Acta Med Okayama 1999; 53(1): 27-30

The Impact of Propylthiouracil Therapy on Lipid Peroxidation and Antioxidant Status Parameters in Hyperthyroid Patients

Arzu Seven[a]*, Ertuğrul Tasan[b], Hüsrev Hatemi[b] and Gülden Burcak[a]

Departments of [a]Biochemistry and [b]Internal Medicine, Cerrahpasa Medical Faculty, Istanbul University, Istanbul 34303, Turkey

This study was performed on 17 hyperthyroid patients and 15 healthy controls. The patients were under propylthiouracil (PTU) therapy at a dosage of 3 × 100 mg/day for one month. Blood samples, taken at the beginning and on the 30th day of therapy, were analyzed for hormonal parameters (T_3, T_4, TSH), lipid peroxidation end-product [thiobarbituric acid reactive substances (TBARS)] and antioxidant status parameters: glutathione (GSH), glutathione peroxidase (GSH-Px) and CuZn superoxide dismutase (CuZn SOD). Hyperthyroid patients were observed to have significantly higher TBARS, GSH and CuZn SOD levels than controls ($P < 0.05$, $P < 0.001$, $P < 0.001$, respectively). PTU therapy caused a relief in oxidative stress as reflected by significantly decreased TBARS levels ($P < 0.001$) and a selective modification in the antioxidant status parameters: significant decreases in GSH and CuZn SOD levels ($P < 0.001$) and a significant increase in GSH Px ($P < 0.01$) activity. Our findings suggest a selective modification of the antioxidative profile in hyperthyroidism. PTU should also be considered as an *in vivo* antioxidant, in addition to its antithyroid action.

Key words: propylthiouracil, hyperthyroidism, glutathione, glutathione peroxidase, superoxide dismutase, lipid peroxidation

Free-radical-mediated oxidative stress has been implicated in the development and exacerbation of several degenerative diseases (1-3). Evidence has accumulated in support of a role of oxidative processes in the genesis of hyperthyroidism-induced tissue damage and antioxidants have been added to the list of preventive/therapeutic measures in hyperthyroidism (4, 5).

As is known, the amount of thiobarbituric acid reactive substances is a widely used index of lipid peroxidation, the characteristic feature of free radical toxicity. Activities of oxygen radical-scavenging enzymes are expected to increase as an adaptive response to sustained oxidative stress and are considered as proof of existing free-radical toxicity. However, in the literature great controversy exists as to whether hyperthyroidism is associated with an increase or decrease in the activities of antioxidant enzymes (6, 7).

The purpose of this investigation is twofold: First, to determine the extent of oxidative stress in hyperthyroid patients in comparison to healthy controls and, second, to evaluate the impact of propylthiouracil (PTU) therapy on oxidative stress.

To this end, plasma-thiobarbituric-acid-reactive substances (TBARS) values and erythrocyte glutathione (GSH) levels, CuZn superoxide dismutase (CuZn SOD) and GSH peroxidase (GSH-Px) activities were measured in 17 hyperthyroid patients prior to and on the 30th day of PTU therapy and in 15 healthy controls.

Subjects and Methods

Subjects and design of study. The study was performed on 17 patients and 15 healthy control subjects. Clinical characteristics related to the subjects are reported in Table 1. Twelve patients were diagnosed as Basedow disease, 5 as toxic multinodular goitre. The patients were diagnosed according to T_3, T_4 and TSH values and thyroid scintigraphy. Patients with liver and kidney diseases, stroke, diabetes mellitus, severe vascular diseases (myocardial infarction in previous 12 months or peripheral arterial occlusive diseases), hypertension (systolic blood pressure > 165 mmHg and/or diastolic

* To whom correspondence should be addressed.

Table I Clinical characteristics of the subjects

	Hyperthyroid patients (n = 17)	Control subjects (n = 15)
Sex (male/female)	6/11	10/5
Age (years)	42.19 ± 11.12	38.27 ± 6.70

blood pressure > 95 mmHg) were excluded from the study. Other excluding criteria for the study were regular drug ingestion and antioxidant use (vitamin E, β-carotene, ascorbic acid, glutathione, probucol). The patients were under propylthiouracil therapy at a dosage of 3 × 100 mg/day for 1 month.

Informed consent was obtained from the subjects prior to commencement of the experiment.

Blood sampling and preparation of erythrocyte lysates. Blood samples were taken at the beginning and at the end of one month PTU therapy. Blood was collected after an overnight fast by venipuncture into a 10 ml heparinised tube. After centrifugation at 2500 × *g* for 5 min, the plasma was removed and erythrocytes were washed three times in 5 ml of sterile 9 g/L NaCl solution, hemolyzed by diluting fourfold with water and stored at − 80 °C until biochemical analyses.

Biochemical analysis. T_3, T_4 and TSH values were determined by radioimmunoassay (TKTU1, TKC4, IKNT1 Diagnostic Products Corp., Los Angeles, CA, USA).

Assay of TBARS. TBARS levels were determined according to a modification of the method of Buege and Aust (8). One volume was mixed thoroughly with two volumes of a stock solution of 15 % w/v trichloroacetic acid, 0.375 w/v thiobarbituric acid and 0.25 N hydrochloric acid. The mixture was heated for 30 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation of 1000 × *g* for 10 min. The absorbance of the sample was determined at 535 nm and the TBARS concentration was calculated using $1.56 \times 10^5\,M^{-1}cm^{-1}$ as mol/l extinction coefficient.

Assay of GSH. Reduced GSH concentration was determined according to the method of Beutler *et al.* (9) using metaphosphoric acid for protein precipitation and 5,5'-dithiobis 2-nitrobenzoic acid for color development. GSH concentration was expressed as mg/g Hb.

Assay of GSH-Px activity. The modified method of Paglia and Valentine (10) was used. Enzyme activity was determined from the oxidation of NADPH in the presence of H_2O_2 used as a substrate and monitored spectrophotometrically at 340 nm. Results were expressed in terms of U/g Hb. Hemoglobin concentration was determined by the cyanmethemoglobin method (11).

Assay of CuZn SOD activity. CuZn SOD activity was determined by the method of Sun *et al.* (12). This assay involves inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50 %.

Statistical analysis. Results were expressed as means ± SD. The small paired "t" test was used for comparison of hyperthyroid patients prior to and at the end of one month PTU therapy. Variance analysis was used for comparison of the study groups and 0.05 was selected as the point of significance. Correlation analysis was performed by using Pearson's correlation test.

Results

T_3, T_4 and TSH values of the hyperthyroid patients prior to and on the 30th day of PTU therapy and healthy controls are shown in Table 2.

Hormonal values of the controls were within the normal range, T_4 and T_3 values being significantly lower ($P < 0.001$) and TSH values significantly higher ($P < 0.001$) than hyperthyroid patients prior to PTU therapy.

In hyperthyroid patients, plasma T_4 ($P < 0.001$) and T_3 ($0.02 > P > 0.01$) values were observed to be sig-

Table 2 Values of the hormonal status parameters in the study groups (mean ± SD)

	Control group (n = 15)	Hyperthyroid patients: Prior to PTU therapy (n = 17)	Hyperthyroid patients: 30th day of PTU therapy (n = 17)
T_3 (ng/dl)	1.45 ± 0.84	4.11 ± 2.91^{a***}	2.18 ± 1.13^{b***,c**}
T_4 (ng/dl)	7.82 ± 1.84	17.25 ± 5.23^{a***}	10.13 ± 4.43^{b***,c***}
TSH (uIU/ml)	2.45 ± 0.84	0.12 ± 0.09^{a***}	0.18 ± 0.05^{b***}

a : Prior to therapy *vs* control
b : 30th day of therapy *vs* control
c : Prior to therapy *vs* 30th day of therapy
$^{**}P < 0.01$; $^{***}P < 0.001$
PTU: Propylthiouracil

nificantly decreased at the end of one month PTU therapy. However, T_4 and T_3 values still remained significantly higher ($P < 0.01$) and TSH value significantly lower ($P < 0.001$) than healthy controls.

Plasma TBARS and erythrocytic GSH, GSH Px and CuZn SOD values of the controls and hyperthyroid patients prior to and on the 30th day of PTU therapy are shown in Table 3.

In hyperthyroid patients prior to PTU therapy, plasma TBARS, erythrocyte GSH and CuZn SOD values were found to be significantly higher than controls ($P < 0.05$, $P < 0.001$, $P < 0.001$, respectively). One month of PTU therapy in hyperthyroid patients caused significant decreases in lipid peroxidation and GSH and CuZn SOD levels ($P < 0.001$). However, GSH Px activity was found to be significantly increased at the end of 30-day PTU therapy ($P < 0.01$).

Comparison of PTU-treated hyperthyroid patients with the controls revealed significantly higher GSH, GSH Px and CuZn SOD values for the patient group ($P < 0.001$, $P < 0.01$, $P < 0.05$, respectively).

The correlation analysis performed between hormonal status parameters and peroxidation and antioxidant status parameters revealed a positive correlation between T_4 values and GSH Px activity in hyperthyroid patients prior to PTU therapy ($r = 0$, 55, $0.05 > P > 0.02$).

Table 3 Values of the determined parameters in the study groups (mean ± SD)

		Hyperthyroid patients	
	Control group (n = 15)	Prior to PTU therapy (n = 17)	30th day of PTU therapy (n = 17)
TBARS (μM)	3.40 ± 0.43	4.65 ± 0.45^{a*}	3.45 ± 0.36^{c***}
GSH (mg/gHb)	2.11 ± 0.56	5.63 ± 0.60^{a***}	3.21 ± 0.49^{b***,c***}
GSH Px (U/gHb)	25.75 ± 12.57	29.12 ± 9.95	43.98 ± 18.83^{b**,c**}
CuZn SOD (mg/L)	89.09 ± 13.44	154.01 ± 55.03^{a***}	100.12 ± 48.87^{b*,c***}

a: Prior to therapy *vs* control
b: 30th day of therapy *vs* control
c: Prior to therapy *vs* 30th day of therapy
$*P < 0.05$, $**P < 0.01$, $***P < 0.001$
TBARS: Thiobarbituric acid reactive substances; GSH: Glutathione; GSH Px: Glutathione peroxidase; CuZn SOD: CuZn superoxide dismutase.

Discussion

It is well established that thyroid hormones accelerate the basal metabolic rate and more specifically oxidative metabolism in mitochondria. The accelerated oxidative metabolism results in accelerated production of reactive oxygen metabolites (13, 14). Videla *et al.* (15) suggested that this condition might reflect oxidative stress at the cellular level in tissues which are targets for thyroid hormone action with a calorigenic response. Our findings on plasma TBARS values are in accordance with the foregoing. We observed higher values in the patients than in the controls.

This finding is consistent with the fact that hyperthyroidism induces a substantial fluidity in biological membranes and makes them more prone to peroxidation. The patients displayed a decrease in TBARS values nearly down to control values in response to 30-day of PTU therapy, proven to be effective on the basis of clinical symptoms and hormonal findings. This effect of PTU therapy suggests a relief in oxidative stress, achieved through the antithyroid action of the drug. However, PTU has also been suggested to act as an inhibitor of peroxidation of erythrocyte plasma membrane lipids, at concentrations close to therapeutic levels (16).

The endogenous antioxidant components, GSH and CuZn SOD, which were present at higher levels in the hyperthyroid patients than in the healthy controls, were observed to decrease in response to PTU therapy.

As for GSH Px, it is known that it is present and active in cells and is less inducible in contrast to CuZn SOD which, though present at only low levels, can be readily induced under oxidative stress (17). Our finding of an absence of significant difference in GSH Px activity between the hyperthyroid patients and the controls is in accordance with this fact. On the other hand, thyroid hormones are known to enhance GSH Px catabolism (4). Thus, the improvement of the thyroid status might have led to an increase in GSH Px activity, observed on the 30th day of PTU therapy.

With regard to the maintenance of GSH levels in hyperthyroid patients, the increased erythrocytic GSH level may be due to the direct inducing effect of the hyperthyroid state on the components of the antioxidant defense system. Erythrocytes are known to possess an active machinery for GSH synthesis, thus, GSH content in erythrocytes is considered to be the primary component

of blood total GSH.

The erythrocyte membrane, known to be impermeable to GSH in the physiological state, might have undergone an increase in permeability in the hyperthyroid state (known to induce a substantial fluidity in biological membranes) (18). The altered membrane fluidity/transport in hyperthyroidism may be another explanation for the increased GSH level of erythrocytes (19).

Our findings suggest, firstly, that under conditions of hyperthyroidism GSH and CuZn SOD are induced as part of the cell's defense against prooxidants for the maintenance of oxidative homeostasis. This reflects a selective modification in the erythrocyte antioxidative profile. Secondly, the relief in oxidative stress, achieved by the antithyroid action of PTU, causes a decrease in the need for antioxidant defense. In addition to the antithyroid action of PTU, its relevance as an *in vivo* antioxidant should be considered.

References

1. Ames BN, Shigenaga MK and Hagen TM: Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci USA (1993) **90**, 7915-7922.
2. Halliwell B: Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet (1994) **344**, 721-724.
3. Gutteridge JM: Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem (1995) **41**, 1819-1828.
4. Asayama K and Kato K: Oxidative muscular injury and its relevance to hyperthyroidism. Free Radical Biol Med (1990) **8**, 293-303.
5. Mano T, Sinohara R, Sawai Y, Oda N, Nishida Y, Mokumo T, Asano K, Ito Y, Kotake M, Hamada M, Nakai A and Nagasaka A: Changes in lipid peroxidation and free radical scavengers in the brain of hyper- and hypothyroid aged rats. J Endocrinol (1995) **147**, 361-365.
6. Pareiro B, Rosa LF, Sapi DA, Bechara EJ and Curi R: Control of superoxide dismutase, catalase and glutathione peroxidase activities in rat lymphoid organs by thyroid hormones. J Endocrinol (1994) **140**, 73-77.
7. Fernandez V, Barrientos X, Kipreos K, Valenzuela A and Videla L: Superoxide radical generation, NADPH oxidase activity and cytocrome P-450 content of rat liver microsomal fractions in an experimental hyperthyroid state: Relation to lipid peroxidation. Endocrinology (1985) **117**, 496-501.
8. Buege JA and Aust SD: Microsomal lipid peroxidation. Methods Enzymol (1978) **52**, 302-310.
9. Beutler E, Durgn O and Kelly BM: Improved method for the determination of blood glutathione. J Lab Clin Med (1963) **61**, 882-888.
10. Paglia DE and Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med (1967) **70**, 158-169.
11. Fairbanks V and Klee GG: Biochemical aspects of hematology; in Textbook of Clinical Chemistry, Norbert W Tietz ed, WB. Saunders, Philadelphia (1986) pp1532-1534.
12. Sun Y, Oberley LW and Li A: A simple method for clinical assay of superoxide dismutase. Clin Chem (1988) **34**, 497-500.
13. Morini P, Casalino E, Sblano C and Landriscina C: The response of rat liver lipid peroxidation, antioxidant enzyme activities and glutathione concentration to the thyroid hormone. Int J Biochem (1991) **23**, 1025-1030.
14. Asayama K, Dobashi K, Hayashibe H, Megata Y and Kato K: 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 (1987) **121**, 2112-2118.
15. Videla LA, Sir T and Wolff C: Increased lipid peroxidation in hyperthyroid patients: Suppression by propylthiouracil treatment. Free Radical Res Commun (1988) **5**, 1-10.
16. Faure M, Lissi EA and Videla LA: 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 (1991) **77**, 173-185.
17. Maral J, Puget K and Michelson AM: Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. Biochem Biophys Res Commun (1977) **77**, 1525-35.
18. Sen CK, Atalay M and Hanninen O: Exercise-induced oxidative stress: glutathione supplementation and deficiency. J Appl Physiol (1994) **77**, 2177-2187.
19. Shull S, Heintz NH and Preiosamy M: Differential regulation of antioxidant enzymes in response to antioxidants. J Biol Chem (1991) **226**, 24398-24403.

Received June 25, 1998; accepted August 27, 1998.