

Antioxidants and methimazole in the treatment of Graves' disease: effect on urinary malondialdehyde levels

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Received 28 April 2004; received in revised form 24 August 2004; accepted 24 August 2004

Abstract

Background: We have postulated that metabolic oxidation could be the source of signs and symptoms of hyperthyroidism. The present study was designed to evaluate urinary malondialdehyde levels in Graves' disease and compare this oxidative stress biomarker with the clinical evolution of patients suffering this illness.

Methods: We evaluated the concentration of urinary and serum malondialdehyde (MDA) in 36 patients with Graves' disease. Patients were treated with the antithyroid drug methimazole (MMI; Group A) or antioxidant mixture (200 mg vitamin E, 3 mg β -carotene, 250 mg vitamin C, 1 mg Cu, 7.5 mg Zn, 1.5 mg Mn, and 15 μ g Se; Group B).

Results: MDA concentrations were higher in hyperthyroid patients compared to euthyroid controls, and a positive correlation was observed between serum and urinary MDA levels. Group A decreased urinary MDA to control values. There was a positive correlation between the clinical score and the heart rate of patients with urinary MDA before and during the treatment with MMI (Group A). Similar results were observed after treatment with the antioxidant mixture.

Conclusions: Urinary MDA might be a good parameter in the follow-up of patients during MMI treatment. We proposed that oxidative stress correlates with signs and symptoms of hyperthyroidism.

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Keywords: Hyperthyroidism; Methimazole (MMI); Malondialdehyde

1. Introduction

Graves' disease is characterized by low levels of TSH and high levels of T3 and T4 hormones. One of the main effects of thyroid hormones (T3, T4) is the increase in mitochondrial respiration, which

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causes a hypermetabolic state with generation of free radicals [1]. Increased oxidative stress with high free radical levels has been described previously in animal models of hyperthyroidism and in patients with Graves' disease [2–6]. Tissue damage in hyperthyroidism might be mediated by an increase in free radicals. Lipoperoxidation and O₂ consumption increase in T3-treated rats [7]. In our endocrinology unit, we demonstrated the benefits of applying an antioxidant treatment as an adjuvant therapy of Graves' disease when patients were treated with methimazole (MMI) and a mixture of antioxidants (including vitamins C and E) [8]. The main effect of MMI is to inhibit thyroid hormone synthesis in the thyroid gland. However, MMI has also been reported to exert antioxidant effects eliminating H₂O₂ in vitro [9].

There are a variety of methods for the assessment of oxidative stress, some of which can be applied in the clinical laboratory. However, it is worth remembering that the analysis of a single parameter does not account for the entire phenomenon of oxidative stress; several reports show that urinary malondialdehyde (MDA) levels rise in response to increasing lipid peroxidation in vivo. MDA evaluation in urine represents a noninvasive biomarker of oxidative stress [10,11]. We determined urinary MDA levels in the treatment of hyperthyroid patients with the antithyroid agent methimazole (MMI), in order to evaluate if MDA could be a useful biomarker.

2. Materials and methods

2.1. Patients and drugs

We studied 36 hyperthyroid patients (Graves Basedow), 28 females and 8 males from 22 to 60 years old, all of them Caucasian. Normal controls (20 euthyroid subjects) of similar age and sex were also studied. The Hospital Ethical Committee has approved these studies, and informed consent was obtained from each subject. Five of the hyperthyroid patients presented anti-TPO autoantibodies.

Methimazole (MMI) was from Gador. Levothyroxine sodium tablets were from Química Montpellier. Patients did not receive levothyroxine after the treatment finished. Some patients did not receive methi-

mazole during the first month; they were administered 2 capsules/patient daily of an antioxidant mixture. Each capsule contained 200 mg vitamin E, 3 mg β -carotene, 250 mg vitamin C, 1 mg Cu, 7.5 mg Zn, 1.5 mg Mn, and 15 μ g Se (Larotabe, Roche). After this period, these patients were included in the usual protocol with MMI.

2.2. Biochemical determinations

Hormone concentration (T3, T4, TSH) in sera were determined by radioimmunoassay using a Diagnostic Products Corporation Kit (Los Angeles, CA). The concentration of malondialdehyde (MDA) reacting with thiobarbituric acid (TBA) in sera and urine of 36 patients was determined using an extinction coefficient of $1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$ [12]. Proteins were determined by the method of Bradford [13], with crystalline bovine serum albumin as standard.

2.3. Clinical score diagnosis

A clinical score was established according to the most common symptoms and signs of hyperthyroidism [8]: (a) nervousness/insomnia, (b) heat/sweat, (c) weight loss/diarrhea, (d) tachycardia, and (e) tremor. The presence of one of the symptoms or signs from each item was enough to score 1 point. We selected patients with a clinical score of 5 before treatment. Inclusion of patients in different treatment groups was at random.

2.4. Treatment

Patients were divided into two groups according to the treatment they received. (A) MMI treatment for 8 weeks: 19 patients (15 females and 4 males, 22–60 years old), 3 with exophthalmos and 4 with goiter. (B) Antioxidant mixture (Larotabe) for 4 weeks, and then patients were treated with antioxidant mixture and MMI for another 4 weeks: 17 patients (13 females and 4 males, 24–60 years), 4 with exophthalmos and 6 with goiter. Daily doses: MMI=50 mg/patient, antioxidant mixture (Larotabe)=2 capsules/patient, one at noon and the other at 9:00 P.M.

Table 1
Effect of treatment on signs and symptoms of hyperthyroidism

	Group A (n=19)		Group B (n=17)	
	4 weeks	8 weeks	4 weeks	8 weeks ^a
Nervousness, insomnia ^b	68	10	0	0
Diarrhea ^b	53	5	0	0
Hotness, sweat ^b	63	5	0	0
Weight loss ^b	79	0	0	0
Tremor ^b	84	10	5	0
Clinical score	3-5	0-1	0-1	0

^a Patients were treated with antioxidant mixture+MMI after week 4. All the patients included in the study had a clinical score of 5 before treatment.

^b The results are the percentage of patients presenting the sign or symptom after different treatments. Period of treatment: Group A=MMI 4 or 8 weeks. Group B=Antioxidant mixture 4 weeks.

2.5. Statistics

Results are means±S.D. Statistical analysis was performed by one-way analysis of variance [14]. Due to the results obtained with ANOVA, we performed the Bonferroni test [15].

3. Results

3.1. Clinical parameters

Because some signs and symptoms are difficult to quantify, we decided to evaluate only their presence or absence (Table 1). A double-blind approach was carried out to perform this study. Neither the patient nor the observer knew about the treatment applied during the first 4 weeks. The score of each patient was

always determined by the same physician. The clinical score is a sum of the points obtained with the parameters considered. Patients treated with MMI did not show any decrease in their clinical score after 4 weeks of treatment, and the score only diminished from 5 to 0–1 after 8 weeks. Patients receiving the antioxidant mixture showed a significant improvement in their score after the first 4 weeks (Table 1).

At clinical scores 0–1, the heart rate significantly decreased from 105.4 ± 4.0 pulsation/min before treatment to 77.1 ± 6.1 pulsation/min, $p<0.01$, after MMI treatment (8 weeks treatment), and from 101.4 ± 5.3 pulsation/min to 80.5 ± 3.4 pulsation/min, $p<0.01$, in patients treated with the antioxidant mixture (4 weeks treatment).

3.2. Biochemical parameters

All patients had low serum TSH levels before treatment (Table 2). Although patients were included at random in the groups, thyroid hormone levels of patients in group B were lower than those observed in patients treated with MMI (group A).

At clinical scores 0–1, thyroid hormone concentrations in patients treated with MMI decreased to normal levels. MMI treatment continued for 18–24 months and there was no recurrence during this period. On the other hand, at clinical score 0–1, patients treated with the antioxidant mixture for 4 weeks showed serum thyroid hormone levels similar to those observed before treatment. When MMI was added, thyroid hormonal concentrations significantly reduced after 4 weeks (data not shown). No recurrence was observed after a follow-up of 24 months.

Table 2
Effect of treatment on thyroid hormone levels in sera of hyperthyroid patients

	Before treatment			After treatment		
	Clinical score=5			Clinical score=0–1		
	T3 (nmol/l)	T4 (nmol/l)	TSH (μ U/ml)	T3 (nmol/l)	T4 (nmol/l)	TSH (μ U/ml)
Group A	6.6 ± 2.1	206.0 ± 39.1	0.16 ± 0.10	$2.1\pm 0.7^*$	$74.7\pm 29.3^*$	$1.75\pm 1.30^*$
Group B	4.6 ± 1.3	236.1 ± 52.9	0.44 ± 0.38	4.7 ± 0.8	239.9 ± 38.7	0.38 ± 0.37

The results are expressed as: means±S.D. Group A=MMI 8 weeks. Group B=Antioxidant mixture 4 weeks. Normal level T3: 1.2–3.3 nmol/l. T4: 64.3–167.3 nmol/l. TSH: up to 5 μ U/ml.

* $p<0.05$ vs. values before treatment.

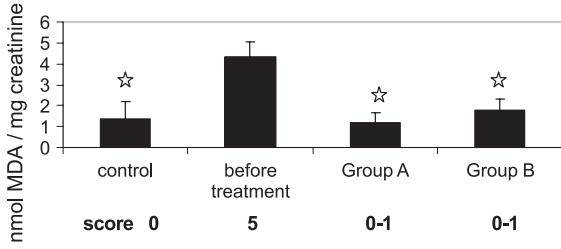


Fig. 1. Urinary MDA content. Control: euthyroidism; before treatment: Hyperthyroidism; Group A: MMI (8-week treatment); Group B: Antioxidant mixture (4-week treatment). Malondialdehyde is expressed in nmol MDA/mg creatinine. $p < 0.01$ vs. values before treatment.

Urinary MDA was significantly higher in hyperthyroidism cases than in euthyroid controls (hyperthyroid patients before treatment: 4.32 ± 0.75 nmol

MDA/mg creatinine vs. control 1.37 ± 0.81 nmol MDA/mg creatinine, $p < 0.01$), but it decreased to values similar to controls when clinical scores reached 0–1 after MMI treatment. Similar results were observed with the antioxidant treatment (Fig. 1). All samples showed normal distribution (Shapiro–Wilks, $p = 0.6382$). Bonferroni test demonstrated that means were not significantly different between the two groups (A and B).

MMI and the antioxidant treatment affected both urinary and serum malondialdehyde contents. There was a positive correlation between urinary and serum MDA in hyperthyroid patients that was maintained after the treatments (Fig. 2a: $r = 0.90$, $p < 0.01$). A positive correlation was also observed between heart rate and urinary MDA ($r = 0.915$, $p < 0.01$) in hyperthyroid patients, before and after treatments (Fig. 2b). We observed a positive correlation between urinary MDA and serum thyroid hormone level (T3 and T4) in hyperthyroidism (Fig. 3).

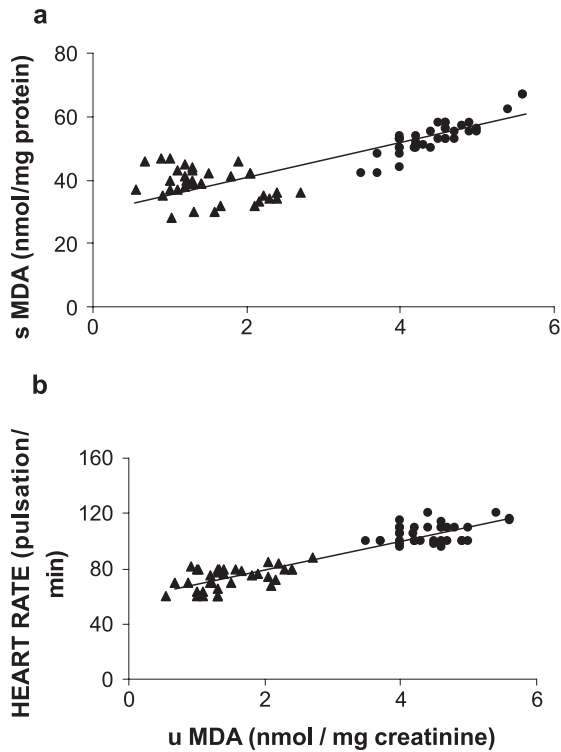


Fig. 2. (a) Correlation between serum MDA content (s MDA) and urinary MDA content (u MDA) in (●) hyperthyroid patients and (▲) treated patients (8 weeks of MMI or 4 weeks of antioxidant mixture); (b) correlation between heart rate and urinary MDA content in (●) hyperthyroid patients and (▲) treated patients (8 weeks of MMI or 4 weeks of antioxidant mixture). Heart rate is expressed in pulsation/min, s MDA is expressed in nmol/mg protein and u MDA is expressed in nmol/mg creatinine.

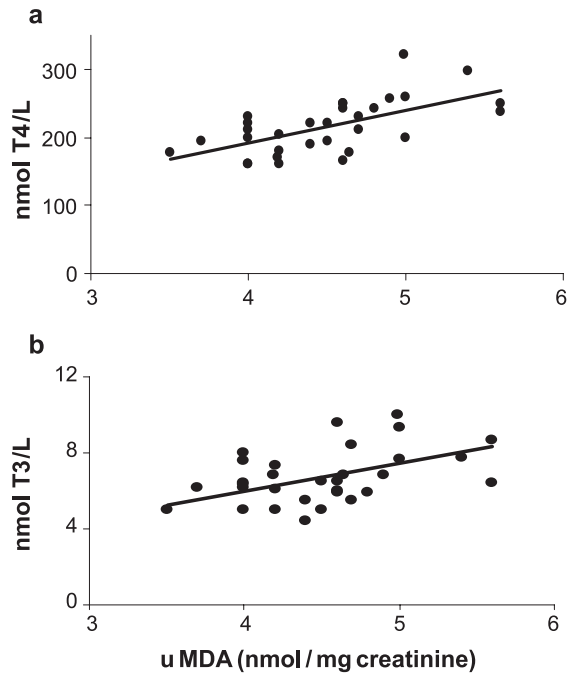


Fig. 3. (a) Correlation between serum T4 level (T4) and urinary MDA content (u MDA) in (●) hypothyroid patients; (b) correlation between serum T3 level (T3) and urinary MDA content (u MDA) in (●) hyperthyroid patients. T3 is expressed in nmol/l (normal level: 1.2–3.3 nmol/l), T4 is expressed in nmol/l (normal level: 64.3–167.3 nmol/l), u MDA is expressed in nmol/mg creatinine.

4. Discussion

Free radical-mediated oxidative stress has been related to the etiopathogenesis of several autoimmune disorders such as Graves' disease [2,4,16]. The damage produced by the reactive oxygen species (ROS) resulted from an imbalance between these toxic molecules and antioxidant capacity [17]. Lissy et al. [4] observed a correlation between urinary MDA levels and urinary chemiluminescence in hyperthyroid patients. In agreement with this observation, preliminary results obtained by our laboratory indicated an increase in urinary bioluminescence in hyperthyroid patients that decreased after treatment with MMI or the antioxidant mixture described in this report. MDA levels can be used as a screening method for the diagnosis and follow-up of several diseases. Control values for urinary MDA were similar to those of Lissy et al. [4] and Feillet-Coudray et al. [18].

Cardiac MDA content in aged rats significantly increased during hyperthyroidism. This alteration could be a biochemical effect of thyroid hormone in cardiac tissue [19]. It is not known whether similar changes occur in humans during clinical thyroid disease. Such changes may interfere with cardiac contractility, alter cardiac electrophysiology, and contribute to the cardiomyopathy associated with thyroid disease. According to our results, there is a positive correlation between heart rate and urinary MDA in hyperthyroid patients. This correlation is sustained after MMI or antioxidant treatment. We also observed a correlation between thyroid hormone levels and urinary malondialdehyde in hyperthyroid patients. Therefore, we postulated that elevated thyroid hormone levels trigger signs and symptoms of hyperthyroidism through the increase of free radicals. It has been shown that free radicals are increased in Graves' disease [20]. When this increase is neutralized, urinary MDA decreases and patients improve clinically, although antioxidant treatment has no effect on the production of thyroid hormones. Although biochemical data determined that the group treated with the antioxidant mixture might have milder hyperthyroidism than the MMI group, all the patients included in this study had a clinical score 5 before the treatment. We randomly distributed these patients into two treatment groups (MMI or antiox-

idant mixture); both urinary MDA and hyperthyroidism signs and symptoms similarly decreased in the two groups.

We observed a reduction in urinary MDA after MMI treatment. The antithyroid drugs methimazole (MMI) and propylthiouracil (PTU) are used to treat patients with Graves' hyperthyroidism. Their main function is to inhibit thyroid hormone synthesis in the thyroid gland. One of their targets is thyroid peroxidase which blocks the iodination of tyrosine residues and the coupling of iodotyrosines into iodothyronines [21]. Videla reported that PTU treatment reduced urinary MDA, in agreement with our results using MMI. Other authors reported that methimazole treatment resulted in free radical normalization [22]. High concentrations of thyroid hormone could alter oxygen metabolism in cells and stimulate free radical production [23]; MMI might abolish or at least reduce this oxygen radical production. We believe that urinary MDA content might be a useful tool to monitor patients who had completed MMI treatment.

MDA levels significantly increased in erythrocytes from patients with hyperthyroidism [24]. Videla et al. [2], Dumitriu et al. [25], and Guerra et al. [8] reported a significant increment in plasmatic MDA levels of hyperthyroid patients. The positive correlation between urinary and serum MDA in hyperthyroid patients suggests that urinary MDA concentration might be used as a non-invasive method to MDA levels in hyperthyroidism. Because we demonstrated that clinical score correlates with urinary MDA, it would be useful to include urinary MDA as an objective parameter in our clinical score.

Acknowledgement

These studies were supported by grants from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), from Agencia Nacional de Promoción Científica y Tecnológica PICT No. 98-3406, from University of Buenos Aires and by Roche S.A. José A. Burdman, Liliana N. Guerra, and MC Ríos de Molina are Career Scientific Researcher in CONICET. The Hospital Ethical Committee has approved these studies.

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