

CLINICAL STUDY

Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis

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Abstract

Objective: Selenium (Se) in the form of selenocysteine is an essential component of the family of the detoxifying enzymes glutathione peroxidase (Gpx) and of the iodothyronine selenodeiodinases that catalyse the extrathyroidal production of tri-iodothyronine (T₃). Thus, Se deficiency may seriously influence the generation of free radicals, the conversion of thyroxine (T₄) to T₃ and the autoimmune process. Therefore, we performed a randomised, placebo-controlled prospective study to investigate the effects of Se treatment on patients with autoimmune thyroiditis (AIT).

Design and methods: Sixty five patients aged 22–61 years (median age 48 years) with AIT were recruited into two groups. Group I (Gr I) (*n* = 34) was treated with selenomethionine (Seme) 200 µg, plus L-thyroxine (LT₄) to maintain TSH levels between 0.3–2.0 mU/l, whereas group II (Gr II) (*n* = 31) received LT₄ plus placebo over a period of 6 months. Moreover, the pharmacokinetics of Seme were studied in 10 patients and eight volunteers at baseline and 2 h, 4 h, 6 h and 24 h after oral administration of a 200 µg tablet of Seme. Finally, Se levels were measured at the end of the study in some patients of both groups and their results were correlated with thyroid hormone levels.

Results: In the pharmacokinetics study, basal serum concentration of Se (75±6 µg/l) was within the reference range (70–125 µg/l), it promptly increased at 2 h, peaked at 4 h (147±17 µg/l; *P* < 0.0001) and it was abundant in serum at 24 h. In Gr I, antibodies against thyroid peroxidase (anti-TPO) levels showed an overall decrease of 46% at 3 months (from 1875±1039 U/l to 1013±382 U/l; *P* < 0.0001) and of 55.5% at 6 months. In Gr II the overall decrease of anti-TPO amounted to 21% at 3 months and to 27% at 6 months (from 1758±917 U/l to 1284±410 U/l; *P* < 0.005). There were no significant changes of antibodies against thyroglobulin levels between the groups. At the end of this study Se levels were found to be statistically significantly increased in Gr I (*n* = 9/34) compared with Gr II (*n* = 11/31) (97±8.4 vs 79±8; *P* < 0.01) but no correlation with thyroid hormone was found.

Conclusions: Seme is proven to be rapidly absorbed by the gastrointestinal tract. It appears to be useful as adjunctive therapy with LT₄ in the treatment of AIT. The exact mechanism(s) is not very well determined, it might enhance the activity of detoxifying enzymes and enforce the defense against oxidative stress.

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Introduction

Selenium (Se) in the form of selenocysteine, an analogue of cysteine in which sulphur is replaced by Se, is an essential component of the glutathione peroxidase enzymes (Gpx) and of thioredoxin reductase (TR) which protects tissues from oxidative damage by reduction of hydrogen peroxide (H₂O₂) (1). Another important class of selenoproteins are the iodothyronine selenodeiodinases, D1 and D2, which are responsible for the production of biologically active tri-iodothyronine (T₃) via 5'-deiodination in the various extrathyroidal tissues (2). Se in plasma is incorporated in selenoprotein P, that may serve as a transport protein for Se and facilitate whole body Se distribution (3).

There are scarce data regarding the effects of Se supplementation on thyroid function. In a trial conducted in northern Zaire, a severely Se and iodine deficient area, two months of treatment with 50 µg selenomethionine (Seme) resulted in a spectacular fall in serum thyroxine (T₄) that could only be partially recovered after iodine supplementation (4). In animal models, long term Se nutritional deficiency induced only marginal effects on the thyroid T₄ and T₃ content and on the activity of 5'-D1 in the thyroid gland, revealing a resistance of the gland to Se deficiency (5). In contrast, peripheral 5'-D1 and Gpx activity in the thyroid were markedly decreased (6).

Currently, a study from South Germany, an area with mild iodine and Se deficiency, reported that Se

substitution with sodium selenite led to improvement of inflammatory activity in patients with autoimmune thyroiditis (AIT) (7). However, it is difficult to extrapolate these results to a wider population, as there is a broad variation of Se concentrations in the European population that may reflect dietary habits, bioavailability of Se compounds, racial differences or various analytical methods. South Greece and more precisely the region around Athens (Attiki) is Se and iodide sufficient (8, 9). For the above mentioned reasons, and since there are no data available, we conducted this investigation to assess the effects of long-term treatment with Se combined with L-thyroxine (L-T₄) on the autoimmune and thyroid function of patients with AIT.

Subjects and methods

Sixty five patients (mean age 47.8 years, range 22 to 61 years), 56 female and 9 male, with AIT (antibodies against thyroid peroxidase (anti-TPO) > 100 U/l) and mild thyroid failure (MTF) characterized by normal free (F)T₄ and T₃ and increased serum thyroid stimulating hormone (TSH) levels (TSH > 4 mU/l) were randomized into two groups. Group I (Gr I) (*n* = 34) was treated with L-T₄ in a titrated dose to maintain TSH from 0.3 to 2.0 mU/l combined with Se in the form of 200 µg Seme (Lamberts, Athens, Greece) administered once daily. Group 2 (Gr II) (*n* = 31) received L-T₄ plus placebo. None of the patients were undergoing treatment with anti-depressive drugs, anti-psychotic drugs, or preparations containing vitamins or trace elements. The investigation was performed over a period of 6 months. Serum concentrations of FT₄, T₃, TSH, anti-TPO and antibodies against thyroglobulin (anti-Tg) were measured at baseline and after 3 and 6 months of treatment. Moreover, to detect any correlation with thyroid hormone, serum Se levels were measured arbitrarily in 9/34 patients of Gr I and 11/31 of Gr II.

To obtain some kinetic data of Seme, blood samples were collected at 0, 2, 4, 6 and 24 h after the intake of a tablet containing 200 µg Seme in 10 patients and 8 volunteers. Informed consent was obtained from all participants in this study.

Measurements

Serum Se levels were determined in duplicate with a graphite furnace atomic absorption spectrometer with the Zeeman background correction (Spectra 300, Varian, Australia) by using a standard addition method. Samples were compared with the standard curve by linear least-squares fit analysis. The detection limit for Se was 7.0 µg/l (0.090 µmol/l). Within run and run-to run coefficients of variation (CV) for Se were 1.9% and 4.4% respectively.

Serum concentrations of anti-Tg and anti-TPO were measured using IRMA (DiaSorin, Salugia, Italy) with a cut-off value for anti-Tg at 100 U/l, determined as the first standard point, and for anti-TPO at 100 U/l. All values below these cut-off points were graded as negative. The intra-assay CV values were 4.5% for anti-Tg and 4.8% for anti-TPO. The interassay CV values were 4.1% and 9.1% respectively.

Serum T₃ levels were determined using Amerlex MT₃ RIA Kits (Johnson and Johnson Clinical Diagnostics Ltd, Amersham, UK). The intra-assay CV value was 5%, whereas the interassay CV value was 5.8%. FT₄ was measured using a one-site chemiluminescence immunometric method (FT₄-Estimate, Nichols, San Juan Capistrano, CA, USA). The normal range for FT₄ was 9–25 pmol/l. The mean intra-assay CV was 5.6% and the interassay CV was 8.5%. TSH concentrations were determined with TSH third-generation assay (Nichols). The normal values ranged from 0.3 to 4.0 mU/l. The mean intra-assay CV at 0.01 mU/l was 12% and at 1.29 mU/l was 4.6%. The interassay CV at 0.02 mU/l and at 1.28 mU/l was 15.0% and 5.5%, respectively.

Statistics

All results are presented as mean ± s.d. Comparisons between baseline and post-selenium serum concentrations were performed by the analysis of variance (ANOVA) for repeated measures. Differences between the groups during the treatment period were analyzed by the Mann–Whitney nonparametric test. To study the relationship between TSH, FT₄, T₃ and Se concentrations, a linear regression was used. A *P* value of < 0.05 was considered significant.

Results

Serum concentrations of TSH, FT₄ and T₃ of both groups during the study period are presented in Table 1. There were no statistically significant changes between the two groups at any time during the study.

In the pharmacokinetic study, basal serum concentrations of Se were found to be within the reference range in patients and volunteers (75 ± 6 µg/l vs 77 ± 7 µg/l, non-significant). Se increased 2 h after intake (122 ± 14 µg/l; *P* < 0.0001 vs baseline), peaked at 4 h in both groups (147 ± 17 µg/l; *P* < 0.0001 vs baseline) and it was abundant in serum at 24 h (102 ± 10 µg) (Fig. 1).

In Gr I anti-TPO levels exhibited an overall decrease of 46.0% at 3 months and of 55.5% at 6 months. In a further analysis, by stratifying the patients according to the percentage decrease of anti-TPO levels, 18/34 (53%) in Gr I exhibited a marked decrease of anti-TPO by 73% at 3 months and by 86% at 6 months, whereas 12/34 (35%) showed a decrease of 22% at

Table 1 TSH, FT₄, T₃ and anti-Tg serum concentrations (mean±s.d.) in patients with autoimmune thyroiditis treated either with selenomethionine plus LT₄ (Gr I) or with LT₄ plus placebo (Gr II) over a period of 6 months.

	Months					
	Gr I			Gr II		
	0	3	6	0	3	6
TSH mU/l (nr: 0.3-4 mU/l)	9.8±3.6	1.4±0.6	0.7±0.2	9.5±3.8	1.1±0.5	0.8±0.3
FT ₄ pmol/l (nr: 9-25 pmol/l)	12.4±1.1	15.9±1.2	16.7±1.3	11.9±1.2	16.4±1.7	16.8±1.5
T ₃ nmol/l (nr: 0.9-2.7 nmol)	1.7±0.2	2.1±0.2	2.2±0.2	1.7±0.2	2.2±0.2	2.2±0.2
Anti-Tg (< 100 U/l)	1724±412	1672±392	1638±404	1807±482	1750±386	1697±417

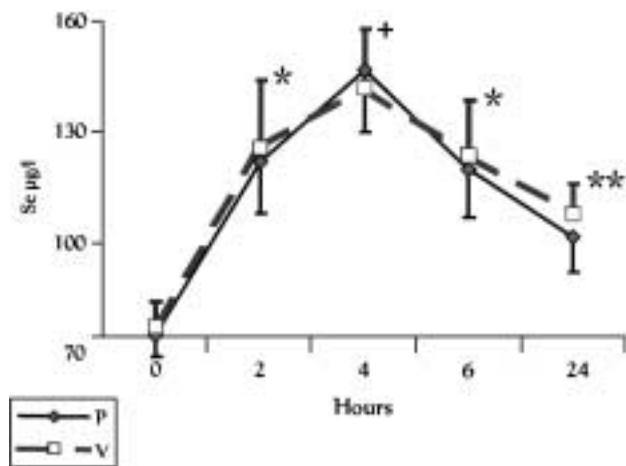


Figure 1 Selenium concentrations in serum after oral intake of 200µg selenomethionine in 10 patients with autoimmune thyroiditis (P) and 8 volunteers (V). * *P* < 0.0001 vs t₀; + *P* < 0.0010 vs t₀; ** *P* < 0.05 vs t₀.

3 months and 28% at 6 months and 4/34 (12%) did not show any decrease. In absolute numbers anti-TPO levels decreased from 1875±1039 U/l to 1013±382 U/l (*P* < 0.0001) at 3 months and to 844±227 U/l (*P* < 0.05 vs 3 months) at 6 months (Table 2). In Gr II an overall decrease of anti-TPO by only 21% at 3 months and by 27% at 6 months was registered. In an analysis by subdividing the patients, 22/31 (71%) showed a decrease of anti-TPO amounting to 29% at 3 months and to 32% at 6 months of treatment. In contrast 7/31 (22%) presented a decrease of only 13% at 3 and of 22% at 6 months. In absolute numbers anti-TPO levels decreased from

1758±917 U/l to 1389±520 U/l at 3 months and to 1284±410 U/l (*P* < 0.001) at 6 months (Table 2).

Anti-Tg levels decreased very slightly in both groups without reaching any significant level (Table 1). No side effects have been observed. In Gr I, 25/34 (73.5%) patients reported a satisfactory improvement of mood and sleep and stated less fatigue. In Gr II 15/31 (48.4%) affirmed an amelioration of behavior and tiredness. No correlations could be found between TSH, FT₄, T₃ and serum Se concentrations.

Discussion

In our study Se levels promptly increased in serum after oral administration of Seme indicating a good absorption of the supplement. The data do not allow us to draw any conclusions about bioavailability, as it depends on the conversion of absorbed Se into a biologically active form and tissue retention. Nevertheless, the increase of Se may lead to the presumption that Seme is rapidly transformed to an active form. Although there is some evidence suggesting that Seme is retained more efficiently than inorganic selenate or selenite, the retained fraction may not all be bioavailable (10).

The most important finding of this study was the striking reduction of anti-TPO levels in patients treated with Seme and LT₄ in a borderline Se sufficient region. The mechanism(s) of action of Se on the immune system is not well determined. Several enzymatic systems, such as dismutases and Gpx, have evolved to circumvent the electron spin restriction of O₂ reduction and prevent the accumulation of very reactive free radicals in the various organs such as superoxide

Table 2 Overall decrease in percentage of serum anti-TPO concentrations after 6 months of treatment with selenomethionine plus LT₄ (Gr I) or with LT₄ and placebo (Gr II) over a period of 6 months.

	Months					
	Gr I			Gr II		
	0	3	6	0	3	6
Anti-TPO (< 100 U/l)	1875±1039	1013±382*	844±227**	1758±917	1389±520	1284±410***

* *P* < 0.0001 vs t₀; ** *P* < 0.05 vs t₃; *** *P* < 0.001 vs t₀.

anion radical (O_2^-), hydroxyl radical (OH^-) and H_2O_2 (11). H_2O_2 in the thyroid gland may easily cross the apical membrane to the luminal site where it reacts with TPO for the iodination of Tg (12). It is well known that the iodination of Tg not only requires H_2O_2 and TPO but also iodide in close proximity (13). Thus, Gpx activity at the apical membrane may reduce the substrate for Tg iodination decreasing the H_2O_2 production. The scavenging activity of Gpx is regulated by the production rate of O_2^- and H_2O_2 (14). In contrast, the maintenance of the scavenging capacity during states of increased O_2^- flux is dependent on the nutritional levels of Se, as low serum levels appear to be reflected in low Gpx activity (15). However, the normal basal serum Se concentrations in our study do not exclude low intrathyroidal Se levels and reduced scavenging activity in AIT. In this hypothesis selenoprotein P might have a crucial role (16). Furthermore, Se supplementation may additionally increase TR activity. TR is a strong defender against oxidative stress and it has been reported high in acute and reduced in chronic phase of some diseases (17).

The reduction of anti-TPO in our study was very prominent in the first 3 months in the group treated with Se and it was further sustained after 6 months of treatment. One explanation could be a lower baseline intrathyroid Se concentration and decreased scavenging activity possibly due to a prolonged inflammatory process that has been reverted by Se supplementation. Thus, administration of Seme 200 μ g per day over 6 months may increase intrathyroid Se levels without saturating this biological compartment. The decrease of anti-TPO levels was slightly more profound compared with the study by Gärtner *et al.* (7). Some reasons for this could be that the initial values of anti-TPO were higher in our patients and/or differences in nutritional Se intake.

We could not detect any difference regarding anti-Tg levels between both groups, in contrast to previous report by other authors (7). The discrepancies might be due to differences in iodine intake.

The levels of thyroid hormone were not affected by Seme treatment, indicating a sufficient Se intake in these patients. This is consistent with other data showing that Se treatment of patients with thyroiditis did not affect thyroid hormone synthesis (18). Furthermore, Se is not a limiting factor for peripheral T_4 to T_3 conversion in patients with congenital hypothyroidism and borderline Se intake (19). Thus, Se intake does not cause any change in thyroid hormone metabolism as 5/DI-I remains unchanged in populations with sufficient iodine and Se intake.

Finally, Seme treatment was very well tolerated. The striking majority of the patients reported an improvement in mood and well-being. This is supported by other researchers who showed that healthy men who consumed dietary Se at high levels (226 μ g/day) had a significant improvement of their moods as compared

with those consuming 32 μ g Se per day in their diets (20). This may be due to changes in dopamine and/or serotonin metabolism in the CNS (21).

Conclusively, our study demonstrates that Seme supplementation combined with lT_4 may enhance immunocompetence without affecting thyroid hormone metabolism. However, further research is required to clarify the exact mechanism of action and demonstrate if modification of Se dietary intake, especially in areas with selenium deficiency, may also strengthen immunocompetence.

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