/L selenium supplementation group. EI+1.0 mg/L Se: 1.0 mg/L selenium supplementation group.

Serum Thyroid Hormone Level

Serum thyroid hormone level is shown in Fig. 4. Compared with control group, the serum TT_4 level increased (P<0.05) and the serum TT_3 level decreased (P<0.01) significantly in EI groups. In both selenium supplementation groups, the increased TT_4 and the decreased TT_3 were inhibited significantly.

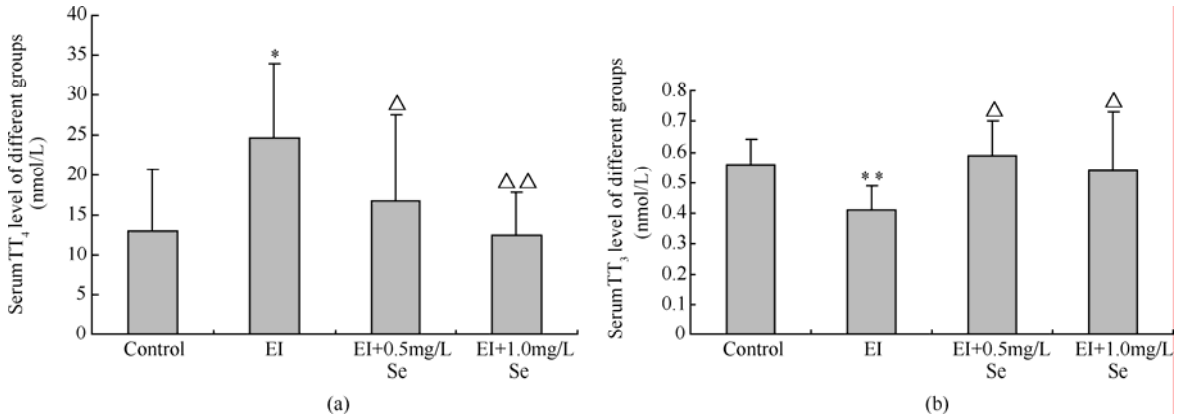

FIG. 4. Serum thyroid hormone levels of TT_4 (a) and TT_3 (b) in different groups. Each bar represents the mean and SE in a group of eight animals. $^{*}P$<0.05 *vs* control group. $^{**}P$<0.01 *vs* control group. $^{\triangle}P$<0.05 *vs* EI group, $^{\triangle\triangle}P$<0.01 *vs* EI group. EI: excessive iodine intake group. EI+0.5 mg/L Se: 0.5 mg/L selenium supplementation group, EI+1.0 mg/L Se: 1.0 mg/L selenium supplementation group.

D1 Activity and mRNA Expression in Liver and Kidney

Figure 5 demonstrates the D1 activity in the liver and kidney. Compared with control group, the mean enzyme activity was inhibited by 33% in the liver and by 30% in the kidney due to excessive iodine intake. 0.5 mg/L selenium supplementation enhanced the D1

activity in the liver and kidney significantly (P<0.05). But in 1.0 mg/L selenium supplement group, no further increase in D1 activity was observed.

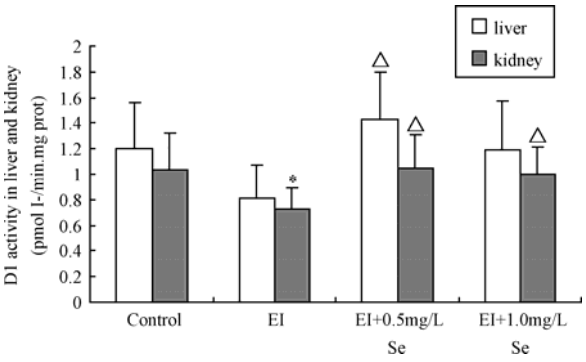

FIG. 5. D1 activities in liver and kidney of different groups. Enzyme activity was expressed as pmol of I^- released per mg protein per minute of reaction. Each bar represents the mean and SE in a group of eight animals. $^*P$<0.05 *vs* control group. $^\triangle P$<0.05 *vs* EI group. EI: excessive iodine intake group. EI+0.5 mg/L Se: 0.5 mg/L selenium supplementation group. EI+1.0 mg/L Se: 1.0 mg/L selenium supplementation group.

Hepatic and renal D1 mRNA expression was investigated by RT-PCR analysis. As shown in Fig. 6, excessive iodine intake obviously decreased D1 mRNA expression both in liver and in kidney. Compared with control group, hepatic and renal D1 mRNA expressions were reduced by 86% and 55%, respectively. Meanwhile in two selenium supplementation groups, hepatic and renal D1 mRNA expressions were remarkably up-regulated. However, D1 mRNA expression in the liver was reduced by increasing the selenium supplement level, but differed from that in kidney.

DISCUSSION

In this study, the obviously increased median urinary iodine concentration indicated that iodine intake in EI group was excessive. For selenium status, not only selenium excretion in urine, but also selenium storage in liver of mice treated with excessive iodine decreased significantly. As a valuable marker of selenium nutritional status[27], decreased GSH-Px activity in liver was also observed.

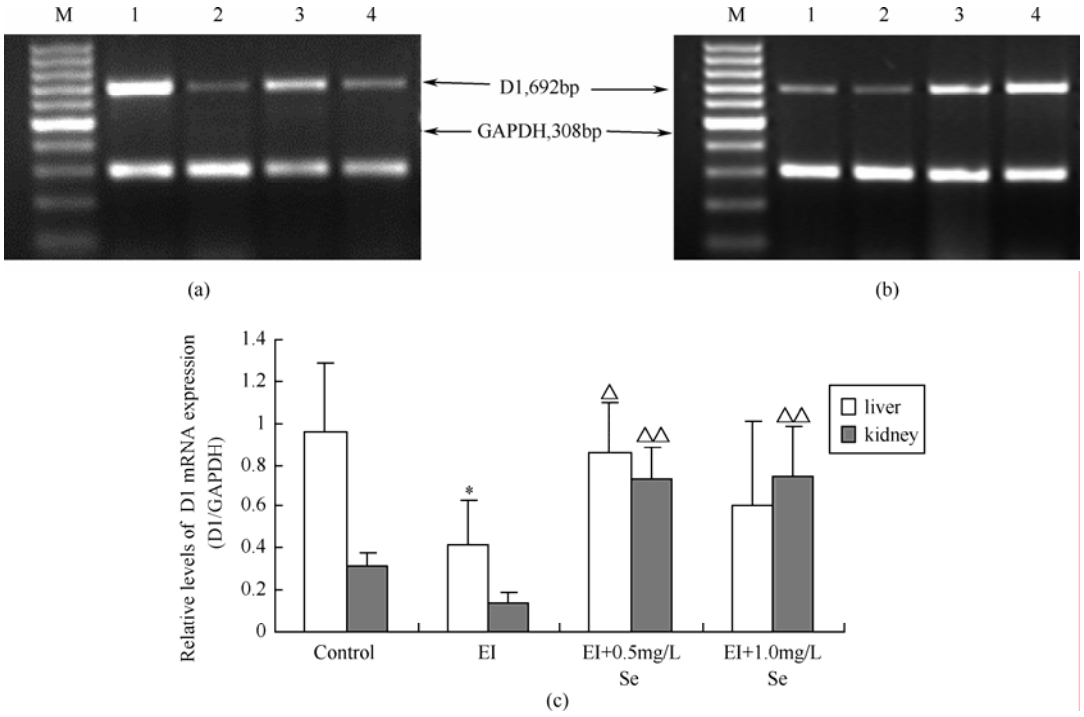

FIG. 6. Agarose gel electrophoretogram of D1 RT-PCR product in liver (a) and kidney (b). Lane M: Marker; lane 1, NC group; lane 2, EI group; lane 3, EI+0.5 mg/L Se group; and lane 4, EI+1.0 mg/L Se group.(c) mRNA expression of D1 in liver and kidney revealed by RT-PCR. Each bar represents the mean and SE in a group of four (liver) or three (kidney) animals. $^*P$<0.05 *vs* control group. $^\triangle P$<0.05 *vs* EI group. $^{\triangle\triangle}P$<0.01 *vs* EI group. EI: excessive iodine intake group. EI+0.5 mg/L Se: 0.5 mg/L selenium supplementation group. EI+1.0 mg/L Se: 1.0 mg/L selenium supplementation group.

In physiological condition, thyroid follicles continuously produce H_2O_2 for thyroid hormone synthesis throughout lifetime, and excessive H_2O_2 is degraded by GSH-Px, thus protecting the thyroid

from H_2O_2-induced damage[28]. When iodine intake is excessive, the oxidative and antioxidative balance is upset by generation of redundant H_2O_2 and other free radicals. If the imbalance persists for a long time, a large mount of GSH-Px and selenium is consumed, resulting in relative selenium deficiency. The fact that there exist different conditions of Se retention in different tissues, and also strong compensatory ability of antioxidative enzyme activity, may explain the contradictory results of different investigations[29] on the activities of GSH-Px in animals with excessive iodine intake. Due to the limited availability of the tissues, we did not analyze the related indexes in thyroid. But in the liver, after excessive iodine intake for 5 months, Se content and GSH-Px activity were reduced by 27% and 47%, respectively. Based on the results obtained, it can be concluded that excessive iodine intake results in relative selenium deficiency in experimental animals. Moreover, oxidative stress induced by selenium deficiency may be involved in the damage caused by excessive iodine.

We also found that in mice supplemented with selenium, hepatic selenium content and GSH-Px activity increased obviously. However, increasing selenium supplementation dose from 0.5 mg/L to 1.0 mg/L was not accompanied with a rise but a slight decrease in hepatic GSH-Px activity, suggesting that selenium supplementation contributes to the amelioration of selenium status in excessive iodine intake mice, but high dose of selenium may have the opposite effect.

Previous animal studies revealed that excessive iodine intake could result in an increased serum T_4 and a decreased or unchanged serum T_3. Similar changes were also observed in the present study. Meanwhile, D1 activity, which is responsible for generating most of the circulating T_3, reduced 33% in the liver and 30% in the kidney. The inhibition of 5′-deiodinase activity may be the main cause of iodine-induced thyroid hormone abnormality. However, few studies have been carried out to explore the mechanism of 5′-deiodinase activity inhibition by excessive iodine. A number of substances, agents, or conditions influence the rate of D1 synthesis, and selenium status is one of the most important factors. Most investigators believe that selenium influences deiodinase synthesis in the form of selenocysteine at translation level. But DePalo *et al*.[30] found that selenium deficiency decreases D1 activity in the liver and kidney, and decreased renal D1 activity is at companied with decreased D1 mRNA, which does not occur in the liver. In our study, not only in the liver, but also in the kidney, D1 mRNA expression in mice intaking excessive iodine markedly reduced, suggesting that relative selenium deficiency induced by excessive iodine intake possibly regulates the D1 activity at transcription level and that besides selenium, other factors, such as direct effect of high level iodine or T_4 may be involved in the regulation of D1 mRNA expression. In the present study, hepatic D1 enzyme activity and mRNA were reduced by 33% and 86%, respectively, and renal D1 enzyme activity and mRNA were reduced by 30% and 55%, respectively. Percentage changes in the activity of D1 were not always the same as the changes in its mRNA levels, suggesting that other processes, including mRNA stability, translation and protein turnover, may be involved in the regulation of the ultimate level of enzyme activity.

With supplementation of 0.5 mg/L selenium, both D1 activity and mRNA expression in liver and kidney increased. In contrast with EI group, serum thyroid hormone levels tended to be normal, namely, TT_4 level decreased and TT_3 level increased. However, in 1.0 mg/L selenium supplementation group, D1 activity and mRNA level in the liver, unlike that in the kidney, showed no further increase. Different selenium retention in different tissues is one possible explanation for this result. For liver, 1.0 mg/L selenium supplementation may elicit side effect.

In conclusion, relative selenium deficiency induced by excessive iodine intake plays an important role in iodine-induced thyroid hormone abnormality and thyroid dysfunction. Adequate selenium nutrition supports efficient thyroid hormone synthesis and metabolism, and supplementation of selenium might be a potential dietary therapy to protect against the damage or disease caused by excessive iodine exposure. Regulation of D1 activity at mRNA level is one of the mechanisms. However, other mechanisms, such as changes in other selenoenzymes, regulation of D1 expression at protein level, and optimal selenium dose, need further investigation.

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(*Received March* 2, 2005 *Accepted July* 4, 2006)