

Activity of antioxidative enzymes and concentration of malondialdehyde as oxidative status markers in women with newly diagnosed Graves-Basedow disease and after thiamazole therapy leading to euthyroidism

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Abstract: Introduction. Hyperthyroidism in the course of Graves-Basedow disease leads to intensification of oxidative processes and increased production of free oxygen radicals. It results in abnormal oxidative status of the organism. **Objectives.** Aim of this work was to assess the dynamics of oxidative status changes in women with Graves-Basedow disease before and after treatment with thiamazole. **Patients and methods.** Studies were carried out in 20 women with newly diagnosed hyperthyroidism in the course of Graves-Basedow disease and in 15 healthy women. Measurements of activity of antioxidant enzymes – superoxide dismutase (cytosolic copper/zinc isoform – Cu/ZnSOD, mitochondrial manganese isoform – MnSOD and extracellular copper/zinc isoform – EC-SOD) and glutathione peroxidase (GPx), as well as of malondialdehyde (MDA) concentration were performed twice, i.e. before the treatment and after 3–7 months of thiamazole therapy (euthyroidism). **Results.** Before the treatment, higher MnSOD plasma activity and lower EC-SOD activity were observed in women with hyperthyroidism in comparison with the control group, whereas the erythrocyte Cu/ZnSOD activity did not differ between the groups. Besides, women with hyperthyroidism had higher GPx activity in red blood cells. In this group studies have demonstrated higher plasma MDA levels, without any differences between the groups in MDA levels in red blood cells. After thiamazole therapy no differences could be demonstrated in MnSOD, EC-SOD, Cu/ZnSOD and GPx activities and MDA level between the groups. **Conclusions.** Women with hyperthyroidism in the course of Graves-Basedow disease experience abnormal oxidative status of the organism, and induction of euthyroidism after therapy with thiamazole results in resolution of these abnormalities.

Key words: Graves-Basedow disease, oxidative status

INTRODUCTION

Graves-Basedow disease is characterised by elevated levels of thyroid hormones – thyroxine (T_4) and triiodothyronine (T_3), which results in increased basal metabolism, increased oxygen consumption and production of large quantities of reactive oxygen species (ROS) [1–3].

In normal conditions there is an equilibrium between the ROS formation and activity of endogenous antioxidant systems. ROS are produced in all cells, depending on the intensity of aerobic metabolism, especially in activated neutrophils, monocytes, smooth muscle cells and in endothelial cells [4]. ROS with the highest biological activity include the hydroxyl radical ($OH\cdot$), superoxide anion radical ($O_2^{\cdot-}$) and

hydrogen peroxide (H_2O_2). Disequilibrium between ROS production and inactivation leads to oxidative stress. ROS also cause injury to the basic cell structures. They readily react with macromolecules, such as lipid, protein and DNA molecules, which results in degradation of cell membranes and excessive activation or inactivation of enzymes. The ultimate ef-

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Table 1. Characteristics of the study groups

	Before treatment	After treatment	
Age (years)	42.2 ±1.2	43 ±12	44 ±13
TSH (mU/l)	0.02 ±0.002*	1.16 ±0.3	1.4 ±0.57
fT ₃ (pmol/l)	24.9 ±8.05*	6.9 ±1.31	5.7 ±0.94
fT ₄ (pmol/l)	51.7 ±15.31*	15.9 ±1.85	15.7 ±1.18
TPOAb (IU/ml)	553.8 ±23.8*	352.1 ±35.4*	<50
TgAb (IU/ml)	572.1 ±121.4*	413.4 ±35.5*	<100
TRAb (U/l)	6.6 ±0.37*	3.4 ±0.23*	0.2 ±0.02
BMI (kg/m ²)	24.6 ±3.2	24.7 ±2.9	24.9 ±2.8
RBC (T/l)	4.5 ±0.3	4.6 ±0.4	4.3 ±0.4
Hb (mmol/l)	8.1 ±0.7	8.1 ±0.6	8.6 ±0.9
WBC (G/l)	6.7 ±1.8	7.0 ±1.6	7.1 ±1.3

*p <0.001 vs. the control group

Abbreviations: BMI – body mass index, fT₃ – free triiodothyronine, fT₄ – free thyroxine, GB – Graves-Basedow disease, Hb – hemoglobin, RBC – red blood cells, TPOAb – thyroid peroxidase antibodies, TRAb – TSH receptor antibody, TSH – thyrotropic hormone, WBC – white blood cells

fects of ROS activity include mutations, metabolic dysfunction and cell ageing. They in turn are a cause of development of inflammatory processes, oncogenesis and impaired organ functioning [5,6].

Among substances able to neutralise ROS there are ascorbic acid, α -tocopherol, carotenes, and also enzymes – including superoxide dismutase (SOD; E.C.1.15.1.1), glutathione peroxidase (GPx; E.C.1.11.1.9) and catalase (CAT; E.C.1.11.1.6). In humans 3 isoenzymes of superoxide dismutase can be found: cytosolic copper/zinc isoform – Cu/ZnSOD (SOD-1), mitochondrial manganese isoform – MnSOD (SOD-2) and extracellular copper/zinc isoform – EC-SOD (SOD-3). SOD catalyses the reaction of superoxide anion radical (O_2^-) to H_2O_2 and molecular oxygen (O_2). EC-SOD, similarly to Cu/ZnSOD, exhibits also a peroxidase activity, inactivating H_2O_2 [7-8]. CAT and GPx are principally intracellular enzymes, therefore EC-SOD plays a predominant role in H_2O_2 degradation in extracellular space [7]. GPx is an enzyme containing a selenium ion as a cofactor [9], and for the catalysed reaction it requires reduced glutathione (GSH), which is provided by glutathione reductase (GR, E.C.1.6.4.2) [10]. Both CAT and GPx degrade H_2O_2 , however GPx has higher affinity to hydrogen peroxide, which suggest it's dominating role in the majority of conditions with low levels of H_2O_2 [11].

Studies from recent years point at contribution of oxidative stress in development of autoimmune diseases [12]. Experimental data suggest that impaired ROS metabolism and beginning of the immune response in Graves-Basedow disease can be closely related [13]. Burch et al. [14] have demonstrated that anti-TSH receptor antibodies react with SOD from orbital fibroblasts, which possesses a fragment homologous to the TSH receptor. It has been proven that patients with Graves-Basedow disease have increased frequency of anti-SOD antibodies in comparison to healthy individuals.

It has been also demonstrated that ROS enhance the proliferation of fibroblasts sampled from the retrobulbar space in patients with infiltrative orbitopathy, and this effect is inhibited by thiamazole [15].

Aim of this work was to assess the dynamics of oxidative status changes in women with newly diagnosed Graves-Basedow disease before and after treatment with thiamazole.

PATIENTS AND METHODS

20 women from the Upper and Lower Silesia with newly diagnosed hyperthyroidism in the course of Graves-Basedow disease were enrolled in the study. The diagnosis was established based on clinical and biochemical criteria (Table 1). All patients had clinical symptoms and signs of thyrotoxicosis, elevated free thyroxine (fT₄) and free triiodothyronine (fT₃) levels, decreased TSH levels and significant titres of TSH receptor antibodies (TRAb). TSH levels were determined in women from the studied group at 4–6-week intervals. Measurements of activity of antioxidant enzymes and malondialdehyde (MDA) levels were carried out twice – before and after 3–7 months of thiamazole treatment, immediately after confirmation of biochemical features of euthyroidism.

The control group consisted of 15 women with normal thyroid gland function, as demonstrated by measurements of fT₄, fT₃ and TSH concentrations (Table 1). Patients with ischaemic heart disease, bronchial asthma, heart failure, renal insufficiency, liver diseases and other diseases requiring chronic pharmacotherapy, as well as these with positive history of smoking, alcohol and drug abuse were excluded from the study. Women participating in the study had no dietary restrictions. The study protocol has been approved by the Lo-

Table 2. Parameter of oxidative stress in patients with Engraving's disease and in the control group

	Before treatment	After treatment	
EC-SOD (NU/ml)	6.9 ±1.8*	10.7 ±2.0	11.48 ±2.12
CuZnSOD (NU/mg Hb)	25.29 ±3.85	24.8 ±2.5	26.9 ±2.12
MnSOD (NU/ml)	7.7 ±1.43*	4.4 ±0.55	4.69 ±0.78
GPx (IU/g Hb)	27.1 ±1.2*	15.2 ±1.1	14.5 ±0.7
MDA (p) µmol/l	3.25 ±0.53*	2.3 ±0.7	2.16 ±0.31
MDA (e) µmol/g Hb	1.95 ±0.4	1.8 ±0.82	1.91 ±0.6

*p <0.001 vs. the control group

CuZnSOD – cytosolic copper/zinc superoxide dismutase, e – red blood cells, EC-SOD – extracellular copper/zinc superoxide dismutase, GPx – glutathione peroxidase, MnSOD – mitochondrial manganese superoxide dismutase, p – plasma, SOD – superoxide dismutase, MDA – malondialdehyde

cal Bioethical Committee of ŚAM in Katowice (Protocol No. NN-013-50/00/02), and the participants gave their written informed consent to take part in the study.

Blood for tests was sampled on empty stomach in morning hours to test tubes containing EDTA, centrifuged immediately and measurements were performed on the same day. SOD activity was determined according to the Oyanagui method and expressed in nitrite units (NU/ml) in plasma and in nitrite units/g of hemoglobin (NU/g Hb) in red blood cells [16]. GPx activity in erythrocytes was measured according to a Paglia and Valentine method taking advantage of enzymatic conjunction with glutathione reductase, using H₂O₂ as a substrate [17]. MDA level in plasma and erythrocytes was determined with a colorimetric method according to Ohkawa using the reaction with thiobarbituric acid. Extinction was read at the wavelength of 270 nm; (absorbance) and 522 nm (emission). MDA level was read from a standard curve [18].

The obtained results were presented as arithmetic means ± standard deviations. One-way analysis of variance was performed with previous control of variance homogeneity with a Fisher test. A Newman-Keul test was used for post-hoc analysis. p <0.05 was adopted as statistically significant.

RESULTS

Characteristics of the investigated groups is presented in table 1. In all patients with hyperthyroidism the pretreatment fT₄ and fT₃ levels were significantly higher, and TSH lower compared to the control group.

In women with hyperthyroidism a higher pretreatment plasma MnSOD activity was detected in comparison with the control group (7.7 ±1.43 vs. 4.69 ±0.78 NU/ml; p <0.001) with lower EC-SOD activity (6.9 ±1.8 vs. 11.48 ±2.12 NU/ml, p <0.001). Besides, women with hyperthyroidism in comparison with the control group had higher GPx activity in red blood cells (27.1 ±1.2 vs. 14.5 ±0.7 IU/g Hb; p <0.001). Cu/ZnSOD activity in erythrocytes did not differ between the groups (25.29 ±3.85 vs. 26.9 ±2.12

NU/mg Hb) (Table 2). The test also demonstrated that women with hyperthyroidism, compared to the control group, had higher plasma MDA levels (3.25 ±0.53 vs. 2.16 ±0.31 µmol/l; p <0.001), whereas no differences were demonstrated between the groups in MDA levels in red blood cells (1.95 ±0.4 vs. 1.91 ±0.6 µmol/g Hb (Table 2).

After thiamazole treatment the TSH, fT₄ and fT₃ levels returned to normal values (Table 1). Thiamazole therapy also led to decreased MnSOD, GPx activity, elevated EC-SOD activity and normalisation of MDA levels (Table 2).

DISCUSSION

Results of this work demonstrate that women with manifest hyperthyroidism in the course of Graves-Basedow disease experience oxidative disequilibrium, which is supported by significant differences in activity of antioxidant enzymes – MnSOD and EC-SOD in plasma and GPx in red blood cells in comparison with the control group. It is accompanied by intensification of the lipid peroxidation processes, which is reflected by elevated MDA plasma levels in hyperthyroid women.

Results of previous studies on activity of antioxidant enzymes in patients with hyperthyroidism are equivocal [19]. The majority of works point at increased Cu/ZnSOD and GPx activity in erythrocytes patients with fully symptomatic hyperthyroidism in the course of Graves-Basedow disease [20], and even in patients with the subclinical form of the disease [21]. However contradictory results were obtained in some studies [22]. In this work we detected increased MnSOD activity in erythrocytes in patients with hyperthyroidism, which can be explained by the fact that T₃ and T₄ can stimulate the activity of MnSOD in order to protect mitochondria against oxidative injury by the superoxide anion radical [23]. It is confirmed by the results of experimental studies, which demonstrated that T₄ supplementation results in increased cerebral MnSOD activity [24].

In our work we demonstrated reduced EC-SOD activity in hyperthyroid patients. It is probably associated with lack of enzyme release in order to protect the epithelium against the superoxide anion radical generated in excess [15]. On the other hand, the observed elevated plasma MDA level can likewise contribute to decreased activity of EC-SOD by formation of cross-links with this protein [11]. EC-SOD deficiency results in increased production of hydrogen peroxide, which can additionally inactivate the enzyme. In the study, in women after induction of euthyroidism with thiamazole no differences were observed in EC-SOD plasma activity in both groups. It may result from decreased levels of thyroid hormones, but the antioxidant effect of thiamazole should also be considered. This drug inhibits the synthesis of thyroid hormones, but additionally shows antioxidant and immunomodulating effect on thyrocytes and other cells of the immune system [25].

Function of intracellular GPx is degradation of H_2O_2 and hydroperoxides of free fatty acids, whereas in plasma GPx catalyses degradation of H_2O_2 and hydroperoxides of phospholipids. In addition GPx exert a protective effect on membrane phospholipids by inhibiting their peroxidation processes [4]. According to hypothesis proposed by Seven et al. increased ROS production may lead to elevated GPx activity [26]. This thesis is confirmed by elevated activity of GPx in red blood cells demonstrated in this work. On the other hand, the observed lack of changes of MDA levels in erythrocytes is consistent with previous studies, suggesting low susceptibility of red blood cells to oxidative stress in hyperthyroidism [27]. Because of the fact that proteins are not synthesised *de novo* in erythrocytes, it can be suspected that these cells contain high reserves of enzymatic protein levels; therefore on one hand it is possible to activate antioxidant enzymes in response to ROS activity, and on the other hand – correction of losses caused by oxidative stress. Reduction of antioxidant potential of red blood cells occurring in thyrotoxicosis is explained by more rapid degradation of enzymatic proteins [28]. In this work we demonstrated that thiamazole therapy was followed by normalisation of GPx activity. Similar results were obtained by Abalivich et al. [29].

Changes in activity of antioxidant enzymes are accompanied by intensification of lipid peroxidation processes, which is confirmed by elevated MDA plasma levels that we observed in women with symptomatic hyperthyroidism. In quantitative terms, MDA is the most important component among reactive aldehydes originating from lipid peroxidation. For this reason, it is commonly considered as an index of oxidative stress severity. It seems that elevated MDA concentration can be caused by 2 factors: first – excessive tissue oxygenation in consequence of increased cellular respiration caused by thyroid hormones; it should be kept in mind that women enrolled in the study suffered from an autoimmune thyroid disease, therefore the effect of immune-mediated factors should be considered in addition to the hormones. *In vitro* studies on thyrocytes obtained from persons with Graves-Basedow disease demonstrated that activated immune system cells

present in intrathyroid inflammatory infiltrations release cytokines, which enhance production of toxic metabolites in cell membranes of neutrophils [30,31].

Previously published data indicate that, in patients with symptomatic hyperthyroidism, high levels of lipid peroxidation products correlate with levels of T_3 and T_4 hormones [20,32-34]. In experimental studies it was demonstrated that animals with induced thyrotoxicosis had more intense processes of lipid peroxidation, as evidenced by increased MDA content in the myocardium [35]. On the other hand, Cetinkaya et al. [21] demonstrated elevated MDA plasma levels in patients with subclinical hyperthyroidism. Studies by Bianchi et al. [33] in addition demonstrate that the process of free-radical oxygenation of phospholipids building plasmatic membranes of muscle cells are most likely the cause for development of thyrotoxic myopathy and cardiomyopathy – most common complications of hyperthyroidism. Increased production of ROS, as a consequence of more intense aerobic metabolism induced by thyroid hormones, likewise leads to oxidative injury of skeletal muscles [33].

The degree of cell injury in Graves-Basedow disease correlates with the oxidative stress level, which in turn depends upon the effectiveness of therapy used and antioxidant defensive capabilities of the organism [25]. In this work we demonstrated that, similarly to activity of antioxidant enzymes, MDA plasma level normalises after thiamazole treatment. Studies by Guerra et al. [36] in addition show decreased quantity of MDA eliminated with urine in patients with Graves-Basedow disease after thiamazole therapy. Therefore we hypothesise that urinary MDA content can be a useful index during monitoring of patients that completed thiamazole therapy [36].

According to the obtained results we cannot unambiguously conclude whether the observed biochemical effects, i.e. normalisation of activity of antioxidant enzymes and MDA concentrations, result from direct effects of thiamazole, or is the consequence of achieving euthyroidism. It would be also interesting to trace whether after shorter treatment, that is before euthyroidism has been achieved, the effect of thiamazole or reduction of excessive thyroid hormones on the measured parameters could be observed. Such information, similarly to the analysis of behaviour or activity of antioxidant enzymes and MDA levels during and after propylthiouracil treatment, would provide more data substantiating the conclusions about possible effect of these agents on the oxidative status. Solving these problems can be a subject of further studies on oxidative status in patients with Graves-Basedow disease.

In conclusion one can express an opinion that women with symptomatic hyperthyroidism in the course of Graves-Basedow disease experience oxidative equilibrium disorders, manifesting with elevated activity of MnSOD in plasma, as well as of GPx and CuZn/SOD in red blood cells, and reduced plasma activity of EC-SOD. The observed elevated plasma malondialdehyde level points at intensification of lipid peroxi-

dation processes in these patients. Achieving euthyroidism after thiamazole therapy is associated with normalization of oxidative status parameters.

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