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SHORT-TERM THYROXINE ADMINISTRATION LEADS TO LIPID PEROXIDATION IN RENAL AND TESTICULAR TISSUES OF RATS WITH HYPOTHYROIDISM

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Thyroid dysfunction brings about pathological changes in different organs of the body. The aim of the present study was to examine how experimental hypothyroidism and additional short-term high-dose thyroxine administration (one-week) affected lipid peroxidation in renal and testicular tissues of rats. The study was carried out on 30 male Spraque-Dawley rats. The experimental animals were divided into 3 groups as control, hypothyroidism and hypothyroidism + thyroxine administration. Both malondialdehyde (MDA) and glutathione (GSH) levels were lower in renal and testicular tissues of the hypothyroidism group than the control and hypothyroidism + thyroxine administration groups and the levels in hypothyroidism + thyroxine administration group were higher than those in the control and hypothyroidism groups ($p < 0.001$). Results of the study demonstrate that hypothyroidism reduced oxidant stress in kidney and testis tissues, but short-term, high-dose thyroxine administration in addition to hypothyroidism increased oxidant stress in the same tissues of rats.

Keywords: Hypothyroidism – thyroxine administration – oxidant stress – rat

INTRODUCTION

Free radicals formed in high amounts, products of radicals and non-radical molecules in the body are hazardous for the health of the body and they can cause damage in all cellular structures [6]. Increases in thyroid hormones can affect oxygen metabolism and stimulate the formation of free radicals in mitochondria [24]. Reactive oxygen types play an important role in physiological mechanisms [14]. However, extremely reactive oxygen types lead to oxidative stress [14]. Thyroid hormones act on the mitochondria by regulating the energy metabolism [12]. Several researchers had reported that the increase in metabolic activity in hyperthyroidism resulted in cellular damage [2, 9, 29].

It was reported in previous studies that not only hyperthyroidism, but also hypothyroidism caused changes in oxidant and antioxidant systems [4, 24, 25]. Mano et al. [18] studied oxidant and antioxidant system activities in cerebral tissues of rats

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with either hypothyroidism or hyperthyroidism and determined that the free radicals formed in hyperthyroidism and hypothyroidism were counteracted by eliminating mechanisms. Glutathione peroxidase levels increased in another study examining oxidant and antioxidant system activities in cardiac tissues of rats with either hypothyroidism or hyperthyroidism [18]. Yet another study established that propylthiouracil (PTU) treatment protected the liver against injury stimulated by ethanol by reducing oxygen consumption [23] and reduced lipid peroxidation in the brain [8]. However, Rahaman et. al. [22] reported that there was an increase in the activities of antioxidants such as superoxide dismutase and catalase, but glutathione levels decreased during the hypothyroidism.

It was shown that hyperthyroidism stimulated by L-thyroxine increased susceptibility to lipid peroxidation in female rats, but could not prevent mitochondrial damage in cardiac tissue despite increased GSH levels [13]. The same study demonstrated that propylthiouracil administration to rats reduced mitochondrial DNA damage. Another change caused by thyroid hormones was renal hypertrophy which was observed in hyperthyroidism [16] and it was reported that renal tissue had structural impairments in hypothyroidism induced by thyroidectomy [19]. It was also shown that renal hypertrophy stimulated by *in vivo* thyroxine application was associated with an increase in mitotic index [17].

An overall evaluation of the related reports showed that the number of studies about the effects of thyroid gland dysfunctions on testicular and renal tissues was limited. The present study was conducted to determine how experimental hypothyroidism and short-term high-dose L-thyroxine administration in addition to hypothyroidism affected lipid peroxidation in both kidney and testis tissues of rats.

MATERIALS AND METHODS

This study was conducted at Selcuk University Experimental Medicine Research and Application Center. The study protocol was approved by the ethics committee of the this institution. The study included 30 male rats of Spraque-Dawley species, aged 8 weeks, weighing 250–280 g. All rats were kept under the same experimental conditions. They were fed with standard rat pellet and given tap water. The rats were kept under 12 h/12 h dark/light cycle. Experimental groups were formed as follows:

1-Intact control group (n = 10): The group that was not subjected to any procedure.

2-Hypothyroid group (n = 10): Rats in this group were given propylthiouracil (10 mg/kg/day) for 2 weeks.

3-Hypothyroid + Hyperthyroid group (n = 10): Rats in this group were given propylthiouracil (Sigma) by intraperitoneal (10 mg/kg/day) for 2 weeks plus L-thyroxine (Sigma) (1.5 mg/kg/day) was given by intraperitoneal for 1 week.

At the end of the 3 weeks, animals were decapitated. Kidney and testes tissue samples were collected and stored at –80 °C until analysis.

Tissue malondialdehyde (MDA) analysis

The wet weight of the tissue samples at pH 7.4 were measured, then divided into pieces and transferred into tubes and homogenized to about 10% in 150 mM KCl at 4 °C using a Misonix's Microscam ultrasonic cell disruptor. Two ml of 8% $HClO_4$ was added to the homogenate and centrifuged at 3000 rpm for 25 min., 3 ml of 1% H_3PO_4 and 1 ml of 0.675% tiobarbituric acide (TBA) were added to 0.5 ml supernatant and incubated in a 90 °C water bath for 45 min. After cooling of mixture, the MDA-TBA complex was extracted with 4 ml of *n*-butanol and its absorbance was read against an *n*-butanol blank, as described earlier [28]. Levels of MDA were expressed as nmol/g protein.

Tissue glutathione (GSH) analysis

The tissue was homogenized in 150 mM KCl at 4 °C as described for MDA, centrifuged at 3000 rpm for 15 min. The level of GSH in the samples was measured by Ellman's method [7] as described early. Tissue protein was obtained by the biuret's method.

Eight ml of pH 6.8 phosphate buffer, 78 ml of 1 N NaOH, and 100 µL Ellman's solution were added to 200 µL of the supernatant and read at 412 nm after standing for 5 min. The activity (a) was calculated from a standard containing 15.34 g GSH/100 ml. GSH levels were presented as µmol/g protein.

Statistical analysis

The statistical analysis was performed with the use of the SPSS statistical program. The results were expressed as mean ± SD. Kruskal-Wallis analysis of variance was used for comparison between groups and Mann-Whitney U-test was applied to those with $p < 0.05$.

RESULTS

Results obtained of MDA levels in the renal tissue were 43.80 ± 0.98, 33.30 ± 0.60 and 45.83 ± 0.65 in the control group, hypothyroidism group and hypothyroidism + + thyroxine administration group. The comparison among groups showed that MDA levels in hypothyroidism + thyroxine administration group were higher than those in the other two groups ($p < 0.001$) and the levels in the control group were higher than those in the hypothyroidism group ($p < 0.001$, Table 1). As for GSH levels in renal tissue, it was seen that hypothyroidism + thyroxine administration group had the highest levels (35.54 ± 0.37) and hypothyroidism group (15.74 ± 0.57) had the lower renal GSH levels, when compared with the control group (19.72 ± 0.49) ($p < 0.001$, Table 2).

Table 1
Tissue MDA levels in control and experimental groups

Groups	Kidney (nmol/g protein)	Testis (nmol/g protein)
Control (n = 10)	43.80 ± 0.98^{b}	66.90 ± 0.43^{b}
Hypothyroidism (n = 10)	33.30 ± 0.60^{c}	38.05 ± 0.99^{c}
Hypothyroidism + Thyroxine (n = 10)	45.83 ± 0.65^{a}	93.45 ± 0.49^{a}

Notes: Different letters in same column are significant as statistic ($P < 0.001$); a > b > c.

Table 2
Tissue GSH levels in control and experimental groups

Groups	Kidney (µmol/g protein)	Testis (µmol/g protein)
Control (n = 10)	19.72 ± 0.49^{b}	24.54 ± 0.54^{b}
Hypothyroidism (n = 10)	15.74 ± 0.57^{c}	13.77 ± 0.79^{c}
Hypothyroidism + Thyroxine (n = 10)	35.54 ± 0.37^{a}	45.35 ± 0.53^{a}

Notes: Different letters in same column are significant as statistic ($P < 0.001$); a > b > c.

Evaluation of MDA levels in testis tissues of experimental groups showed that hypothyroidism + thyroxine administration group (93.45 ± 0.49) had higher MDA levels than the control (66.90 ± 0.43) and hypothyroidism (38.05 ± 0.99) groups ($p < 0.001$, Table 1). It was seen that this parameter decreased in hypothyroidism group in comparison to the control group ($p < 0.001$, Table 1). GSH levels in testis tissue were 24.54 ± 0.54, 13.77 ± 0.79 and 45.35 ± 0.53 in control, hypothyroidism and hypothyroidism + thyroxine administration groups, respectively. The comparison of GSH levels among groups showed that hypothyroidism + thyroxine administration group had the highest levels and it was followed by the control group and hypothyroidism group, respectively ($p < 0.001$, Table 2).

DISCUSSION

The results obtained indicated that propylthiouracil administration for two weeks reduced lipid peroxidation in kidney and testis tissues of rats. However, it was observed that short-term L-thyroxine administration in addition to hypothyroidism reversed the decrease in lipid peroxidation observed in hypothyroidism. Various methods have been used as markers of oxidative stress in the organism in other studies [27]. However, the most frequently used method is the determination of MDA levels [27]. In our study, determination of MDA levels which a marker of oxidant

damage caused in tissues by hypothyroidism and short-term thyroxine administration in addition to hypothyroid was employed.

While evaluating our study about renal tissue, we observed that both MDA and GSH levels decreased as a result of hypothyroidism. Although studies about the effects of thyroid hormones on oxidant and antioxidant systems in various tissues of the body report on different findings, it was noted that oxidant damage increased in hyperthyroidism and that the tissue was protected against lipid peroxidation during hypothyroidism [2, 10]. In their study, Rahaman et. al. [22] reported that glutathione levels decreased as a result of hypothyroidism induction in growing rats and this finding was parallel to decreased GSH levels we obtained in hypothyroidism group in our study. In a similar study, Mano et. al. [18] examined lipid peroxidation and antioxidant system activities in cerebral tissues of rats with either hypothyroidism or hyperthyroidism. They found that the level of lipid peroxides measured with an indirect method, decreased in hyperthyroidism but did not change in hypothyroidism. These findings were inconsistent with those of our study. However, these differences may be explained by periods of administration, use of different substances to induce hypothyroidism and use of experimental animals from different age groups. In the above study, methimazole was employed to induce hypothyroidism, whereas we used propylthiouracil for that purpose. That study which included rats at 1.5 years of age, while ours enrolled 2-months-old rats. It was stated in different studies that oxidant and antioxidant system activities of animals changed in an age-dependent manner [1, 25]. A supportive finding to our results was reported by Faure et al. [8], who determined that lipid peroxidation decreased in hypothyroidism induced by propylthiouracil administration. Likewise, it was reported in another study that hypothyroidism prevented oxidant damage in renal failure induced by ischemia [21]. In their study, Galkina et al. [11] examined the effects of different thyroxine isomers on free radical formation in rat cerebral cortex and expressed that the antioxidant activity of D-thyroxine was about two and a half times higher than that of L-thyroxine. Oziol et al. [20] reported similar findings in their study and showed increases in oxidant and antioxidant system activities depending on structural differences of thyroid hormones. In the present study, we have found that one-week L-thyroxine administration in addition to hypothyroidism increased oxidant and antioxidant system activity in renal tissues of rats, but that the oxidant damage caused could not be prevented in spite of the increase in antioxidant system activity. A similar finding to the effect that thyroxine administration remarkably prolonged the decrease in oxidant damage in hypothyroidism was reported by Venditti et al. [29].

When we evaluated oxidant and antioxidant system activities in testis tissues of rats with hypothyroidism, we observed decreases in both MDA and GSH levels. We also found that short-term thyroxine treatment following hypothyroidism elevated MDA and GSH values to a level above control values.

It is known that in general thyroid hormones increase metabolic systems of the body. Therefore, it was stated that an increase in reactive oxygen types inevitably leads to lipid peroxidation [3, 10, 29]. It is noted that the suppression of oxidant systems in hypothyroidism stems from a decrease in the production of mitochondrial

reactive oxygen types as a result of reductions in both oxygen consumption rate of tissues and food intake in hypothyroidism [15]. However, Gredilla et al. [13] reported that lipid peroxidation increased in hyperthyroidism, but did not change in hypothyroidism. Tapia et al. [26] noted that lipid peroxidation increased during short-term T_3 administration. It was stated in a study by Choudhury et al. [5] that such oxidative stress parameters as hydrogen peroxide and protein carbonyl increased in testis homogenates of rats as a result of hypothyroidism and that T_3 administration to rats with hypothyroidism did not significantly altered hydrogen peroxide levels, but further increased protein carbonyl content. In our study, however, short-term L-thyroxine administration in addition to hypothyroidism resulted in an increase in the levels of MDA, a marker of stress parameters, and this aspect of our study is inconsistent with the findings of Choudhury et al. [5]. Nevertheless, hypothyroidism was followed by T_3 administration in the concerned study and by thyroxine administration in ours, by which difference the inconsistency may be explained. It was stated that thyroid hormones with different structures influenced oxidant and antioxidant systems in different ways [20]. As opposed to our study, it was reported that hepatic MDA levels did not change as a result of short-term L-thyroxine administration [24]. However, we think that this opposite finding is attributable to differences in application.

An overall evaluation of the results of our study showed that both MDA and GSH levels were suppressed in renal and testicular tissues of rats with hypothyroidism induced by 2-weeks propylthiouracil administration. Besides, one-week L-thyroxine administration in addition to hypothyroidism increased lipid peroxidation in tissues and this increase could not be hindered despite the increase in GSH levels.

REFERENCES

1. Amer, M. A. (2002) Modulation of age-related biochemical changes and oxidative stress by vitamin C and glutathione supplementation in old rats. *Ann. Nutr. Metab. 46,* 165–168.
2. Asayama, K., Dobashi, K., Hayashibe, H., Megata, Y., Kato, K. (1987) Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. *Endocrinology 121,* 2112–2118.
3. Asayama, K., Kato, K. (1990) Oxidative muscular injury and its relevance to hyperthyroidism. *Free Radic. Biol. Med. 8,* 293–303.
4. Berzinska-Slebodzinska, E. (2001) Fever induced oxidative stress: the effect of thyroid status and the 5'-monodeiodinase activity, protective role of selenium and vitamin E. *J. Physiol. Pharmacol. 52,* 275–284.
5. Choudhury, S., Chainy, G. B. N., Mishro, M. M. (2003) Experimentally induced hypo- and hyperthyroidism influence on the antioxidant defence system in adult rat testis. *Andrologia 35,* 131–140.
6. Droge, W. (2002) Free radicals in physiological control of cell function. *Physiol. Rev. 82,* 47–95.
7. Elman, G. L. (1959) Tissue sulfhydryl groups. *Arch. Biochem. Biophys. 82,* 70–77.
8. Faure, M., Lissi, E. A., Videla, L. A. (1991) Evaluation of the antioxidant properties of thyroid hormones and propylthiouracil in the brain-homogenate autoxidation system and in the free radical-mediated oxidation of erythrocyte membranes. *Chem. Biol. Interact. 77,* 173–85.

9. Fernandez, V., Barrientos, X., Kipreos, K., Valenzuela, A., Videla, L. A. (1985) Superoxide radical generation, NADPH oxidase activity, and cytochrome P-450 content of rat liver microsomal fractions in an experimental hyperthyroid state: relation to lipid peroxidation. *Endocrinology 117,* 496–501.
10. Fernández, V., Llesuy, S., Solari, L., Kipreos, K., Videla, L. A. (1998) Boveris A. Chemiluminescent and respiratory responses related to thyroid hormone-induced liver oxidative stress. *Free Radic. Res. Commun. 5,* 77–84.
11. Galkina, V., Prokopenko, V. M., Putilina, F. E., Eshchenko, N. D., Arutyunyan, A. V. (2001) The effects of thyroxine isomers on free-radical oxidation processes in subcellular fractions of rat cerebral cortex. *Neurosci. Behav. Physiol. 31,* 463–465.
12. Goglia, F., Silvetri, E., Lanni, A. (2002) Thyroid hormones and mitocondria. *Biosci. Rep. 22,* 17–32.
13. Gredilla, R., Lopez-Torres, M., Portero-Otin, M., Pamplona, R., Barja, G. (2001) Influence of hyper- and hypothyroidism on lipid peroxidation, unsaturation of phospholipids, glutathione system and oxidative damage to nuclear and mitochondrial DNA in mice skeletal muscle. *Mol. Cell Biochem. 221,* 41–48.
14. Karbownik, M., Lewinski, A. (2003) The role of oxidative stress in physiological and pathological processes in the thyroid gland; possible involvement in pineal-thyroid interactions. *Neuroendocrinol. Lett. 24,* 293–303.
15. Katzeff, H. L., Powell, S. R., Ojamaa, K. (1997) Alterations in cardiac contractility and gene expression during low-T3 syndrome: prevention with T3. *Am. J. Physiol. 273,* E951–E956.
16. Kobori, H., Ichihara, A., Miyashita, Y., Hayashi, M., Saruta, T. (1998) Mechanism of hyperthyroidism-induced renal hypertrophy in rats. *J. Endocrinol. 159,* 9–14.
17. Lopez-Torres, M., Romero, M., Barja, G. (2000) Effect of thyroid hormones on mitochondrial oxygen freee radical production and DNA oxidative damage in the rat heart. *Mol. Cell. Endocrinol. 168,* 127–134.
18. Mano, T., Sinohara, R., Sawai, Y., Oda, N., Nishida, Y., Mokumo, T., Asano, K., Ito, Y., Kotake, M., Hamada, M. (1995) Changes in lipid peroxidation and free radical scavengers in the brain of hyper- and hypothyroid aged rats. *J. Endocrinology 147,* 361–365.
19. Mogulkoc, R., Canpolat, L., Baltacı, A. K., Yılmaz, B., Kelestimur, H. (1999) Histological examination of various tissues in propylthiouracil and thyroidectomy-induced hypothyroidism in the rat. *Med. Sci. Res. 27,* 801–805.
20. Oziol, L., Faure, P., Vergely, C., Rochette, L., Artur, Y., Chomard, P., Chomard, P. (2001) *In vitro* free radical scavenging capacity of thyroid hormones and structural analogues. *J. Endocrinol. 170,* 197–206.
21. Paller, M. S. (1986) Hypothyroidism protects against free radical damage in ischemic acute renal failure. *Kidney Int. 29,* 1162–1166.
22. Rahaman, S. O., Ghosh, S., Mohanakumar, K. P., Das, S., Sarkar, P. K. (2001) Hypothyroidism in the developing rat brain is associated with marked oxidative stress and aberrant intraneuronal accumulation of neurofilaments. *Neurosci. Res. 40,* 273–279.
23. Ross, A. D., Varghese, G., Oporto, B., Carmichael, F. J., Israel, Y. (1995) Effect of propylthiouracil treatment on NADPH-cytochrome P450 reductase levels, oxygen consumption and hydroxyl radical formation in liver microsomes from rats fed ethanol or acetone chronically. *Biochem. Pharmacol. 49,* 979–989.
24. Sewerynek, E., Wiktorska, J., Lewinski, A. (1999) Effects of melatonin on the oxidative stress induced by thyrotoxicosis in rats. *Neuroendocrinol. Lett. 20,* 157–161.
25. Shinohara, R., Mano, T., Nagasaka, A., Hayashi, R., Uchimura, K., Nakano, I., Watanabe, F., Tsugawa, T., Makino, M., Kakizawa, H., Nagata, M., Iwase, K., Ishizuki, Y., Itoh, M. (2000) Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status. *J. Endocrinol. 164,* 97–102.
26. Tapia, G., Cornejo, P., Fernandez, V., Videla, L. A. (1999) Protein oxidation in thyroid hormone-induced liver oxidative stress: relation to lipid peroxidation. *Toxicol. Lett. 106,* 209–214.
27. Tsai, L. Y., Lee, K. T., Tsai, S. M., Lee, S. C., Yu, H. S. (1993) Changes of lipid peroxide levels in blood and liver tissue of patients with obstructive jaundice. *Clin. Chim. Acta 215,* 41–50.

28. Uchiyama, M., Mihara, M. (1977) Determination of malondyaldehyde precurser in tissues by thiobarbituric acid test. *Anal. Biochem. 86,* 271–278.
29. Venditti, P., Balestrieri, M., Di, Meo, S., De Leo, T. (1997) Effect of thyroid state on lipid peroxidation, antioxidant defences, and susceptibility to oxidative stress in rat tissues. *J. Endocrinol. 155,* 151–157.
30. Venditti, P., De Rosa, R., Di Meo, S. (2003) Effect of thyroid state on susceptibility to oxidants and swelling of mitochondria from rat tissues. *Free Radic. Biol. Med. 35,* 485–494.
31. Venditti, P., De Rosa, R., Di Meo, S. (2002) Effect of thyroid state on susceptibility to oxidants and swelling of mitocondria from rat tissues. *Cardiovasc. Res. 56,* 76–85.