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Hypercholesterolemia and tissue-specific differential mRNA expression of type-1 5'-iodothyronine deiodinase under different selenium status in rats

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ABSTRACT

Type-1 5'-iodothyronine deiodinase (5'-DI) is responsible for conversion of T_4 to T_3. Selenium (Se) is an integral part of this enzyme. Keeping in view the strong association between atherosclerosis and hypothyroidism, the present study examined the behavior of 5'-DI in liver, aorta and thyroid during hypercholesterolemia following different Se status, i.e., Se deficiency (0.02ppm), adequate (0.2ppm) and excess dose (1ppm) in SD male rats. Animals were fed a control or high-cholesterol diet (2%) for 1 and 2 months. 5'-DI activity and mRNA expression was measured by RIA and RT-PCR respectively. In liver and aorta, 5'-DI expression significantly decreased with the Se-deficient and the high-cholesterol diet. The trend was opposite in thyroid, i.e., mRNA expression increased significantly during selenium deficiency and with a high-cholesterol feeding. But with 1ppm Se supplementation, the 5'-DI expression increased in all the three tissues. The present study indicates that hypercholesterolemia along with selenium deficiency is co-responsible for differential regulation of 5'-DI enzyme in thyroidal vs. extrathyroidal tissues. Distinct regulation of 5'-DI in the thyroid reflects the clinical importance of this selenoprotein during hypercholesterolemia as this enzyme is essential for T_3 production, which further has a vital role in the maintenance of lipid metabolism.

Key terms: selenium, hypercholesterolemia, 5'-DI, cholesterol, RT-PCR.

INTRODUCTION

Type-1 5'-iodothyronine deiodinase (5'-DI) has a pivotal role in controlling the supply of T_3 (the biologically active form of thyroid hormone) to the tissues. Under normal circumstances, more than 80% of plasma T_3 is derived from 5'-monodeiodination of T_4 (Vander Geyten et al., 2005), mainly in the liver and kidney, where this reaction is catalyzed by 5'-DI (Alvarez et al., 2005). Thyroid also express 5'-DI activity, and studies have shown that it is identical to the hepatic 5'-DI (Green, 1978). The activity of 5'-DI has been shown to be decreased in a hypothyroid state and elevated during hyperthyroidism (Wassen et al., 2004).

Selenium (Se), an essential element and potent antioxidant, has an important role in the control of thyroid hormone metabolism. Evidence from animal, clinical and *in vitro* cell culture studies suggested a clear Se-dependent expression of 5'-DI varying with Se availability (Korhle, 1999). As selenium is an integral part of this enzyme (Behne et al., 1990; Berry et al., 1991), 5'-DI activity rapidly decreases with Se deficiency (Backett et al., 1987).

Hypercholesterolemia has been shown to induce hypothyroidism (Wojcicki et al., 1991; Frank et al., 2004). Thyroid hormones (T_3 and T_4) have a vital role in the maintenance of normal lipid metabolism, and abnormal thyroid status can lead to biochemical and pathological changes that are potentially injurious to the cardiovascular system (Pingitore et al., 2005). Studies have demonstrated that almost 10% of asymptomatic hypercholesterolemic patients have subclinical hypothyroidism

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(Michalopoulou et al., 1998). Thyroid hormone replacement therapy improved the cardiac function in several patients (Siegmund et al., 2004). Several studies indicate that Se deficiency is associated with hypercholesterolemia (Kang et al., 2000; Lee et al., 2003). Keeping in view the important role of Se in hypercholesterolemia and its association with 5'-DI, the purpose of present study was to explore the response of 5'-DI enzyme in liver, aorta and thyroid under different Se status during experimental hypercholesterolemia.

MATERIALS AND METHODS

Animals

Male Sprauge-Dawley rats (100g-body weight) were used in the present study. Animals were obtained from the Central Animal House, Panjab University, Chandigarh.

Treatment Protocol

Animals were acclimatized to the laboratory animal room and initially divided into three groups: group I (Se-deficient diet fed); group II (Se-adequate diet fed); and group III (Se-excess diet fed). Feed and water were given *ad libitum*. This Se diet was given to the animals for 10 days in order to achieve the required Se status. The animals in these three groups were further divided into two each, *viz*.: group Ia (Se-deficient control), group Ib (Se-deficient + high-cholesterol diet fed); group IIa (Se-adequate control), group IIb (Se-adequate + high-cholesterol diet fed); group IIIa (Se-excess control), group IIIb (Se-excess + high-cholesterol diet fed). Treatment protocol was for 1 and 2 months.

Diet preparation

Se deficient diet: Yeast-based synthetic Se-deficient diet (0.02ppm Se) was prepared in the laboratory (Burk, 1987). It contained torula yeast (inactivated) 30%, sucrose 56.99%, corn oil 6.67%, mineral mix 5%, vitamin mix 1%, dlmethionine 0.3% and vitamin E 0.04%.

Se-supplemented diets: Se-adequate and Se-excess diets were prepared by supplementing the Se-deficient diet with 0.2 ppm and 1ppm of Se as sodium selenite (Sigma Chemicals).

High-cholesterol diet (HCD): 2% of cholesterol (Loba-Chemie, India) was added to the respective diets of the HCD groups.

After completion of the diet feeding schedule, the rats were kept on fasting for 10hrs, anesthetized and exsanguinated. Serum and tissue (liver, aorta and thyroid) samples were collected from each animal. Tissues were snap frozen in liquid nitrogen. Serum total cholesterol and triglycerides levels were estimated by enzymatic colorimetric kits obtained from HUMAN Gmbh (Germany). Various parameters were evaluated as detailed below.

Selenium estimation

Selenium level was estimated by fluorimetric method (Hasunuma et al., 1982), based on the principle that Se content in serum or tissue on acid digestion is converted to selenous acid. The reaction between selenous acid and aromatic-o-diamines, such as 2,3-diaminonaphthalene (DAN), leads to the formation of 4,5-benzopiazselenol, which displays brilliant lime-green fluorescence when excited at 366nm in cyclohexane. Fluorescence emission in extracted cyclohexane was read on a fluorescence spectrophotometer using 366nm as the excitation wavelength and 520nm as the emission wavelength.

Se-dependent glutathione peroxidase activity

Glutathione peroxidase (GSH-Px) activity was assayed by the coupled enzyme procedure with glutathione reductase, using H_2O_2 as substrate (Paglia and Valentine, 1967). The assay was carried out in the post-mitochondrial fraction (PMF) of liver as already published by the authors (Dhingra et al., 2003) and the activity expressed as μmoles of NADPH oxidized/min/mg protein. Total protein estimation was done in all the samples (Lowry et al., 1951).

T_3 and T_4 levels

Serum T_3 and T_4 estimation was done by radioimmunoassay (RIA) kits procured

from BARC, Mumbai (Cat. No. RIAK-4/4A and RIAK-5/5A for T_3 and T_4 respectively).

5'-DI activity

5'-DI activity in liver, aorta and thyroid was estimated by radioimmunoassay (Behne et al., 1990).

mRNA analysis

5'-DI mRNA expression was analyzed in liver, aorta and thyroid by using RT-PCR kit (QIAGEN Inc., USA).

RNA isolation

Total RNA from tissues was extracted using TRI REAGENT (Molecular Research Center, Inc., Ohio, USA). The integrity and size distribution (quality) of RNA was examined by formaldehyde agarose gel electrophoresis.

RT-PCR

2µg of total RNA template from the different groups after treatment with DNase I (Ambion) was used in RT-PCR reaction. To the reaction mixture, we added 10µl of 5X QIAGEN One Step RT-PCR buffer (2.5mM MgCl2 as final concentration), 2µl of dNTP mix (10mM of each dNTP), 5µl of each coding (+) and noncoding (-) gene-specific primers (from 10µM stock), 2µl QIAGEN One Step RT-PCR Enzyme Mix, 1µl RNase inhibitor (1U/µl) and, finally, 25µl of PCR-grade RNase-free water (provided in the kit) to reach a total volume of 50µl, which was mixed gently by vortex and centrifuged. The PCR reaction was performed in the thermal cycler (Techne Ltd., England) under the following conditions: the RT reaction was performed at 50ºC for 50 min; initial PCR activation was done at 95ºC for 15 min, followed by 35 cycles of 94ºC (denaturation) for 45 sec, 58.8ºC (annealing) for 45 sec, 72ºC (extension) for 1 min. Finally, the reaction mixture was incubated at 72ºC for 10 min to extend any incomplete single strands.

The optimal oligonucleotide primer pair for 5'-DI was selected with the aid of software Gene Runner (Informax Ltd., USA) and primer sequences for ß-actin were taken from the literature (Kimura et al., 1998). The primer sequence (5' to 3') for rat 5'-DI gene coding (+) strand was TCTGGGATTTCATTCAAGGC, the noncoding strand was TAGAGCCTCTCAGGCAGAGC. For rat ß-actin, the gene coding (+) strand was AGAGCTATGAGCTGCCTGAC, and the noncoding (-) strand was CTGCATCCTGTCAGCCTACG. The lengths of the RT-PCR products were 346bp and 236bp respectively for 5'-DI and ß-actin.

Final PCR products were analyzed on 1.5% agarose gel electrophoresis using 10 mM TE buffer. 5µl of PCR product was used from each tube. Densitometric analysis of the bands was done by UviBandMap software (Uvitech, England).

The above-described RT-PCR reactions for 5'-DI and ß-actin were determined on the basis of a series of experiments to test the reaction conditions, such as amount of RNA linearity, cycle linearity, primer concentrations etc. The final RT-PCR conditions were shown to be in the linear range of amplification for both the genes.

Statistical analysis

Data is expressed as mean ± SD. Difference between different groups was tested using student's t-test for unpaired values.

RESULTS

Selenium levels

Selenium levels in the serum and liver decreased significantly ($p<0.001$) in Se-deficient groups (Ia and Ib) and increased in 1ppm Se-supplemented diet fed groups (IIIa and IIIb) in comparison to the respective adequate groups (IIa and IIb). Significant decrease ($p<0.001$) in the Se level was observed in HCD-fed groups as compared to respective controls in all the three Se status, i.e., deficient, adequate and excess groups. In deficient groups (Ia and Ib) and in HCD fed adequate group, Se level decreased significantly ($p<0.001$), whereas in 1ppm Se supplemented groups, the level increased significantly ($p<0.001$) after 2 months in comparison to 1-month data (Fig. 1).

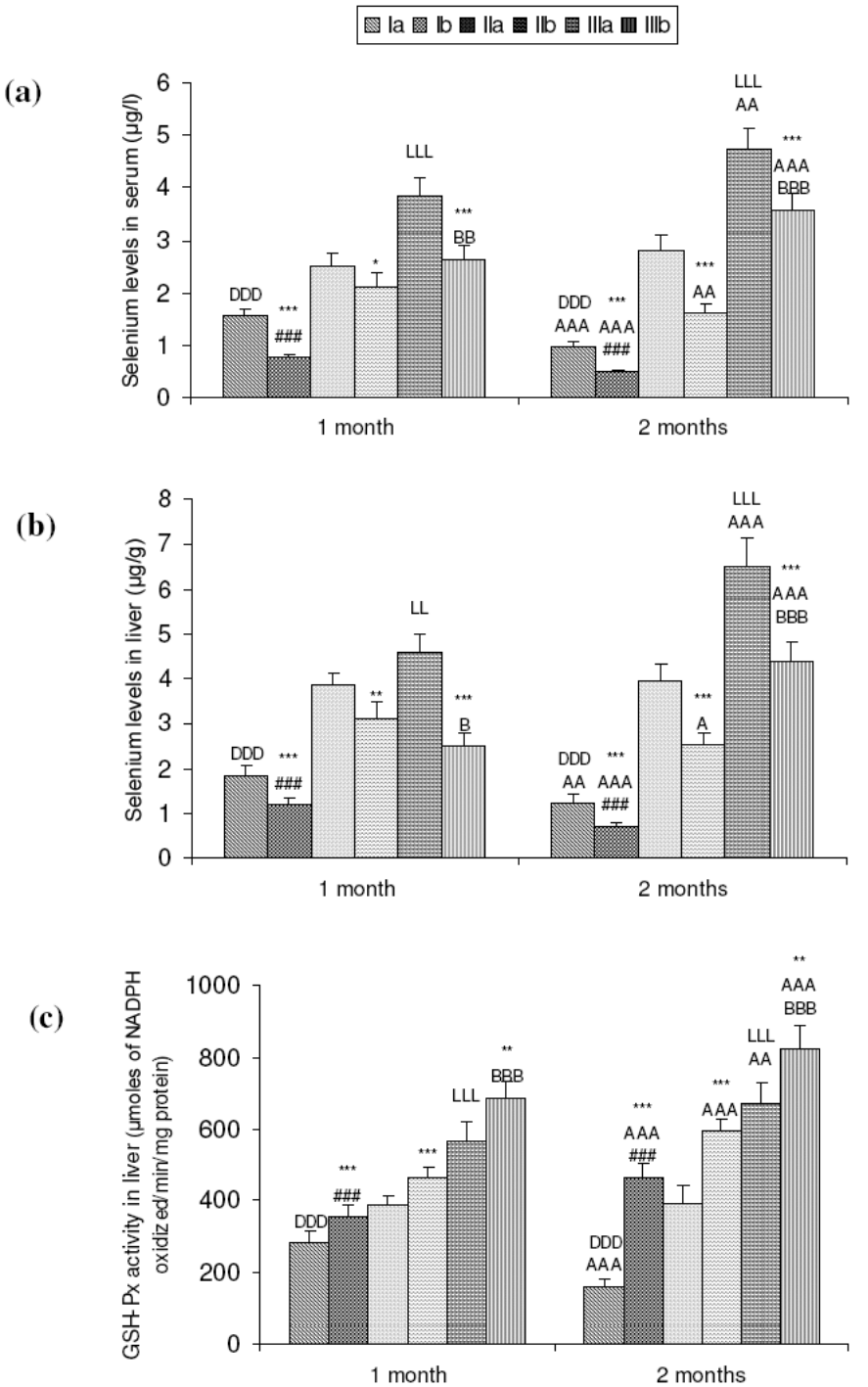

Figure 1: Selenium levels in serum and liver (a and b), hepatic GSH-Px levels (c), after 1 and 2 months, in different groups: Ia. Se-deficient control; Ib. Se-deficient+HCD; IIa. Se-adequate control; IIb. Se-adequate+HCD; IIIa. Se-excess control; IIIb. Se-excess+HCD,. Data is represented as mean ± SD from 6 observations. *p<0.05, **p<0.01, ***p<0.001 represent comparison between control and HCD groups; Ap<0.05, AA p<0.01, AAAp<0.001 comparison between 1 and 2 months; DDDp<0.001 comparison between groups Ia and IIa; ###p<0.001 comparison between groups Ib and IIb; LLp<0.01, LLLp<0.001 comparison between groups IIa and IIIa; Bp<0.05, BBp<0.01, BBBp<0.001 comparison between groups IIb and IIIb.

GSH-Px activity

GSH-Px activity in liver decreased significantly ($p<0.001$) in Se deficiency (Ia and Ib), and it increased with 1ppm Se supplementation (IIIa and IIIb) in comparison to the respective adequate groups (IIa and IIb). With HCD feeding, significant increase ($p<0.001$) was observed in all the groups in comparison to respective controls. In the Se-deficient control group, GSH-Px level decreased, whereas in HCD-supplemented Se-deficient and Se-adequate as well as Se-excess fed groups, the level increased significantly ($p<0.001$) after 2 months as compared to 1-month data (Fig. 1).

Lipid profile

In all the three Se status groups on HCD feeding, significant increase ($p<0.001$) in cholesterol and triglycerides concentration was observed in comparison to the respective control groups. In Se-deficient groups (Ia and Ib), lipid level increased, and with 1ppm Se supplementation, it decreased significantly ($p<0.001$) in comparison to respective adequate groups (IIa and IIb). In both the Se-deficient groups (Ia and Ib) and in HCD-fed adequate group, lipid levels increased significantly ($p<0.001$), whereas they decreased with Se supplementation after 2 months in comparison to 1 month (Table 1).

T3 and T4 levels

Levels of T_3 decreased and T_4 increased significantly ($p<0.001$) with HCD feeding in comparison to respective controls in all the three Se status groups. In Se deficiency (Ia and Ib), T_3 decreased and T_4 level increased in comparison to respective adequate groups, whereas the reverse trend was observed with 1ppm Se supplementation, i.e., the T_3 level increased and the T_4 level decreased significantly. In both the Se-deficient groups and in the HCD-fed adequate group (IIb) T_3 decreased and T_4 increased significantly, and in the Se-supplemented groups, T_3 increased and T_4 decreased after 2 months in comparison to 1 month (Table 1).

Type-I iodothyronine deiodinase (5'-DI) activity

5'-DI activity was estimated in liver, aorta, and thyroid. The activity decreased significantly ($p<0.001$) in HCD-fed animals in comparison to controls in liver and aorta. In Se deficiency, there was a significant ($p<0.001$) decrease in the activity in comparison to respective adequate groups. The trend was just opposite in thyroid in Se deficiency as well with HCD feeding: the activity increased significantly ($p<0.001$). With 1ppm Se supplementation, the activity increased in all the tissues. The 5'-DI activity decreased in Se-deficient groups (Ia and Ib), as well as in HCD-fed, adequate group, whereas it increased in Se-supplemented groups after 2 months in comparison to 1-month data in liver and aorta. In thyroid, the activity increased in all the groups (except IIa) after 2 months in comparison to 1-month data (Table 2).

Type-I iodothyronine deiodinase (5'-DI) mRNA expression

RT-PCR products of expected size, i.e., 346bp and 236bp, were obtained for 5'-DI and ß-actin, respectively. mRNA expression in liver and aorta decreased ($p<0.001$) in Se-deficient groups (Figs. 2 and 3) in comparison to adequate groups (IIa and IIb). With HCD feeding, the mRNA expression decreased ($p<0.001$) in liver and aorta in all the selenium status groups (Figs. 2 and 3). However, as with enzyme activity, in thyroid tissue the trend was the opposite, i.e., mRNA expression increased in selenium deficiency, as well as with HCD feeding ($p<0.001$) (Fig. 4). With selenium supplementation, the mRNA expression increased significantly ($p<0.001$) in all the tissues. The 5'-DI mRNA expression decreased in Se-deficient groups (Ia and Ib) as well as in HCD-fed adequate group, whereas it increased in Se-supplemented groups after 2 months in comparison to 1-month data in liver and aorta. In thyroid, the expression increased in all the groups (except IIa), after 2 months in comparison to 1 month (Figs. 2, 3 and 4).

TABLE 1

Total cholesterol, triglycerides, and T_3 and T_4 levels in serum after 1 and 2 months of control and high-cholesterol diet (HCD) feeding schedule.
(Data is represented as mean ± SD from 6 observations)

	Se deficient		Se adequate		Se excess	
	Control Ia	HCD Ib	Control IIa	HCD IIb	Control IIIa	HCD IIIb
Cholesterol in serum (mg/dl)						
1 month	107.87±6.87^{DDD}	246.54±11.96$^{\#\#***}$	83.62±5.45	215.23±13.45***	74.43±6.28^{L}	184.29±11.73$^{***\ BB}$
2 months	120.23±9.53^{DDDA}	295.14±13.43$^{\#\#***AAA}$	85.23±5.24	269.82±10.67^{***AAA}	62.73±5.38^{AALLL}	165.34±10.60$^{***BBB\ A}$
Triglycerides in serum (mg/dl)						
1 month	89.38±7.14^{DD}	241.13±15.71$^{\#\#\ ***}$	76.24±5.82	210.41±16.86***	63.40±5.74^{LL}	180.43±13.47^{***BB}
2 months	105.40±8.39^{AADDD}	273.19±19.64$^{\#\#\ ***A}$	77.40±6.34	238.64±15.45^{***A}	52.83±4.36^{AALLL}	148.36±11.43^{***BBBAA}
T3 in serum (ng/dl)						
1 month	29.91±2.67^{DDD}	21.85±1.97$^{***\#\#\#}$	38.42±2.09	31.88±2.08***	48.13±2.99^{LLL}	38.69±2.77$^{***\ BBB}$
2 months	22.14±2.44$^{DDD\ AAA}$	14.99±1.53$^{***A\ AA\#\#\#}$	38.61±2.34	24.88±2.72$^{***\ AAA}$	58.58±2.95^{AAALL}	46.75±3.15$^{***AAA\ BB}$
T4 in serum (μg/dl)						
1 month	4.36±0.20^{DDD}	5.18±0.25$^{***\#\#}$	3.51±0.30	4.57±0.29***	3.10±0.15^{L}	3.63±0.25$^{***\ BBB}$
2 months	5.28±0.25^{DDDAAA}	5.91±0.24$^{**AAA\#\#\#}$	3.60±0.23	5.28±0.32^{***AA}	2.63±0.16^{AAALLL}	3.12±0.16^{***AAABBB}

** $p<0.01$, *** $p<0.001$ represent comparison between control and HCD groups; ^{A} $p<0.05$, ^{AA} $p<0.01$, ^{AAA} $p<0.001$ comparison between 1 and 2 months; ^{DD} $p<0.01$, ^{DDD} $p<0.001$ comparison between groups Ia and IIa; $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ comparison between groups Ib and IIb; ^{L} $p<0.05$, ^{LL} $p<0.01$, ^{LLL} $p<0.001$ comparison between groups IIa and IIIa; ^{BB} $p<0.01$, ^{BBB} $p<0.001$ comparison between groups IIb and IIIb.

TABLE 2

Type-I 5'-iodothyronine deiodinase (5'-DI) activity in liver, aorta, and thyroid after 1 and 2 months of control and high-cholesterol diet (HCD) feeding schedule.
(Data is represented as mean ± SD from 6 observations)

	Se deficient		Se adequate		Se excess	
	Control Ia	HCD Ib	Control IIa	HCD IIb	Control IIIa	HCD IIIb
5'-DI in liver (pmoles of T3 liberated/min/mg of protein)						
1 month	6.73 ± 0.65^{DD}	$5.29\pm0.48^{***\#\#\#}$	8.64 ± 0.83	$7.14\pm0.63^{**}$	10.69 ± 1.59^{L}	$8.86\pm0.75^{*\ BB}$
2 months	$5.42\pm0.54^{DDD\ AA}$	$4.21\pm0.51^{**AA\#\#\#}$	8.97 ± 0.69	$6.12\pm0.51^{***AA}$	18.26 ± 2.11^{AAALLL}	$12.23\pm1.34^{***AAA\ BBB}$
5'-DI in aorta (pmoles of T3 liberated/min/mg of protein)						
1 month	5.28 ± 0.42^{DD}	$4.03\pm0.33^{***\#\#\#}$	6.13 ± 0.51	$5.21\pm0.46^{**}$	9.34 ± 0.87^{LLL}	$7.45\pm0.62^{**\ BBB}$
2 months	$3.91\pm0.28^{DDD\ AAA}$	$3.24\pm0.26^{***AAA\#\#\#}$	6.25 ± 0.45	$4.15\pm0.39^{***\ AA}$	13.21 ± 1.24^{AAALLL}	$10.24\pm1.14^{**AAA\ BBB}$
5'-DI in thyroid (pmoles of T3 liberated/min/mg of protein)						
1 month	7.15 ± 0.68^{D}	$8.87\pm0.75^{**\#\#}$	6.04 ± 0.61	$7.28\pm0.57^{**}$	9.46 ± 0.82^{LLL}	$11.51\pm1.13^{**BBB}$
2 months	9.22 ± 0.73^{DDDAAA}	$11.14\pm1.16^{**AA\#}$	6.47 ± 0.55	$9.34\pm0.87^{***AAA}$	13.82 ± 1.16^{AAALLL}	16.47±1.21**AAABBB

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ represent comparison between control and HCD groups; ^{AA} $p<0.01$, ^{AAA} $p<0.001$ comparison between 1 and 2 months; ^{D} $p<0.05$, ^{DD} $p<0.01$ ^{DDD} $p<0.001$ comparison between groups Ia and IIa; $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ comparison between groups Ib and IIb; ^{L} $p<0.05$, ^{LLL} $p<0.001$ comparison between groups IIa and IIIa; ^{BB} $p<0.01$, ^{BBB} $p<0.001$ comparison between groups IIb and IIIb.

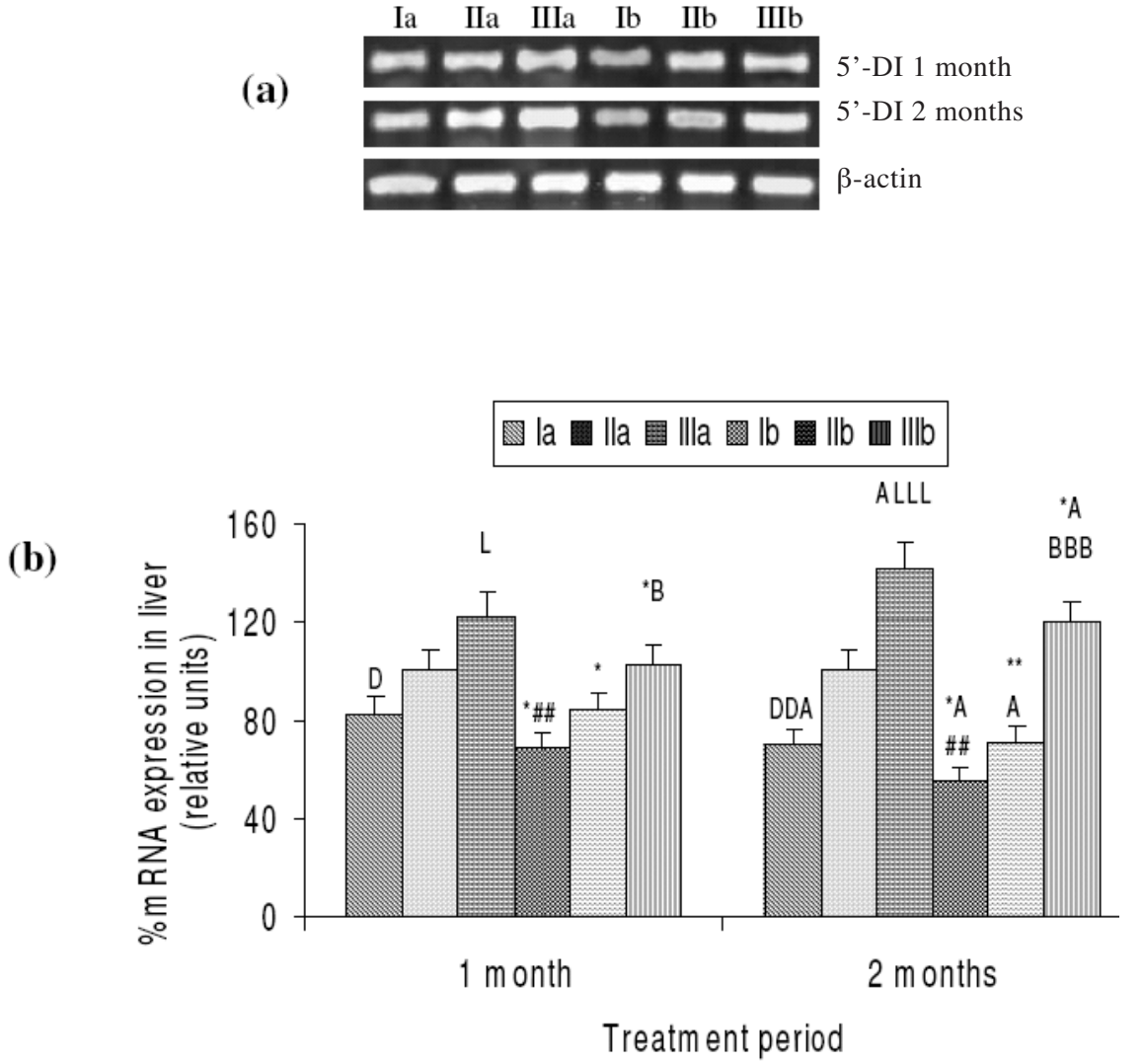

Figure 2: 5'-DI mRNA analysis in liver, after 1 and 2 months, in different groups: Ia. Se-deficient control; IIa. Se-adequate control; IIIa. Se-excess control; Ib. Se-deficient+HCD; IIb. Se-adequate+HCD; IIIb. Se-excess+HCD. (a) mRNA expression by RT-PCR. (b) Expression was quantified by densitometric analysis. Data is expressed as mean ± SD from 4 observations. *p<0.05, **p<0.01 represent comparison between control and HCD groups; Ap<0.05 comparison between 1 and 2 months; Dp<0.05, DDp<0.01 comparison between groups Ia and IIa; ##p<0.01 comparison between groups Ib and IIb; Lp<0.05, LLLp<0.001 comparison between groups IIa and IIIa; Bp<0.05, BBBp<0.001 comparison between groups IIb and IIIb.

DISCUSSION

Present data demonstrate that HCD feeding leads to Se depletion in rats (Lee et al., 2003; Dhingra et al., 2005). In Se deficiency, significant increase in cholesterol and triglyceride level was observed in comparison to the animals fed adequate Se after 1 and 2 months (Table 1). Low Se levels are associated with increased platelet aggregation and thromboxane A2 production along with decreased prostacyclin production, all of which may be linked with cardiovascular disease (Huang et al., 2002). With 1ppm Se supplementation, lipid levels decreased significantly in comparison to groups fed adequate Se (Kang et al., 2000). The Se

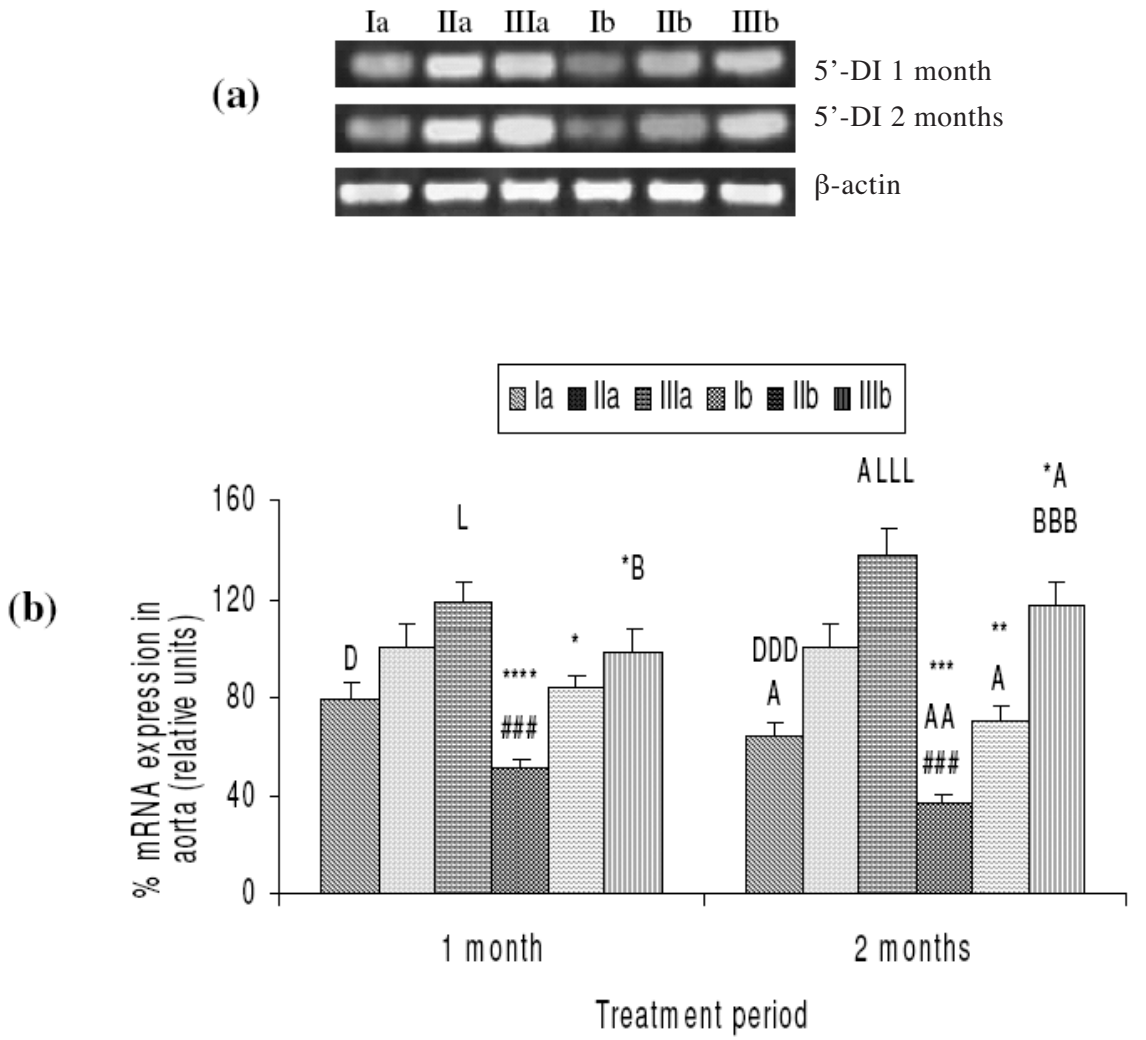

Figure 3: 5'-DI mRNA analysis in aorta, after 1 and 2 months, in different groups: Ia. Se-deficient control; IIa. Se-adequate control; IIIa. Se-excess control; Ib. Se-deficient+HCD; IIb. Se-adequate+HCD; IIIb. Se-excess+HCD. (a) mRNA expression by RT-PCR. (b) Expression was quantified by densitometric analysis. Data is expressed as mean ± SD from 4 observations. *p<0.05, **p<0.01, ***p<0.001 represent comparison between control and HCD groups; Ap<0.05, AAp<0.01 comparison between 1 and 2 months; Dp<0.05, DDDp<0.001 comparison between groups Ia and IIa; ###p<0.001 comparison between groups Ib and IIb; Lp<0.05, LLLp<0.001 comparison between groups IIa and IIIa; Bp<0.05, BBBp<0.001 comparison between groups IIb and IIIb.

supplementation leads to an increase in HDL cholesterol fraction (Wojcicki et al., 1991). HDL cholesterol may downregulate the total cholesterol via reverse cholesterol transport to the liver, i.e., HDL fraction increases the cholesterol elimination from tissues including smooth muscle cells in the aorta wall and facilitates cholesterol transport to the liver, thus preventing its deposition and the formation of atheromatous plaque (Pelton et al., 2005).

The present results demonstrated that in Se-deficient groups, hepatic glutathione peroxidase (GSH-Px) activity decreased significantly in comparison to adequate groups (Fig. 1). In the Se-deficient control group, after 2 months the GSH-Px activity decreased in comparison to 1 month, so these observations

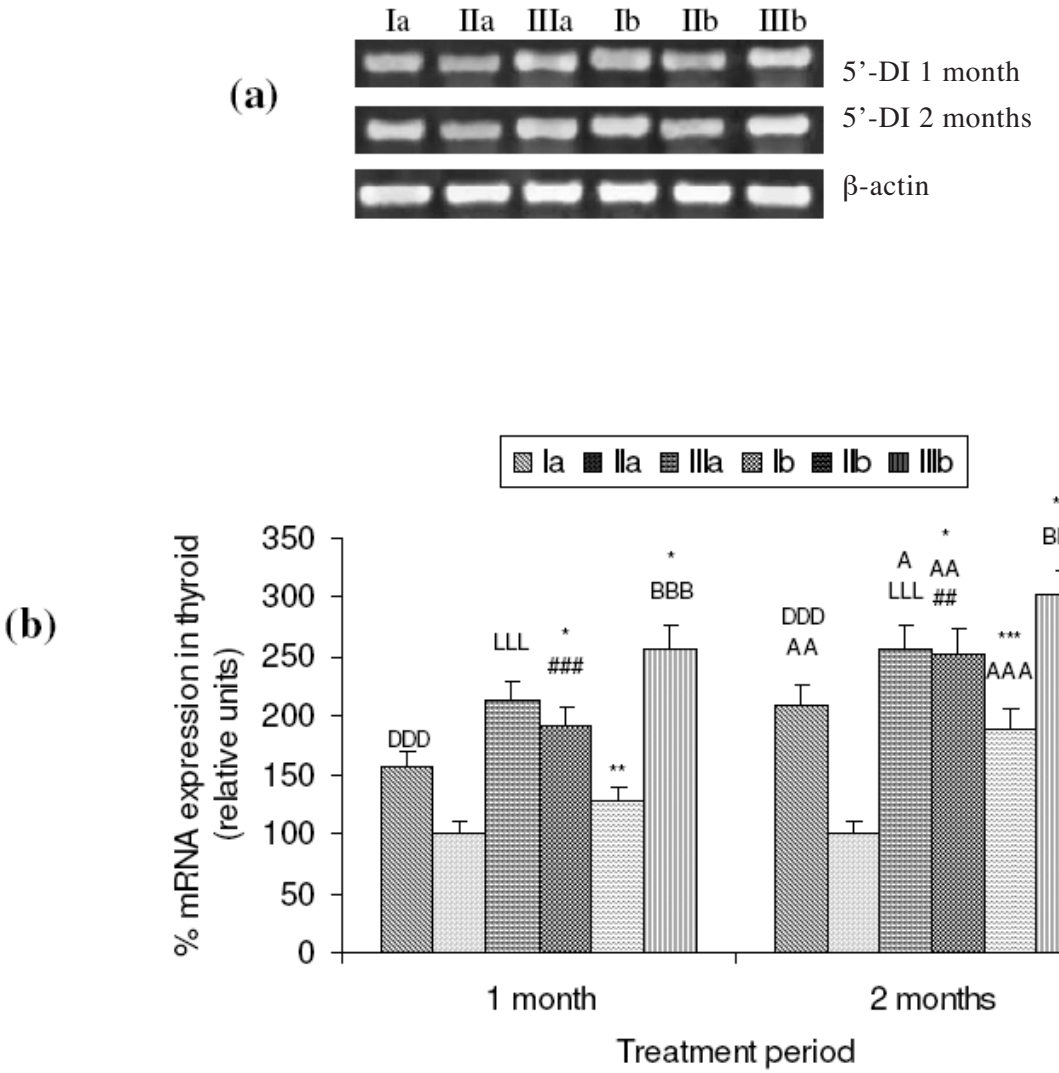

Figure 4: 5'-DI mRNA analysis in thyroid, after 1 and 2 months, in different groups: Ia. Se-deficient control; IIa. Se-adequate control; IIIa. Se-excess control; Ib. Se-deficient+HCD; IIb. Se-adequate+HCD; IIIb. Se-excess+HCD. (a) mRNA expression by RT-PCR. (b) Expression was quantified by densitometric analysis. Data is expressed as mean ± SD from 4 observations. *p<0.05, **p<0.01, ***p<0.001 represent comparison between control and HCD groups; Ap<0.05, AAp<0.01, AAAp<0.001 comparison between 1 and 2 months; DDDp<0.001 comparison between groups Ia and IIa; ##p<0.01, ###p<0.001 comparison between groups Ib and IIb; LLLp<0.001 comparison between groups IIa and IIIa; BBBp<0.001 comparison between groups IIb and IIIb.

confirm the Se deficiency (Arthur et al., 1993), which is associated with decreased GSH-Px activity. With HCD feeding, GSH-Px activity increased in all the groups. Further, it can be interpreted from our results that with cholesterol-rich diet feeding in all the Se status groups, GSH-Px activity increased after 2 months in comparison to 1-month data. This finding can be attributed to the increased lipoperoxidative stress associated with the HCD feeding, which concurs with the literature reporting elevation of GSH-Px activity as associated with a minor increase in oxidative stress (Kang et al., 2000). This increase in the Se-dependent GSH-Px with HCD feeding explains the decrease in the Se levels during hypercholesterolemia, as observed in the present study.

The levels of T_3 decreased and $_{T4}$ increased significantly in Se deficiency as well as with HCD feeding (Table 1). This finding is probably due to the decreased conversion of T_4 to T_3 in the liver due to decreased 5'-DI expression during Se deficiency (Arthur el al., 1991; Dhingra et al., 2004). As observed in Se deficiency, increased plasma T_4 levels usually upregulate the hepatic 5'-DI expression (Berry et al., 1990). In the current study, although plasma T_4 levels are increased in Se deficiency, hepatic 5'-DI expression is diminished, presumably because the hepatic stores of Se are insufficient to allow the synthesis of 5'-DI. However, the trend was the opposite with Se supplementation up to 1ppm, i.e., T_3 levels increased and T_4 levels decreased; probably, with Se supplementation, increase in 5'-DI expression upregulated the T_4 to T_3 conversion.

With the selenium-deficient diet, 5'-DI activity as well as mRNA expression in liver and aorta decreased (Bermano et al., 1995) in comparison to the Se-adequate groups (Table 2; Figs. 2 and 3). On high-cholesterol diet feeding, 5'-DI activity as well as mRNA expression decreased in liver and aorta. This finding could be due to the fact that hypercholesterolemia might have led to depletion in the Se pool, which is needed for normal 5'-DI expression. Another reason for this finding could be that hypercholesterolemia might be leading to hypothyroidism, which in turn downregulates the 5'-DI levels in liver and other peripheral tissues (Verhoelst et al., 2004). Furthermore, we have found a significant increase in 5'-DI activity as well as mRNA expression in liver and aorta with 1ppm Se supplementation. In cell culture experiments, Gross et al. (1995) have shown the clear dependence of 5'-DI expression on Se supply: enzyme activity as well as mRNA levels rapidly increased with the increase in Se concentration.

In contrast to liver and aorta, thyroidal 5'-DI activity and mRNA expression increased significantly with the high-cholesterol diet feeding as well as in Se deficiency (Table 2; Fig. 4). This increased expression of thyroidal 5'-DI in Se deficiency confirms that thyroid is a higher priority tissue than liver and aorta for Se when intake of the element is very low, which is consistent with the fact that thyroid has the ability to retain a significant pool of trace element in Se deficiency (Behne et al., 1988). With 1ppm Se supplementation, 5'-DI activity and expression were further increased in thyroid. Beech et al. (1995) demonstrated that human thyrocytes grown in primary culture in a Se-free medium were able to retain the trace element to maintain the 5'-DI enzyme activity (the expression was further increased by the addition of Se to the diet). The increased 5'-DI expression in thyroid during a hypercholesterolemic state suggests that there is some compensatory mechanism that might get activated when 5'-DI expression is downregulated in other peripheral tissues (liver and aorta). So, this tissue-specific differential behavior of 5'-DI might be maintaining the T_3 level in the body during hypercholesterolemia. T_3 is involved at the transcriptional level in the cardiovascular system. T_3 enters into the cardiomyocytes through T_3-binding nuclear receptors and interacts with specific transcriptional activators to modify the transcription rate of specific target genes (Brent, 1994). Thyroid hormones also decrease peripheral vascular resistance by promoting relaxation in vascular smooth-muscle cells (Park et al., 1997). Napoli et al. (2001) reported that thyroid hormones exert profound effects on the vascular system by improving both endothelium-dependent and -independent mechanisms.

The present data indicate that hypercholesterolemia along with the Se deficiency is co-responsible for tissue-specific differential expression of 5'-DI enzyme, i.e., the expression decreased in liver and aorta whereas it increased in thyroid. Until now, no direct association between 5'-DI behavior and hypercholesterolemia has been observed. Thus, longitudinal studies to clarify the potential association between 5'-DI expression and hypercholesterolemia under different Se status must be performed.

Further studies must be undertaken to explore the therapeutic role of Se supplementation in hypercholesterolemia through its dependent enzyme 5'-DI.

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