METHIMAZOLE PROTECTION AGAINST OXIDATIVE STRESS INDUCED BY HYPERTHYROIDISM IN GRAVES' DISEASE

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Objective. To examine the dynamics of oxidative stress in patients with hyperthyroidism before and during the treatment with methimazole using the measurement of conjugated dienes, malondialdehyde, and Schiff bases in blood serum.

Methods. In eight female patients with diagnosed Graves disease and 8 healthy control subjects (7 females and one male) several parameters of oxidative stress (the level of conjugated dienes [CD], malondialdehyde [MDA] and Schiff bases [SB]) were estimated before and during the treatment with methimazole (Metizol -POLFA) as well as by hormonal and immunological tests. In addition, serum levels of TSH, free thyroxine (FT_4), free triiodothyronine (FT_3), antibodies against thyroperoxidase (anti-TPO) and thyroglobulin (anti–Tg) and low density lipoprotein-cholesterol fraction (LDL-Ch) were estimated.

Results. We observed increased concentrations of free thyroxine (FT_4) and free triiodothyronine (FT_3), as well as of antithyroperoxidase (anti-TPO) and antithyroglobulin (anti-Tg) antibodies. At the same time, TSH level was significantly suppressed. The concentrations of thyroid hormones and of TSH normalised after the methimazole treatment. The examined parameters, i.e., CD, MDA, and SB, were evaluated as proportions of each of them to the level of low density lipoproteins-cholesterol fraction (LDL-Ch). This fraction of cholesterol contains many polyunsaturated fatty acids, being a substrate for the peroxidation of lipids. Additionally, the CD/MDA ratio was calculated.

Conclusions. The increase of the CD/LDL ratio in Graves hyperthyroidism and its normalisation in the course of the treatment with methimazole suggests that the drug can be protective against the oxidative stress induced by overproduction of thyroid hormones. The ratio of CD/MDA decreased in all the patients, as compared to the control group, showing a high speed of lipid peroxidation.

Key words: Hyperthyroidism – Methimazole – Oxidative stress – Malondialdehyde – Conjugated dienes – Schiff bases

Graves disease is a well known autoimmune process in which T lymphocytes become sensitised to the genuine thyroid antigens and stimulate B lymphocytes to synthesise antibodies against these antigens. One of such antibodies is directed against the TSH receptor in the thyroid follicular cell membrane (Drexhage and Rotterdam 1995) and has the ability to stimulate thyroid cells to an increased function and growth.

In Graves disease, the thyroid gland is infiltrated by mononuclear cells and macrophages which, after stimulation, can release cytokines. The intrathyroid cytokine production during Graves disease is not only restricted to the thyroid infiltrating mononuclear cells but it may also involve thyroid follicular cells (Grubeck-Loebenstein et al. 1989). Activated mononuclear cells and macrophages are able to produce

Table 1
The concentration of TSH, thyroid hormones (FT_3, FT_4) and of antithyroperoxidase (Anti-TPO) and antithyroglobulin antibodies (ATg) in the patients with Graves' disease (MGB), treated with methimazole. Means ± SEM. Significance: * P<0.005, ** P<0.01, *** P<0.05 versus the control group.

	Controls	MGB – time O	MGB – after 1 month	MGB – after 3 months	MGB – after 6 months
FT3 (pg/mL)	3.00 ± 0.20	16.96 ± 2.81*	3.75 ± 0.49	2.17 ±0.27	2.49 ± 0.09
FT4 (ng/mL)	1.24 ± 0.06	4.19 ± 0.49*	1.27 ± 0.14	0.69 ± 0.06	0.88 ± 0.07
TSH (μIU/mL)	1.15 ± 0.21	0.14 ± 0.27	0.04 ± 0.01	3.65 ±0.82**	4.32 ± 1.42***
ATg (IU/mL)	39.3 ± 6.3	1144.2 ± 935.7	1208.6 ± 1036.6	879.4 ± 751.5	81.4 ± 31.8
Anti-TPO (U/mL)	16.0 ± 7.9	760.6 ± 318.6	339.8 ± 159.7	443.4 ±298.1	136.2 ± 61.9

interleukin 1 (IL-1), interleukin-6 (IL-6) and the tumour necrosis factor (TNF) (Celik et al. 1995), which have prooxidant and proinflammatory properties (Schreck and Baeuerle 1991; Schreck et al. 1991). Additionally, high concentrations of thyroid hormones may change the metabolism of oxygen in the cells and stimulate the production of free radicals (Weetman et al. 1992).

In the course of hyperthyroidism, oxidative stress and the peroxidation of lipids can be generated (Verditti et al. 1997). It is worth mentioning that some of the antithyroid drugs have antioxidant effects (Hicks et al. 1992; Heufelder et al. 1992). Among different intra- and extrathyroid mechanisms of the action of thyrostatic drugs, an emphasis should be placed on their possible immunosuppressive effects. These effects may directly influence thyroid follicular cells, cause a direct suppression of the TSH receptor antibody formation, or provide indirect effects, mediated via heat shock proteins, oxygen free radicals and the immune system (Wartofsky 1993). It was shown that both methimazole and propylthiouracil abolished or reduced the oxygen radical production by complement-attacked thyroid cells and decreased cytokine production (Weetman et al. 1992). The main action of thionamides is to inhibit the organification of iodide and the coupling of iodotyrosines, thus blocking the synthesis of thyroid hormones (Orgiazzi and Mornex 1990). Propylthiouracil has an additional effect, inhibiting the conversion of T_4 to T_3 in peripheral tissues (Chiovato et al. 1995).

The aim of this study was to examine the dynamics of oxidative stress in patients with hyperthyroidism before and during the treatment with methimazole using the measurement of conjugated dienes, malondialdehyde, and Schiff bases in blood serum.

Subjects and Methods

Eight female patients and eight healthy volunteers (7 females, 1 male) regarded as controls gave an informed consent to take part in the study. None of the subjects had received any medications prior to or during the study or had any other systemic illness.

All the participating patients revealed Graves disease, according to the previously published criteria (Volpe 1991). The serum levels of thyrotropin (TSH), free thyroxine (FT_4), free triiodothyronine (FT_3), antibodies against thyroperoxidase (anti-TPO) and thyroglobulin (anti-Tg), lipid fractions of triacylglycerols and cholesterol were estimated in all subjects before the treatment with methimazole and 1, 3 and 6 months after that treatment.

The levels of total cholesterol (TCh), triglycerides (TG) and of the high density lipoproteins-cholesterol (HDL-Ch) fraction were measured according to Lopes-Virella et al. (1977); Bucolo and David (1973), and Davies et al. (1979), respectively. The value of LDL-Ch fraction was calculated by means of Friedewald's formula

$$\text{LDL} - \text{Ch} = \text{TCh} - \text{Ch} - \frac{\text{TG}}{5}$$

Concentrations of TSH, thyroid hormones and antithyroid antibodies were determined by imunoenzymatic method (EIA).

In the present study, the following products of lipid peroxidation were examined in serum, both before

Table 2
The concentration of total cholesterol (TCh), fractions LDL-Ch, HDL-Ch and of triglycerides (TG)] in the patients with Graves' disease (MGB), treated with methimazole. Data are means ± SEM. Statistical significance: * P<0.005, **<0.05 versus the control group.

	Controls	MGB – time O	MGB – after 1 month	MGB – after 3 months	MGB – after 6 months
TCh (mg/dL)	204.0 ± 13.7	180.0 ±12.0	221.0 ± 14.5	257.0 ± 14.0	248.0 ± 18.9
LDL-Ch (mg/dL)	51.2 ± 4.0	40.8 ± 2.1*	45.0 ± 1.8	47.6 ± 2.27	46.7 ± 2.6
HDL-Ch (mg/dL)	138.2 ± 10.3	111.6 ± 10.6	151.1 ± 11.6	167.7 ± 16.3	173.2 ± 16.3
TG (mg/dL)	73.0 ± 8.3	128.0 ± 18.7	126.0 ± 14.3**	142.0 ± 13.4**	141.0 ± 15.9

methimazole administration and after 1, 3 and 6 months of that treatment: the level of conjugated dienes (CD), malondialdehyde (MDA), and Schiff bases (SB) (Nowak et al. 1993; Nowak et al. 1995; Piotrkowski et al., 1996). The results were expressed as their proportions to the levels of LDL-Ch, which indicated the highest amounts of polyunsaturated fatty acids, i.e., the source of free radicals. The analysis of variance (ANOVA) was applied to estimate statistical significance. All the calculations were performed with Microsoft-EXCEL 5.0 computer software.

Results

The patients with Graves disease were diagnosed, on the basis of the outcome of clinical examination and biochemical parameters. TSH concentration (Tab. 1) was significantly lower in the patients before the treatment than in the control group, increasing in the course of methimazole treatment. We showed significantly increased concentrations of FT_3 and FT_4 (Tab.1). Those concentrations gradually decreased during the treatment with methimazole. The concentrations of anti-TPO and anti-Tg antibodies were elevated in cases of hyperthyroidism, but the differences were not significant, probably because of the high values of standard deviations (SD) (Tab. 1). We have demonstrated the following changes in cholesterol and in its fractions: 1. decreased concentration of TCh, HDL-Ch and LDL-Ch and a normalisation of these parameters during the methimazole treatment; 2. on the other hand, the concentration of TG was elevated not only during hyperthyroidism but also after the normalisation of thyroid hormone concentrations (Tab. 2).

In order to examine the oxidative processes, we analysed the ratios of CD (Fig. 1), MDA (Fig. 2) and SB (Fig. 3) to LDL-Ch. Additionally, we measured the ratio of CD/MDA (Fig. 4). Malondialdehyde is the last product of lipid peroxidation. We have shown a significant elevation of MDA/LDL in the patients with hyperthyroidism. In the course of methimazole treatment that ratio normalised. The CD/LDL and SB/LDL ratios were also slightly elevated in patients with hyperthyroidism but the increase was not significant. The ratio of CD/MDA was decreased at all the examined time points in all the patients, as compared to the control group, showing a high speed of lipid peroxidation.

Discussion

In this study we found a combination of the suppressed TSH level, which – together with the elevation of FT_4 and FT_3 – provided for the diagnosis of hyperthyroidism. Additionally, we observed high concentrations of antithyroid antibodies and all the other clinical manifestations, allowing to diagnose Graves disease. Methimazole administration, in a dose of 60 mg/day, reduced the elevated levels of thyroid hormones. A suppression of high concentrations of antithyroid antibodies followed the normalisation of thyroid hormones. Our results are concordant with the results of other authors, e.g., of Orgiazzi and Millot (1994) who have suggested that, beyond the inhibition of thyroid hormone synthesis, the antithyroid drugs appear to possess the ability to interfere with the immunological abnormalities involved in Graves hyperthyroidism: they cure 50 % of the patients provided their administration is maintained for at least

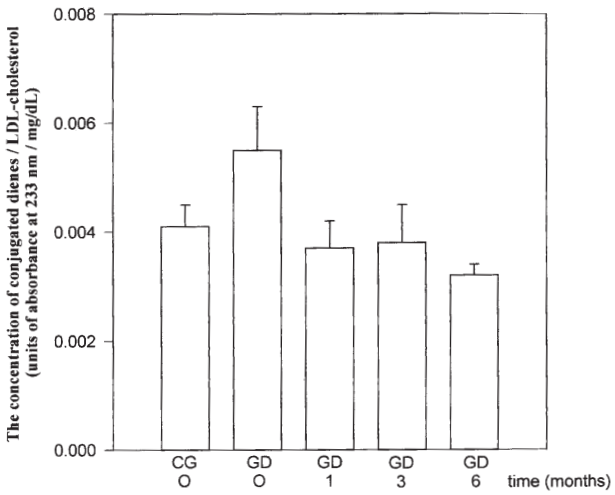

Fig. 1 Ratio of the concentration of conjugated dienes (CD) to LDL-Ch in patients with Graves disease before and after 1, 3 and 6 months of treatment with methimazole. Means ± SEM.

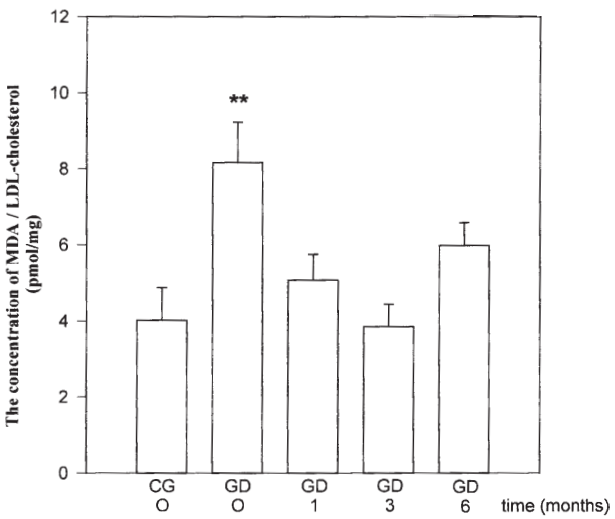

Fig. 2 Ratio of the concentration of malonyldialdehyde (MDA) to LDL-Ch in patients with Graves disease before and after 1, 3 and 6 months of treatment with methimazole. Means ± SEM. Statistical significance: ** = P<0.05

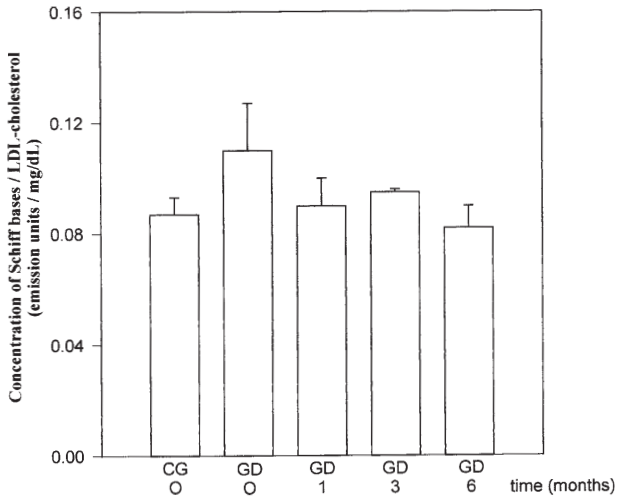

Fig. 3 Ratio of the concentration of Schiff bases (SB) to LDL-Ch in patients with Graves disease before and after 1, 3 and 6 months of treatment with methimazole. Means ± SEM.

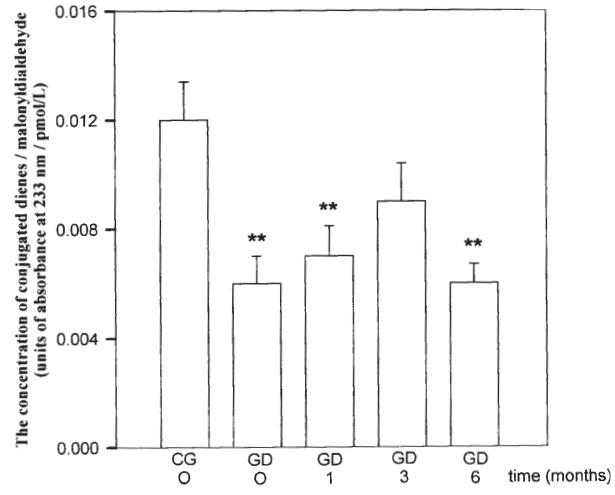

Fig. 4 Ratio of the concentration of CD to MDA in patients with Graves' disease before and after 1, 3 and 6 months of treatment with methimazole. Means ± SEM. Statistical significance: ** = P<0.05

12 months and they significantly decrease the concentrations of antithyroid antibodies in most of the patients. The potential immunomodulatory effects of antithyroid drugs seem to involve a thyroid depletion of iodine, which might reduce antigen expression and the scavenging of reactive free radicals, generated from oxygen and/or iodine during peroxidation (Orgiazzi and Millot 1994).

Alterations of the lipid profile are a well-known phenomenon in thyroid dysfunctions. Thyroid hor-

mones regulate the lipid metabolism through various mechanisms but the key role is played by the LDL receptor pathway (Spandrio et al. 1993). In our study, we showed a decreased concentration of TCh, LDL-Ch and HDL-Ch in the patients with hyperthyroidism, as compared to healthy controls. During the treatment with methimazole those parameters were normalised. The levels of TG were significantly higher in the patients, as compared to the healthy group, remaining unchanged during the treatment. This is in agreement with the results of other authors. Thus, Spandrio et al. (1993) showed that in hyperthyroid patients TCh and LDL-Ch increase after methimazole treatment, while TG levels remain unchanged. Before the treatment with antithyroid drugs, however, decreased concentrations of TCh, LDL-Ch and HDL-Ch were observed (Hoppichler et al. 1995).

Hypermetabolism induced by thyrotoxicosis is well known to aggravate free radical mediated tissue injury (Johnson and Turnbull 1984; Roodyn et al., 1965). In rats with experimentally-induced thyrotoxicosis, Fernandez et al. (1985) observed an increased generation of oxygen free radicals in the rat liver microsome fraction. Pereira et al. (1994) demonstrated that thyroid hormones regulated the activities of antioxidative enzymes in lymphoid organs in rats. The activated mononuclear cells and macrophages infiltrate the thyroid gland in Graves disease, as well as the retrobulbar and pretibial space in Graves ophthalmopathy and pretibial myxedema, respectively (Heufelder et al. 1992). Reactive oxygen species are generated in tissues by these cells (Babior and Woodman 1990). One of them is a superoxide anion radical – a precursor of the highly toxic hydroxyl radical – which is produced after the activation of NADPH oxidase.

In the body, there is a system of endogenous antooxidants and antioxidative enzymes. Mano et al. (1995) have shown the increased concentrations of catalase, Mn-superoxide dismutase (Mn-SOD) and glutathione peroxidase (GSH-Px) in the cerebral cortex of hyperthyroid aged rats, compared to euthyroid controls. The same authors also observed that the concentrations of lipid peroxides, determined indirectly by the measurement of thiobarbituric acid reactants (TBA), were decreased in hyperthyroid rats. The authors suggest that free radicals and lipid peroxides are scavenged to compensate for the changes induced by hyperthyroidism. On the other hand, Rom-Boguslavskaia et al. (1997) studied lipid oxidation in euthyroid and thyrotoxic tissue samples of the human thyroid gland. The authors observed that the content of TBA-active lipid peroxidation products was increased in thyrotoxic tissue and the activity of antioxidant enzymes (calatase, GSH-Px) was decreased. Mano et al. (1997) measured the levels of free radical scavengers and checked superoxide radical generating systems in the human thyroid gland. Thyroid specimens from patients with Graves disease contained significantly higher concentrations of xanthine oxidase (XOD) and GSH-Px, compared to those in the normal thyroid tissue but catalase concentration was lower. Those findings suggest that the levels of free radicals are increased and are scavenged and catalysed in the thyroid of patients with Graves disease. The authors also found that the level of coenzyme Q was reduced in the thyroid tissue of patients with Graves' disease (Mano et al. 1998).

We have shown in the present study that, in case of the hyperthyroid patients with Graves disease, the three examined parameters, i.e., MDA/LDL, CD/LDL and SB/LDL, increased, however only the first one in a significant way. On the other hand, the ratio of CD/MDA decreased in the hyperthyroid patients, as compared to the controls. We believe that our results indicate a high speed of lipid peroxidation during hyperthyroidism.

The increase of the CD/LDL, MDA/LDL and SB/LDL ratios in case of Graves hyperthyroidism and their normalisation in the course of treatment with methimazole, suggest that the drug may protect against oxidative stress, induced by an overproduction of thyroid hormones. There may be different mechanisms of this effect: 1. a decrease of the level of thyroid hormones; 2. an antioxidative action of methimazole; 3. an immunosuppressive effect of methimazole; 4. a limitation of inflammatory processes.

Additionally, there is some information about the influence of methimazole on the endogenous antioxidative system. For example, Ademoglu et al. (1998) observed that hyperthyroidism tends to enhance the lipid peroxide content, to increase glutathione S-transferase activity and to decrease GSH-Px activity, as well as vitamin E and ascorbic acid levels in plasma. The achievement of euthyroidism after methimazole treatment (a dose of 10 mg/day) led to normalisation of

those parameters. Also, SEVEN et al. (1996) has found that superoxide dismutase (SOD), GSH-Px and glutathione (GSH) values significantly increased in hyperthyroid rats, in comparison to the control group. Vitamin E supplementation to hyperthyroid rats induced a significant decrease in GSH-Px activity and a significant increase in GSH level. These findings show that hyperthyroidism increases the components of the antioxidant system in erythrocytes and vitamin E protects against oxidative stress (SEVEN et al. 1996). In their subsequent paper, the authors found that vitamin C supplementation potentialized the antioxidant status in both propylthiouracil-treated hyperthyroid patients and in controls (SEVEN et al., 1998).

In conclusion, the increase of the CD/LDL, MDA/LDL and SB/LDL ratios in Graves hyperthyroidism and the normalisation of those parameters during the treatment with methimazole suggest that the drug can protect against oxidative processes induced by thyrotoxicosis.

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