Emir DÖNDER[1]
Mehmet ÇAY[2]
Necip İLHAN[3]
Gıyasettin BAYDAŞ[4]
Mustafa NAZIROĞLU[2]

Effect of Selenium on "Low T_3 Syndrome" in Hepatic Failure

Departments of [1]Internal Medicine, [3]Biochemistry, and [4]Physiology, Faculty of Medicine, Fırat University, Elazığ-Turkey
Department of [2]Physiology, Faculty of Veterinary, Fırat University, Elazığ-Turkey

Abstract: Triiodothyronine occurs especially in 5'-monodeiodination of thyroxin in peripheral tissues. Deiodination of thyroxin mainly occurs in the liver. The enzyme which catalyzes this reaction is a seleno-enzyme. Deiodinase enzyme activity and the triiodothyronine level decrease in selenium deficiency.

After the occurrence of chronic hepatic failure by carbon tetrachloride in rats, we gave sodium selenite to these rats. Then, we tried to evaluate the changes in thyroid hormone levels.

Liver function tests, thyroxin and triiodothyronine levels, and hepatic 5'-deiodinase and glutathione peroxidase enzyme activities were measured.

Triiodothyronine levels in the carbon tetrachloride group and carbon tetrachloride + selenium group were significantly lower than in the control group ($P<0.01$, $P<0.05$, respectively). In the carbon tetrachloride + selenium group, the level of triiodothyronine was higher than in the carbon tetrachloride group ($P<0.05$).

Deiodination of thyroxin was impaired in hepatic failure. Selenium couldn't prevent the inhibition of hepatic deiodination of thyroxin completely.

Key Words: Hepatic failure, Selenium, Thyroid hormones.

Introduction

Selenium (Se) deficiency has important pathological sequelae in both animals and in man and may cause poor growth, impaired neutrophil function, and increased susceptibility to infections (1–3). In man, lowered Se status has been associated with cardiomyopathy and an increased risk of developing certain cancers (4, 5).

Hepatic enzyme expession changes with Se deficiency, but the mechanism is unclear (6). Thyroid hormones have many effects on hepatic enzyme expression. The relationships between thyroid hormones and Se had been documented by many studies (7–10). All thyroxine (T_4) is obtained from thyroidal synthesis, but over 85% of circulating T_3 is derived from 5'–monodeiodination of T_4 in peripheral tissues. 5'–monodeiodination of T_4 mainly occurs in the liver (11).

Changes in enzyme expression in Se deficiency may also affect the peripheral thyroid hormone metabolism. Deiodination of T_4 to T_3 is especially caused in the liver by Type I 5'–deiodinase enzyme, which is a seleno–enzyme.

Therefore, Se deficiency may decrease the biologically active T_3 level. In non–thyroidal tissues, two types of enzyme are responsible for 5'–monodeiodination of thyroxine. The type I enzyme is present mainly in the liver and kidney and it is inhibited by low concentrations of propylthiouracil. The type II enzyme is found mainly in the pituitary and in the brain (11).

The Se level is very high in the liver, the pituitary, the renal cortex, and the pancreas tissues. The Se level decreases in hepatic failure and in cardiovascular diseases (11).

Decreased T_3 level in hepatic failure has been attributed to various causes (12). Because Se affects deiodination of T_4 to T_3, it is possible that thyroid hormone composition may change in hepatic failure due to Se deficiency. After experimental hepatic failure occurred in rats, we tried to investigate the effect of Se injections on the T_4 metabolism.

Materials and Methods

Forty male Wistar rats were used in this study. The rats were divided into 4 groups.

Group 1 (n=10): Carbon tetrachloride (CCl$_4$) was diluted in refined olive oil at a ratio of 3:2. Diluted CCl$_4$, at a dose of 0.15 ml/100 g, was administered subcutaneously 3 times a week for 6 weeks. By administering CCl$_4$, cirrhosis was induced in these rats (*CCl$_4$ group*). Diagnosis of cirrhosis confirmed by biopsy.

Group 2 (n=10): By administering CCl$_4$, cirrhosis was induced in this group. Then sodium selenite, at a dose of 0.1 mg/kg, was administered intraperitoneally once a day for one week (*CCl$_4$+Se group*).

Group 3 (n=10): Only refined olive oil, at a dose of 0.15 ml/100 g, was administered subcutaneously 3 times a week for 6 weeks (*Control group*).

Group 4 (n=10): Refined olive oil, at a dose of 0.15 ml/100 g, was administered subcutaneously 3 times a week for 6 weeks. Then sodium selenite, at a dose of 0.1 mg/kg, was administered intraperitoneally once a day for one week (*Se group*).

All rats were fed standard rat food and distilled water. 24 hours after the last feeding, all the animals were decapited. Blood samples were taken and centrifuged at 3000 g for 10 minutes. Then, serum was exracted and stored at –20°C. Liver function tests (Serum total bilirubin–T. bilirubin, conjugated bilirubin–C. bilirubin, aspartate aminotransferase–AST, alanine aminotransferase–ALT, lactic dehydrogenase–LDH, alkaline phosphatase–ALP, and gamma–glutamyltranspeptidase–GGT) were evaluated with a Tecnicon RA, XT model autoanalyzer. Serum T$_4$ and T$_3$ levels were measured using radioimmunassay methods. We used DPC test kits–Diagnostic Product Corporation, Los Angeles, California and LKB 1261 Multigamma gamma counter.

Preparation of tissue homogenates

Liver tissues were homogenized in 3 volumes of buffer [0.25 M sucrose/0.05 M–Tris/HCl (pH 7.4)/mM–DDT] using a Teflon–pestle/glass–body homogenizer (6).

Measurement of T$_3$ production

Homogenates (2 ml) were placed into glass tubes and incubated at 37°C for 5 min before the addition of 10 µg of T$_4$ dissolved in 20 µl of phosphate buffer (0.5 M; pH 7.4). At increasing time periods, 0.2 ml portions of the homogenates were put in 0.4 ml of ethanol and mixed for 30 s on a vortex mixer. The ethanolic extracts were then centrifuged at 1500 g for 15 minutes and the T$_3$ concentration of the supernatants was measured with radioimmunoassay after 10–fold dilution with sucrose/Tris buffer (6).

Protein concentration in the homogenates was measured by biuret reaction.

Measurement of Hepatic GSH–Px (Glutathione peroxidase) activity

Liver tissues were homogenized in 3 volumes of 20 mM phosphate buffer containing 150 mM NaCl (pH 7.4) and used for the measurement of GSH–Px activity.

GSH–Px activity was measured, with 0.25 mM–H$_2$O$_2$ as the substrate, in the presence of 5 mM–GSH (13).

The data were expressed as Mean±SD. Differences among groups were compared using the Mann–Whitney U–test.

Results

Diagnosis of cirrhosis was confirmed histopathologically. The results of the liver function tests are shown in Table 1.

GSH–Px activity was measured as an index of selenium deficiency. Hepatic GSH–Px activity was significantly low in the CCl$_4$ group (P<0.001). By administering selenium, hepatic GSH–Px activity increased. Hepatic GSH–Px activity was higher in the CCl$_4$+Selenium group (790.81±78.45 mU/mg protein) than in the CCl$_4$ group (612.15±71.00 mU/mg protein) (P<0.01). By administering selenium, hepatic GSH–Px activity was also increased in the selenium group relative to the control group (P<0.01).

5'–deiodinase (5'DI) enzyme activity decreased 50% in the CCl$_4$ group and 20% in the CCl$_4$+Selenium group. After administering selenium, 5'DI enzyme activity was increased significantly in the selenium group relative to the control group (P<0.05).

Serum T$_4$ level decreased insignificantly in the CCl$_4$ group and increased insignificantly in the CCl$_4$+Selenium group. However, T$_4$ level increased significantly in the Selenium group (P<0.05).

T$_3$ levels in the CCl$_4$ group and CCl$_4$+Se group were significantly lower than in the control group (P<0.01, P<0.05, respectively). In the CCl$_4$+Se group, the level of T$_3$ was higher than in the CCl$_4$ group (P<0.05). These results are shown in Table 2.

In the control group, the correlation between GSH–Px activity and 5'–Deiodinase was r=0.472 (P>0.05) and

Table 1.   Liver function tests.

	Control Group (n=10)	CCl₄ Group (n=10)		CCl₄+Se Group (n=10)		Se Group (n=10)	
AST (U/L)	70.20±12.21	807.15±98.06	A	708.57±100.90	A	73.21±11.05	
ALT (U/L)	50.80±9.70	383.86±67.09	A	354.44±50.71	A	49.01±7.00	
LDH (U/L)	504.67±81.90	2910.54±275.87	A	2900.60±265.98	A	512.32±79.00	
ALP (U/L)	250.29±40.50	600.13±96.23	A	588.13±90.30	A	275.41±34.09	
GGT (U/L)	3.90±0.70	7.80±1.72	A	4.79±0.98	A	4.01±0.40	
T. Bilirubin (mg/dl)	0.24±0.02	3.60±0.65	A	3.03±0.70	A	0.26±0.03	
C. Bilirubin (mg/dl)	0.18±0.01	2.80±0.70	A	2.52±0.45	A	0.17±0.02	

CCl_4 = Carbon tetrachloride

CCl_4+Se = Carbon tetrachloride+selenium

Se = Selenium

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

LDH = Lactic dehydrogenase

ALP = Alkaline phosphatase

GGT = Gamma–glutamyltranspeptidase

T. Bilirubin = Total Bilirubin

C. Bilirubin = Conjugated Bilirubin

A = P<0.001 versus control

Table 2.   Hepatic 5'–Deiodinase and Glutathione peroxidase (GSH–Px) enzyme activities and serum thyroxin (T_4) and triiodothyronine (T_3) values.

	Control Group (n=10)	CCl₄ Group (n=10)		CCl₄+Se Group (n=10)		Se Group (n=10)	
GSH–Px	807.50±95.70	612.15±71.00	B	790.81±78.45		957.48±108.30	A
5'–DI	15.20±1.30	7.70±0.60	C	12.10±1.40	A	20.30±2.00	B
T_4 (µg/dl)	5.37±0.95	4.60±0.87		5.20±0.98		6.50±1.05	A
T_3 (ng/dl)	90.48±18.20	60.12±11.71	B	71.18±10.12	A,D	92.74±13.98	

CCl_4 = Carbon tetrachloride

CCl_4+Se = Carbon tetrachloride+selenium

Se = Selenium

GSH–Px = Glutathione peroxidase (mU/mg protein)

5'–DI=5'–Deiodinase (fmol/min/mg)

T_4 = Thyroxin

T_3 = Triiodothyronine

A = P<0.05 versus control

B = P<0.01 versus control

C = P>0.001 versus control

D = P<0.05 versus CCl_4 group

the correlation between GSH–Px activity and T_3 was r=0.403 (P>0.05).

In the CCl_4 group, the correlation between GSH–Px activity and 5'–Deiodinase was r=0.670 (p<0.01) and the correlation between GSH–Px activity and T_3 was r=0.550 (P<0.05).

In the CCl_4+Se group, the correlation between GSH–Px activity and 5'–Deiodinase was r=0.690

(P<0.01) and the correlation between GSH–Px activity and T$_3$ was r=0.565 (P<0.05).

In the Se group, the correlation between GSH–Px activity and 5'–Deiodinase was r=0.560 (P<0.05) and the correlation between GSH–Px activity and T$_3$ was r=0.510 (P>0.05).

Discussion

T$_4$ produces T$_3$ and rT$_3$. This transformation is in balance under normal conditions. Several clinical states such as starvation, protein calorie malnutrition, acute or chronic illness, cirrhosis of the liver, chronic renal failure, systemic diseases, and exercise are associated with low serum T$_3$ and increased serum rT$_3$ levels due to changes in the T$_4$ metabolism (14–19).

Recently, it was concluded that hyperglucagonemia may be one of the factors responsible for low serum T$_3$ and high serum rT$_3$ concentrations in hepatic cirrhosis. Besides hyperglucagonemia, selenium deficiency occurs in cirrhosis, too. Kabadi et al have suggested that glucagon has a significant effect on the deiodination of T$_4$ (12, 16, 18, 20). The Se level decreases in hepatic cirrhosis. Se is present in the 5'–deiodinase enzyme structure and this enzyme has a role in the monodeiodination of T$_4$. Therefore, 5'–deiodinase enzyme activity decreases in Se deficiency (21, 22).

In this study, we observed that Se didn't affect the serum T$_3$ level in normal rats. But, the T$_3$ level decreased in the group in which hepatic failure was induced with CCl$_4$. The CCl$_4$+Se group had a significantly higher serum T$_3$ level than the CCl$_4$ group (P<0.05), but a lower T$_3$ level than the control group (P<0.05).

5'–deiodinase enzyme activity significantly decreased after hepatic failure. By administering Se, 5'–deiodinase enzyme activity increased. These findings were in parallel with changes observed in the serum T$_3$ level.

Se administered to cirrhotic rats increased GSH–Px activity, 5'–DI activity, and the T$_3$ level. Thus, we think that a low serum T$_3$ level in cirrhosis at least may originate from Se deficiency.

5'–deiodinase enzyme activity decreases in chronically Se deficient rats. Therefore, the serum T$_4$ level increases and the serum T$_3$ level decreases. Menhold et al (8) have observed that hepatic type I 5'–deiodinase enzyme activity significantly decreased in chronically Se deficient rats. They have reported that the T$_3$ level decreased and the T$_4$ level increased in these rats. Beckett et al (23) have demonstrated that Se deficiency for periods of 5 or 6 weeks in rats produced an inhibition of type I 5'–deiodinase enzyme activity in the liver and an inhibition of type II 5'–deiodinase enzyme activity in the brain. Inhibition of type I 5'–deiodinase and type II 5'–deiodinase activities decrease turnover of T$_4$ to T$_3$.

The Se level decreases in hepatic failure and the thyroid hormone metabolism is impaired in Se deficiency. Therefore, Se deficiency may be one of the factors responsible for low T$_3$ hormone levels in hepatic cirrhosis. In our study, we observed that Se, a trace element, played an important role in the thyroid hormone metabolism in cirrhosis. However, we couldn't increase the T$_3$ level to its normal value by administering Se to rats which had hepatic failure. Therefore, we suggest that other factors are also responsible for "Low T$_3$ syndrome" in hepatic failure. Nevertheless, we think that additional Se therapy may be beneficial to the thyroid hormone metabolism in hepatic failure.

References

1. Coombs GF., Coombs SB. Selenium and Cancer. In: Coombs GF., ed. The role of selenium in nutrition, Academic Press, Orlando, 1986, 413–61.

2. L'Abbe MR., Fischer PWF., Cambelli JS., Chavez ER. Effects of dietary selenium on DMBA–induced carcinogenesis in rats fed a diet high in mixed fats. J Nutr 119: 757–65, 1989.

3. Halverson AW. Growth and reproduction with rats fed selenite–Se. Proc S Dakota Acad Sci 53: 167–77, 1974.

4. Willet WC., Morris JS., Pressel S., Taylor JO., Polk BF., Stampfer MJ. Prediagnostic serum selenium and risk of cancer. Lancet 2: 130–4, 1985.

5. Har JR., Exon JH., Whanger PD., Weswig PH. Effect of dietary supplementation on N–2–fluorenyl–acetamide (FAA)–induced cancer in vitamin E supplemented selenium depleted rats. Clin Toxicol 5: 187–94, 1972.

6. Beckett GJ., Beddows SE., Morrice PC., Nicol F., Arthur JR. Inhibition of hepatic deiodination of thyroxine is caused by selenium deficiency in rats. Biochem J 248: 443–7, 1987

7. Beckett GJ., Nicol F., Proudfoot D., Dyson K., Loucaides G., Arthur JR. The changes in hepatic enzyme expression caused by selenium deficiency and hypothyroidism in rats are produced by independent mechanisms. Biochem J 266: 743–7, 1990.

8. Meinhold H., Campos–Barros A., Walzog B., Köhler R., Müller F. Effects of selenium and iodine deficiency on type I, type II, and type III iodothyronine deiodinases and circulating thyroid hormone in rats. Exp Clin Endocrinol 101: 87–93, 1993.

9. Beckett GJ., Russel A., Nicol F., Sahu P., Wolf C., Arthur JR. Effect of selenium deficiency on hepatic type I 5–iodothyronine deiodinase activity and hepatic thyroid hormone level in the rat. Biochem J 282: 483–6, 1992.

10. Chanoine JP., Safran M., Farwel AP., Dubord S., Alex S., Stone S. Effects of selenium deficiency on thyroid hormone economy in rats. Endocrinol 131: 1787–92, 1992.

11. Chopra IJ. A study of extrathyroidal conversion by thyroxine (T4) to 3, 3', 5–triiodothyronine (T3) in vitro. Endocrinol 101: 453–63, 1977.

12. Kabadi UM., Premachandra BN. Low serum T3 and raised reverse T3 levels in hepatic cirrhosis: role of glucagon. J. Gastroenterol 86: 1504–7, 1991.

13. Arthur JR., Morrice PC., Nicol F., Beddows SE., Boyd R., Hayes JD., Beckett GJ. The effects of selenium and copper deficiencies on glutathione S–transferase and glutathione peroxidase in rat liver. Biochem J 248: 539–44, 1987.

14. Burman KD., Smallridge RC., Jones L., Ramos EA. Glucagon kinetics in fasting: Physiological elevation in serum 3, 5, 3'–triiodothyronine increase the metabolic clearence rate of glucagon. J Clin Endocrinol Metab 51: 1158–65, 1980.

15. O'Connel M., Robbins DC., Horton ES., Sims EA., Danforth E. Changes in serum concentration of 3, 3', 5–triiodothyronine and 3, 5, 3'–triiodothyronine during prolonged moderate exercise. J Clin Endocrinol Metab 49: 242–6, 1979.

16. Kabadi UM., Einstein AB., Tucci J. Hyperglucagonemia in hepatic cirrhosis: Its relation to hepatocellular dysfunction and normalization on recovery. J Gastroenterol 790: 143–9, 1984.

17. Bernardi M., Rossane P., Trevisani F., Pesa O. "Low T3 syndrome" in cirrhosis: Effect of beta–blockade. J Gastroenterol 84: 727–31, 1989.

18. Kabadi UM., Premachandra BN. Serum T3 and reverse T3 levels in hepatic cirrhosis: Relation on hepatocellular damage and normalization on improvement in liver dysfunction. J. Gastroenterol 78: 750–5, 1983.

19. Chopra IJ., Chopra U., Smith SR., Reza M., Solomon DH. Reciprocal changes in serum concentrations of 3, 3', 5–triiodothyronine (reserve T3) and 3, 5, 3'–triiodothyronine (T3) in systemic illnesses. J Clin Endocrinol Metab 41: 1043–9, 1975.

20. Kabadi UM., Premachandra BN. Glucagon administration induces lowering of serum T3 and rise in reserve T3 in euthyroid healthy subjects. Horm Metabol Res 17: 667–70, 1985.

21. Arthur JR., Nicol F., Becket GJ. Hepatic iodothyronine 5'–deiodinase: Role of selenium. Biochem J 272: 537–40, 1990.

22. Chanoine JP., Safran M., Farwell AP., Tranter P. Selenium deficiency and type II 5'–deiodinase regulation in the euthyroid and hypothyroid rat: Evidence of a direct effect of thyroxine. Endocrinol 131: 479–84, 1992.

23. Beckett GJ., MacDougall DA., Nicol F., Arthur JR., Inhibition of type I and type II iodothyronine deiodinase activity in rat liver, kidney, and brain produced by selenium deficiency. Biochem J 259: 887–92, 1989.