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Supplemental Selenium Alleviates the Toxic Effects of Excessive Iodine on Thyroid

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Abstract As excessive iodine intake is associated with a decrease of the activities of selenocysteine-containing enzymes, supplemental selenium was hypothesized to alleviate the toxic effects of excessive iodine. In order to verify this hypothesis, Balb/C mice were tested by giving tap water with or without potassium iodate and/or sodium selenite for 16 weeks, and the levels of iodine in urine and thyroid, the hepatic selenium level, the activities of glutathione peroxidase (GSHPx), type 1 deiodinase (D1), and thyroid peroxidase (TPO) were assayed. It had been observed in excessive iodine group that hepatic selenium, the activities of GSHPx, D1, and TPO decreased, while in the groups of 0.2 mg/L, 0.3 mg/L and 0.4 mg/L supplemental selenium, the urinary iodine increased significantly. Compared with the group of excessive iodine intake alone, supplemental selenium groups had higher activities of GSHPx, D1, and TPO. We could draw the conclusion that supplemental selenium could alleviate toxic effect of excessive iodine on thyroid. The optimal dosage of selenium ranges from 0.2 to 0.3 mg/L which can protect against thyroid hormone dysfunction induced by excessive iodine intake.

Keywords Selenium · Iodine · Type 1 deiodinase · Glutathione peroxidase · Thyroid peroxidase · Mouse

Abbreviations

GSHPx	glutathione peroxidase
TPO	thyroid peroxidase
D1	type 1 deiodinase
H_2O_2	hydrogen peroxide
NI	normal controls
EI	excessive iodine group

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IS groups	selenium groups
T_4	thyroxine
T_3	triiodothyronine

Introduction

Iodine is used for biosynthesis of thyroid hormone. An appropriate supply of iodine is essential for the production of thyroid hormone, which is indispensable for normal growth and development of vertebrates. It is well-known that iodine deficiency results in severe goiter and mental retardation [1]. Iodine deficiency has been widespread in China, and this problem had been thoroughly relieved after the introduction of salt iodized program since 1994. On the other hand, iodine excess affects nearly 16 million people in ten provinces of China, and the number has been increasing [2].

Previous studies [3] revealed that the relationship between the iodine intake level and occurrence of thyroid diseases of a population is U-shaped. When excessive iodine is ingested, goiter associated with hypothyroidism [4] or hyperthyroidism [5] may also develop. Whereas the selenium-dependent glutathione peroxidases (GSHPx) protect the thyroid gland against oxidative damage, the selenium-dependent thyronine deiodinases are essential for thyroid hormone metabolism [6]. In parts of China where both selenium and iodine are deficient, hypothyroid cretinism may develop. In order to prevent this particularly serious condition in these areas, the population is supplemented with iodine as well as with selenium. As excessive amounts of both of these elements are toxic, their dosages must be carefully optimized. Since the thyroid gland is particularly sensitive to iodine overdosage, the present study was conducted to determine the optimal selenium amount to prevent disorders of thyroid function due to iodine overdosage. The result of animal studies [7] suggested that iodine-induced thyroid hormone abnormalities were related to the decrease of the activity of selenocysteine-containing enzyme type 1 deiodinase (D1). And the present study was aimed at exploring the effects of supplemental selenium on disorders of thyroid function resulting from excessive iodine intake.

Materials and Methods

Animals

One hundred and forty adult 6–8 week-old female Balb/C mice were purchased from the Laboratory Animal Center of Hubei Provincial Center for Disease Control and Prevention. All procedures involving animal experiments were approved by the Animal Care and Use Committee of Tongji Medical College of Huazhong University of Science and Technology. All mice were divided into seven groups randomly and were housed five per cage in temperature- and light-controlled chambers on a 12: 12-h light–dark cycle and provided with rodent chow and water ad libitum. The contents of iodine and selenium in chow are 300 μg/kg and 0.1 mg/kg, respectively. The iodine and selenium were added into tap water in the form of potassium iodate and sodium selenite. Normal controls (NI) drink normal tap water. Excessive iodine group (EI) received the tap water containing iodine alone. There were five selenium groups (IS groups), and they received the tap water containing 3,000 $\mu g \cdot L^{-1}$ iodine and different levels of selenium (IS1 group, 0.1 mg/L; IS2 group, 0.2 mg/L; IS3 group, 0.3 mg/L; IS4 group, 0.4 mg/L; and IS5 group, 0.5 mg/L). Sixteen weeks later, the mice were killed after overdose of Nembutal. Thyroid, liver, and kidney

were collected and immediately frozen in liquid nitrogen, then stored at −80°C for activity measurements of GSHPx, thyroid peroxidase (TPO), and D1. Five thyroids of NI, EI, and IS2 groups were removed and fixed in 10% formalin for histological microscope measurement.

Determination of Urinary and Thyroidal Iodine Concentration

Urinary samples were obtained in the morning between 8:00 AM and 1:00 PM. Thyroid was weighted and homogenized in 1 ml cold deionized water. Then the urinary samples and thyroid were analyzed for iodine concentration using Cer–Arsenite colorimetric method as modified by Fischer et al. [8]. Urinary creatinine concentrations are determined by alkaline picrate method. The urinary iodine to creatinine ratio (micrograms per gram Cr) was used to estimate urinary iodine concentration.

Determination of Plasma Total Thyroxine and Total Triiodothyronine

At the time of sacrifice, blood samples were collected via cardiac puncture and centrifuged, and the serum was frozen at −20°C for the analysis of thyroxine (T_4) and triiodothyronine ($T_{3)}$ concentrations. Aliquots of 100 μl were assayed for T_3 and T_4 by radioimmunoassay kits obtained from the Chinese Academy of Atomic Energy in Beijing, China.

Determination of Hepatic Selenium and GSHPx Activities in Liver and Thyroid

Fluorimetric assay with 2,3-diaminonaphthalene was used to measure selenium content in liver [9]. GSHPx activities in liver and thyroid were assayed according to the method of L'Abbe et al. [10]. The assay was based on the coupled reaction with glutathione reductase. The unit definition was the amount of enzyme which caused the oxidation of 1 μmol of glutathione per minute at 37°C. Protein concentration was determined according to the method of Lowry method.

Determination of Type 1 Deiodinase Activity

The methods were described in detail earlier [11]. Tissues were homogenized in cold homogenization solution (1 mM dithiothreitol (DTT), 10 mM HEPES, and 320 mM sucrose) at 1:39, 1:24, and 1:19 dilution (*w*/*v*) for liver, kidney, and thyroid, respectively. The mixture including 100 μl of ^{125}I-rT3 incubation solution (0.005 μM^{125}I-r T3, 0.495 μM 5′-L-rT3, 2 mM DTT, 100 mM potassium phosphate buffer, and 1 mM EDTA) and 20 μl of homogenate were heated at 37°C for 11 min. The reaction was stopped by flooded the reaction mixture with 500 μl cold horse serum and 10%(*w*/*v*) acetic acid. After centrifuging, the released $^{125}I^-$ in filtrate was counted in a gamma counter. Total protein content of tissues was determined using a modified Lowry method with reagent obtained from Biorad.

Determination of Thyroid Peroxidase Activity

The method was performed as described previously [12]. Thyroid samples (~10 mg from two mice) were homogenized at 4°C in 2 ml of 5 mM potassium phosphate buffer (pH7.4) containing 200 mM sucrose, 500 units/ml catalase, and 1 mM EDTA. The homogenate was centrifuged at 600 rpm for 10 min at 4°C. The supernatant was collected and then centrifuged at 105,000*g* for 45 min at 4°C. The supernatant was discarded, and the pellet

was resuspended in 250 μl of 200 mM potassium phosphate buffer (pH7.4) then stored at −80°C. The resuspended solution was incubated with 35 mM guaiacol and 300 mM H_2O_2 in 100 mM phosphate buffer (pH7.4) at 37°C, and the oxidation of guaiacol was detected by spectrophotometer at 470 nm wavelength. Protein concentration was measured by using the Lowry method.

Statistical Analysis

Statistical analyses were performed with the SPSS statistical package (version 12.0 for Windows). Because of its skewed distribution, the medians were used to describe the central tendency of iodine concentration in urine and thyroid. The Kruskal–Wallis method was used to test the differences in ranking of urinary iodine concentration. Other data were analyzed by a one-way analysis of variance. Subsequently, multiple comparisons of the means were performed using the least-significant-differences test. Significant level was set at $P<0.05$.

Results

Effect of Excessive Iodine and Selenium Supplement on Thyroid Pathology

Figure 1 shows that, in the NI group, thyroid follicles were medium sized, and follicular cells were simple columnar epithelium. Compared with NI group, thyroid follicles of EI group increased significantly in size and were full of colloid in the chamber, and follicular epithelial cells were thin and flat, which means goiter occurred in EI group. Most of the follicular chamber sizes of the IS2 group were similar to that of the NI group. However, the size of follicular epithelial cells was between that of NI and EI groups (magnification, 200×).

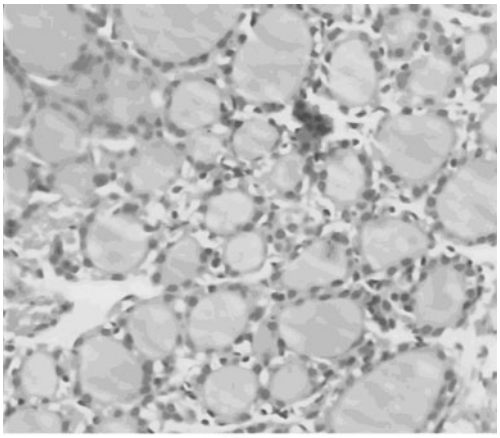
Concentration of Thyroid Hormone in Plasma

Plasma T4 was higher, but T3 was lower in EI group mice in comparison with results obtained in NI. Selenium supplementation restored the thyroid hormone levels. There was no significant difference between the IS groups (Table 1).

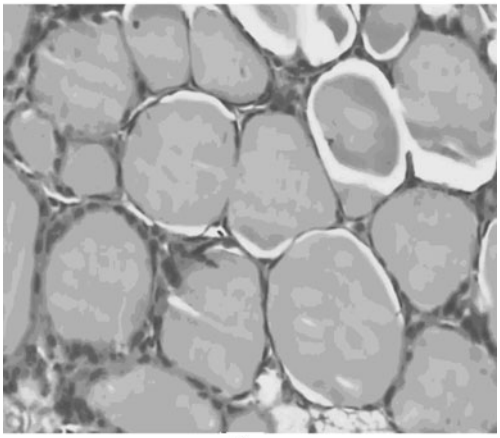
Levels of Iodine in Urine and Thyroid

Analysis of urine samples showed that median urinary iodine level of NI group was 81.0 μg/g Cr. Excessive iodine exposure led to a very significant increase in urinary iodine level. Compared with the EI group, IS1, IS2, IS3, and IS4 groups significantly increased the level of urinary iodine, but there was no significant difference between EI and IS5.

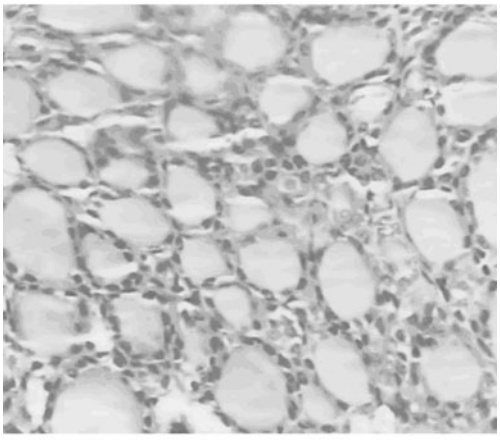
Compared with NI group, the iodine in thyroid of EI and IS increased significantly. Moreover, the iodine in thyroid of EI group was significantly different from that of IS2; this discrepancy could not be observed from the comparison between EI and other IS groups. The iodine level of IS5 (2123.7 μg/g) was the highest in all of the IS groups. There was an increase tendency from IS2 to IS5. The results indicated that selenium supplement could promote iodine excretion and reduce the excessive iodine in thyroid (Table 1).



NI



EI



IS2

Fig. 1 Effect of excessive iodine and selenium supplement on thyroid pathology

Table 1 Concentration of Plasma Thyroid Hormone, Iodine in Urine and Thyroid

Group	T_3 (nmol/L)	T_4 (nmol/L)	Urinary iodine (μg/L)	Iodine in thyroid (μg/g pro)
NI	0.94±0.10	74.45±14.33	81.03	284.89
EI	0.74±0.12[a]	93.25±23.25[a]	4822.58[a]	2974.17[a]
IS1	1.01±0.13[b]	84.81±20.19	6912.79[a,b]	2101.01[a,b]
IS2	0.90±0.11[b]	78.66±18.98	8121.32[a,b]	1014.87[a]
IS3	0.96±0.09[b]	86.57±12.81	6155.87[a,b]	1716.81[a]
IS4	1.01±0.12[b]	80.73±11.76	6180.16[a,b]	1783.81[a]
IS5	0.92±0.13[b]	81.40±24.67	6220.54[a,b]	2123.74[a]

Iodine in urine and thyroid are median; the other values are means±SD for ten mice

[a] Significantly different from the NI group at $P<0.05$

[b] Significantly different from the EI group at $P<0.05$

The Levels of Selenium in Liver and the Activities of GSHPx in Liver and Thyroid

There was no significant difference of selenium in liver between NI and IS groups. Hepatic selenium of the EI group decreased significantly compared with that of NI group. Compared with EI group, hepatic selenium level increased significantly in IS2 group.

Compared with NI group, GSHPx activities in thyroid of IS2 and IS3 group were not significantly different, while those of EI, IS1, IS4, and IS5 groups were decreased significantly (Table 2).

The Activity of TPO

The activity of TPO was inhibited by excessive iodine significantly. Compared with the effect of iodine alone, iodine in combination with selenium increased the activity of TPO, indicating that selenium supplement alleviated the damage of TPO resulted from iodide excess. However, the IS group had decreased tendency although there was no significantly difference between the NI, IS2, and IS3 groups (Table 2).

Table 2 Levels of Selenium in Liver and the Activities of GSHPx and TPO

Group	Selenium in liver (μg/g liver)	GSHPx in liver (U/mg prot)	GSHPx in thyroid (U/mg prot)	TPO (IU/mg pro)
NI	1.26±0.34	191.2±85.0	50.9±8.2	101.97±10.75
EI	0.92±0.45	117.0±59.0[a]	39.5±5.2[a]	54.30±10.64[a]
IS1	1.19±0.49	184.2±120.8	40.4±9.3[a]	90.24±16.52[b]
IS2	1.25±0.28	162.9±96.8	49.5±8.1	94.69±7.61[b]
IS3	1.45±0.10[b]	137.2±57.7	44.4±3.6	91.15±16.40[b]
IS4	1.50±0.32[b]	130.8±40.3	43.5±5.7[a]	69.67±8.32[a,b]
IS5	1.57±0.42[b]	148.7±50.9	40.1±5.7[a]	63.36±7.71[a]

[a] Significantly different from the NI group at $P<0.05$

[b] Significantly different from the EI group at $P<0.05$

The Activities of D1 in Liver, Kidney, and Thyroid

The activities of D1 in liver, kidney, and thyroid were inhibited by iodine excess significantly, and the means of D1 activities of EI group in liver, kidney, and thyroid were decreased 36.6%, 30.4%, and 39.4%, respectively (Table 3).

Discussion

Iodine-induced goiter resulting from excessive iodine intake has been reported in many countries [13–15]. It looms larger and larger in public health of China. However, the mechanism of the toxic effects from excessive iodine intake has not been thoroughly studied; accordingly, the intervention method needs to be discovered.

The constant levels of thyroid hormones in the blood of vertebrates depend on the balance of prohormone thyroglobulin synthesis and degradation [16, 17]. Within the follicular cell, iodide is converted into iodine by the enzyme TPO, which utilizes hydrogen peroxide (H_2O_2) as cofactor and product. TPO catalyzes the incorporation of iodide molecule onto both the three and/or five positions of the phenol rings of tyrosine in thyroglobulin and produced either T_4 or T_3 by coupling iodinated tyrosine rings to another iodinated phenol ring, which is obtained from iodinated tyrosine residues. Then, T_4 and T_3 are secreted into blood under the action of thyrotropic-stimulating hormone. Finally, the majority of thyroid hormone is degraded by the deiodinase in liver and kidney. The iodine from the degradation was reabsorbed or excreted mainly through urine.

TPO and H_2O_2 play important roles in this process. Furthermore, the amount of H_2O_2 is very critical in thyroid hormone biosynthesis. On one hand, H_2O_2 is an essential substrate for the peroxidase reaction. On the other hand, excessive H_2O_2 is harmful to thyroid peroxidase because of its oxidation. H_2O_2 kept on being produced in the process of thyroid hormone biosynthesis; therefore, thyroid needs a very potent anti-oxidative system to maintain a proper level of H_2O_2. GSHPx, which catalyzed H_2O_2 degradation to H_2O, is one of the major actors in the anti-oxidative system [18, 19]. Every mole GSHPx contains 4 g selenium, so selenium deficiency will result in the declination of GSHPx. The experimental results show that the mean values of hepatic selenium and the activity of GSHPx in EI group decrease compared with those in NI group. So, the H_2O_2 from thyroid hormone biosynthesis could not be converted effectively; therefore, the thyroid hormone biosynthesis

Table 3 Activities of D1 in Liver, Kidney, and Thyroid

Group	Liver (I^-/min/mg pro)	Kidney (I^-/min/mg pro)	Thyroid (I^-/min/mg pro)
NI	4.73±0.64	2.14±0.43	17.95±2.91
EI	3.00±0.85[a]	1.49±0.26[a]	10.88±2.72[a]
IS1	3.70±0.88	2.52±0.77[b]	15.22±1.59[b]
IS2	5.11±2.01[b]	1.91±0.33	17.34±8.45[b]
IS3	4.51±3.80[b]	2.16±0.52[b]	15.00±2.72
IS4	3.80±1.17	1.90±0.42	16.39±4.19[b]
IS5	3.26±0.81[a]	1.90±0.28	12.98±4.11[a]

[a] Significantly different from the NI group at $P<0.05$

[b] Significantly different from the EI group at $P<0.05$

will be damaged. There was no significant difference in hepatic selenium and GSHPx between the NI and IS groups, which means that the oxidative/anti-oxidative balance has been maintained in IS groups.

The changes of TPO and GSHPx activities were similar. The excessive H_2O_2 in thyroid would depress the activity of TPO. Supplemental selenium could increase GSHPx activity and correct the unbalanced oxidative/anti-oxidative system. It is the ultimate reason for TPO activity recovery. Supplemental selenium could decrease the level of TPO antibody and the damage of thyroid in the patients with Graves disease [20, 21]. Above all, TPO is not a selenoenzyme, so the effect of supplemental selenium is not direct.

Based on several functional criteria and different protein sequences, 5′-deiodinases is classified into two isoenzymes: type 1 and type 2. D1 is predominantly found in the liver, kidney and thyroid, and is responsible for generating most of the circulating T_3. Beckett et al. [22] first reported that selenium deficiency also caused a depression in the 5′-deiodinase activity in rats fed a purified amino acid diet; but, it is questionable whether supplemental selenium could recover the deactivated D1. In EI group, the activities of D1 in liver, kidney, and thyroid decreased; at the same time, the addition of selenium alleviated the toxic effects of iodine excess on D1. The level of T_4 increased in EI group, while T_3 did not change with T_4. The possible explanation for the result could be due to the change of D1 activity which decreased conversion from T_4 to T_3 and degradation of T_3. After the food containing excessive iodine has been ingested, 80–85% of excessive iodine is excreted into urine. As can be verified by the observation in our study, there is an obvious increase of urinary iodine and decrease of thyroid iodine in IS groups. The reason lies in that increased D1 activity enhanced thyroid hormone degradation, which leads to the loss of iodine. The decline of thyroid iodine relieved its direct damage on mice.

Based on the present investigations, a chain model has been drawn out, i.e., the excessive iodine intake would lead to relative selenium deficiency, as a result, suppressing the activities of the enzymes, such as GSHPx. This is the main reason for the occurrence of goiter. Moreover, the cause and effect mechanism of iodine and selenium is bilateral. By supplemental selenium on the second node of this chain model, on one hand, we can decrease the level of iodine in thyroid; on the other hand, we can increase the activities of GSHPx, TPO and D1, and the goiter could be significantly relieved. The optimal dosage of selenium ranges from 0.2 to 0.3 mg/L which can protect thyroid hormone from dysfunction induced by excessive iodine intake.

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