Effect of experimentally induced subchronic selenosis on thyroid hormones and biochemical indices in calves

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Summary

The present investigation was performed to determine the effect of long-term administration of sodium selenite on the biochemical indices and concentration of thyroid hormones in calves. Sodium selenite solution was drenched at 0.1 and 0.25 mg per kg body weight for 12 weeks. Higher dose produced characteristic symptoms of selenosis whereas mild symptoms were observed with lower dose. The toxic symptoms appeared when blood selenium level was 1.68 ± 0.13 µg per ml. There was a significant ($P<0.05$) increase in the activities of the aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen and creatinine levels in the treated animals. The repeated administration of sodium selenite resulted in a significant p-value decline in thyroxine levels on the 10th week and increase in triiodothyronine on the 8th week of treatment. The findings of the present study suggested that sodium selenite induced selenosis alters thyroid hormone levels in plasma.

Key words: Calves, Selenium toxicity, Thyroid hormones

Introduction

Selenium is capable of exerting multiple actions on the endocrine system by modifying the expression of many selenoproteins (Beckett and Arthur, 2005). The human thyroid gland has the highest selenium content per gram of tissue among all organs. Several selenoproteins such as glutathione peroxidase, type-1 5-deiodinase, thioredoxin reductase and selenoprotein-P are expressed in the thyroidal cells. Activities of selenoenzyme deiodinases lead to the activation of prohormone T_4 to active hormone T_3 as well as the inactivation of T_3 to T_2 (Kvicala and Zamrazil, 2003). Thus, selenium is required for the appropriate thyroid hormone synthesis, activation and metabolism. Deiodinases, are also indispensable for thyroid function and thyroid hormone metabolism (Kohrle, 1996; Rayman, 2000), however, high selenium concentration has inhibitory effect on type-1 5-deiodinase (Kohrle, 1999). So the thyroid hormone metabolism may be subject to alteration depending upon the selenium status. Various studies have shown that selenium deficiency leads to alterations in the peripheral metabolism of thyroid hormone. The information regarding the effect of selenium in excess of requirement on the thyroid hormone levels is limited. Selenium toxicity affects liver and kidney, the two organs that play important role in peripheral thyroid hormone metabolism (Beckett and Arthur, 2005). The present study was undertaken to elucidate the effect of experimentally induced selenosis on the plasma concentration of thyroid hormone levels and biochemical parameters.

Materials and Methods

Nine crossbred male calves, 9–12-month-old, weighing between 70-140 kg were used in the present study. The animals were kept under standard uniform conditions in the departmental animal house. The

animals were maintained on maize, the green fodder of the season and water *ad libitum*. All the animals were dewormed using albendazole at the dose of 7.5 mg per kg body weight prior to the commencement of study. Calves were divided randomly into three groups of three animals each. Group 1 and 2 were administered sodium selenite at 0.1 and 0.25 mg per kg body weight, respectively, for 12 weeks, whereas group 3 served as the control. The specified amount of the selenium salt was dissolved in about 50 ml of tap water and was administered by drenching this solution. The selection of dose was based on earlier work done in our laboratory (Deore *et al*., 2002; Kaur *et al*., 2003). Blood was collected by jugular venipuncture in heparin containing test tubes. Plasma was separated from heparinized blood by centrifuging at 3000 rpm for 15 min, and stored at -20°C. The concentration of selenium in whole blood was determined by spectrophotometric method as described by Cummins *et al*. (1965). Plasma aspartate aminotransferase (AST) was assayed by the method of Reitman and Frankel (1957). Plasma alkaline phosphatase was assayed by using disodium phenyl phosphate as substrate, blood urea nitrogen assayed by the diacetyl monoxime and creatinine by alkaline picrate as procedures described by Wootton (1964). The concentration of triiodothyronine (T_3) and thyroxine (T_4) was estimated using RIA kits (Diasorin, Minnesota USA). The RIA kit was designated for the quantitative determination of triiodothyronine and thyroxine in plasma by a direct assay technique. The sensitivity of the assay for triiodothyronine and thyroxine was 0.09 ng per ml and 0.98 µg per dl, respectively, at 95% confidence limit and intra-assay coefficient of variation was <10%. Data were subjected to statistical analysis as Snedecor and Cochran (1967) using the test of significance at 1 and 5%. All parameters were statistically analysed using Student's t-test. The experimental plan was approved by Institutional Animal Ethics Committee.

Results

The long-term administration of sodium selenite at the dose rate of 0.25 mg per kg body weight produced characteristics symptoms of selenosis, i.e. alopecia, cracking and enlargement of hooves, intra-digital lesions and discoloration of hard palate. Mild symptoms, viz. roughness of hair coat and patchy alopecia were observed at lower doses. The toxic symptoms appeared when the blood selenium level was 1.68 ± 0.13 µg per ml. One of the calves of the lower dose group died in the 2nd week of the post-treatment period because of reasons other than the treatment. The activity of plasma AST and alkaline phosphatase was significantly (P<0.01) increased after 4th and 8th weeks of treatment, respectively; followed by progressive increase in the enzyme activity in the treated animals (Table 1). The long-term administration of sodium selenite in calves also significantly increased blood urea nitrogen (BUN) and plasma creatinine levels. The effect of prolonged administration of sodium selenite on the thyroid hormones of calves is presented in Table 2. In the low dose group, repeated administration of sodium selenite did not produce any significant (P>0.05) alterations in the thyroid hormone levels, however, in the high dose group a significant decline in the thyroxine levels on the 10th week and a significant increase in the triiodothyronine levels on the 8th week of treatment were observed. The T_4/T_3 ratio was also significantly (P<0.01) reduced from 8th to 10th week of treatment.

Discussion

The clinical signs of sub-chronic selenosis appeared within 45-60 days of high-dose sodium selenite administration, when the blood selenium concentration was 1.68 ± 0.13 µg per ml; it was well below 2 µg per ml, the concentration above which clinical signs of selenosis were frequently observed in cattle (Ellis *et al*., 1997). The lower dose produced only subtle signs of toxicosis such as roughness of hair coat and patchy alopecia after 80 days of administration. Such signs could easily be missed on routine clinical examination.

The AST is a cytosolic and

Table 1: Effect of long-term administration of sodium selenite on the blood biochemical indices in calves

Groups	Number of weeks					
	Treatment					Post-treatment
	0	4	8	10	12	3
Plasma aspartate aminotransferase (nmol pyruvate formed/min/ml)						
Control	35.1 ± 0.37	36.3 ± 0.25	35.6 ± 0.60	37.1 ± 0.07	36.0 ± 0.06	37.2 ± 0.27
Group 1	36.1 ± 0.32	37.3 ± 0.24	43.3 ± 0.37**	46.8 ± 0.14**	49.9 ± 0.25**	40.1[a]
Group 2	35.2 ± 0.30	40.2 ± 0.12**	48.6 ± 0.25**	54.1 ± 0.21**	58.3 ± 0.48**	42.2 ± 0.36**
Plasma alkaline phosphatase (nmol phenol liberated/min/ml)						
Control	41.7 ± 0.14	40.9 ± 0.13	41.1 ± 0.10	40.3 ± 0.14	40.9 ± 0.04	41.6 ± 0.04
Group 1	40.1 ± 0.34	41.1 ± 0.23	45.1 ± 0.23**	48.3 ± 0.35**	51.9 ± 0.58**	42.8[a]
Group 2	40.3 ± 0.23	42.5 ± 0.49*	54.6 ± 0.33**	58.0 ± 0.71**	63.2 ± 0.37**	45.1 ± 0.07**
Plasma creatinine (µmol/L)						
Control	53.1 ± 1.77	53.9 ± 0.88	57.5 ± 0.88	57.4 ± 0.87	62.7 ± 0.88	62.7 ± 0.88
Group 1	53.0 ± 2.65	53.9 ± 0.88	62.7 ± 0.88**	67.1 ± 0.88**	70.7 ± 0.88**	65.4[a]
Group 2	53.2 ± 0.88	59.2 ± 0.88	77.8 ± 2.65**	84.0 ± 1.77**	93.7 ± 1.77**	69.8 ± 0.88**
Blood urea nitrogen (mmol/L)						
Control	3.77 ± 0.12	3.83 ± 0.03	3.96 ± 0.05	4.08 ± 0.04	4.18 ± 0.01	4.08 ± 0.08
Group 1	3.83 ± 0.14	4.02 ± 0.05	4.56 ± 0.04**	4.94 ± 0.03**	5.32 ± 0.04**	4.39[a]
Group 2	3.88 ± 0.06	4.18 ± 0.03**	5.44 ± 0.05**	6.25 ± 0.04**	6.99 ± 0.04**	4.63 ± 0.07**

Values given are mean $\pm$ SE of three animals unless otherwise stated; [a] value is the mean of only two animals; *$P<0.05$ and **$P<0.01$

Table 2: Effect of long-term administration of sodium selenite on plasma concentration of thyroid hormones in calves

Groups	Number of weeks					
	Treatment					Post-treatment
	0	4	8	10	12	3
Thyroxine (nmol/L)						
Control	36.6 ± 4.25	37.8 ± 2.58	37.8 ± 1.03	44.9 ± 2.96	42.5 ± 2.83	53.4 ± 6.18
Group 1	36.9 ± 8.75	32.4 ± 3.99	27.9 ± 5.28	49.5 ± 9.40	58.2 ± 11.2	58.7[a]
Group 2	37.7 ± 2.19	27.3 ± 2.70	34.5 ± 5.41	24.3 ± 2.33**	48.6 ± 4.76	39.7 ± 4.76
Triiodothyronine (nmol/L)						
Control	0.98 ± 0.14	1.31 ± 0.09	0.92 ± 0.04	1.07 ± 0.01	1.04 ± 0.05	1.10 ± 0.10
Group 1	1.34 ± 0.15	1.36 ± 0.14	0.90 ± 0.15	1.03 ± 0.23	1.24 ± 0.19	1.39[a]
Group 2	1.21 ± 0.07	1.15 ± 0.04	1.16 ± 0.02*	1.11 ± 0.05	1.16 ± 0.02	1.10 ± 0.03
T_4/T_3 (ratio)						
Control	40.4 ± 1.96	28.9 ± 2.21	41.1 ± 2.50	42.0 ± 3.30	40.9 ± 2.55	48.1 ± 5.1
Group 1	27.5 ± 1.10	23.8 ± 2.75	31.0 ± 1.10**	48.1 ± 3.00	46.9 ± 3.30	42.1[a]
Group 2	31.2 ± 1.82	23.7 ± 2.06	29.7 ± 1.10**	21.9 ± 3.50**	41.9 ± 4.10	36.9 ± 4.1

Values given are mean $\pm$ SE of three animals unless otherwise stated; [a] value is the mean of only two animals; *$P<0.05$ and **$P<0.01$

mitochondrial enzyme widely distributed in tissues and is released from cytoplasm of hepatocytes and myocytes during degenerative changes (Evans and Heath, 1988). The increased plasma activities of AST might be due to these degenerative changes and increased cell membrane permeability (Ramazzotto and Carlin, 1978). Selenosis has been reported to increase the activity of AST in goat (Sashidhar and Prasad, 1993) and buffalo (Dhillon *et al.*, 1990). The increased activity of plasma alkaline phosphatase indicates damage to intestines, liver, bones and kidney (Kaplan and Righetti, 1970). However, there is considerable physiological range of activity of alkaline phosphatase in cattle (Blood *et al.*, 1983; Putman *et al.*, 1986; Kramer, 1989). The increase in BUN and/or creatinine levels generally indicates renal

dysfunctions due to kidney damage (Hook, 1980). The increase in BUN levels may reflect an accelerated rate of protein catabolism rather than decreased elimination of urea, however, dehydration induced pre-renal retention of nitrogenous waste and hepatic dysfunction can also increase blood urea nitrogen levels (Finco, 1989).

The increase in T_3 and corresponding decline in T_4 level recorded in the present study can be attributed to the altered peripheral metabolism of T_4 and this is further supported by the decrease in T_4/T_3 ratio. The selenium status has been reported to affect thyroid hormone metabolism by regulating activity of type I deiodinase (Kohrle, 1999). The activity of this enzyme is decreased in selenium deficient animals but on selenium supplementation the enzyme regains its activity. The concentration of thyroid hormones depends upon their synthesis by thyroid and peripheral metabolism. The conversion of T_4 to T_3 and subsequently to T_2 by deiodination, is controlled by three isoenzyme: type 1D I (liver, kidney and thyroid), type 1D II (brain, brown adipose tissue and pituitary) and type 1D III (brain and placenta) (Beckett et al., 1992). Type 1-5' deiodinase, mainly present in the liver and kidney (Beckett et al., 1992), requires selenium for its optimum activity (Arthur et al., 1990; Beckett et al., 1990) and is responsible for 80% of circularly T_3 levels. So the increase in T_4 at the later stages of the present study, could be due to decreased conversion of T_4 to T_3 as a result of inhibition the activity of type 1-5' deiodinase (Eder et al., 1995). Further work is required to determine the effect of excess selenium on the activities of various deiodinases. The findings of the present study suggested that sodium selenite induced selenosis alters thyroid hormone levels in plasma.

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