IODIDE EXCESS EXERTS OXIDATIVE STRESS IN SOME TARGET TISSUES OF THE THYROID HORMONES

Running title: Iodide excess exerts oxidative stress in some target tissues of the thyroid hormones

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Abstract

Environmental iodine deficiency continues to be a significant public health problem worldwide. On the other hand, iodide excess results principally from the use of iodine-containing medicinal preparations or radiographic contrast media. For this reason we intended to explore iodide excess impairment on prooxidant/antioxidant balance of the thyroid gland, hepatic tissue and in blood and the effect of Selenium administration on oxidative stress markers under the same circumstances. Experiments were performed 10 days long on white, male, Wistar rats, as follows: group1: control- normal iodine supply group 2: high iodine diet, group 3: high iodine diet and Selenium, group 4: high iodine diet and Carbimasole. Oxidative stress markers such as lipid peroxides were determined from thyroid gland, hepatic tissue and in blood. Measuring H+ donor ability of the sera and catalase activity in thyroid gland and in hepatic tissue assessed antioxidant defense. Iodide excess had prooxidant effects, leading to an increased lipid peroxides level and catalase activity in target tissues and in blood and to a decreased H+ donor ability of the sera. Selenium supplementation had opposite effects. Present data allow us to conclude that the alterations due to iodide excess in thyroid gland, hepatic tissue and in blood are mediated through oxidative stress.

Keywords: iodide excess, thyroid hormones, hepatic tissue, oxidative stress

Introduction

Oxidative stress is a general term used to describe a state of damage caused by reactive oxygen species (ROS). This damage can affect a specific molecule or the entire organism. Reactive oxygen species, such as free radicals and peroxides, represent a class of molecules that are derived from the metabolism of oxygen and exist in all aerobic organisms (12). Much of the reactive oxygen species production occurs in mitochondria, via oxidative phosphorilation. Because the mitochondria contains specific receptors for the thyroid hormones, being one of the "favorite" target for them, the concept about a possible relationship between reactive oxygen species production and thyroid pathology has increasing importance (38). When the thyroid hormones production increases hepatic tissue is, also, subjected to oxidative stress because of their action on liver mitochondria and on Kupffer cells (11).

On the other hand, excess iodide displays different effects depending on the intake amount and on the thyroid status at that time, leading to an increase or a decrease in thyroid hormones production.

Because the thyroid gland is subjected to reactive oxygen and iodide species action during thyroid hormones production, the aim of the present study was to investigate the iodide excess-

induced disturbance on prooxidant/antioxidant balance in the thyroid gland, hepatic tissue and blood.

Material and methods

Animals and housing conditions

Wistar rats, male, 90 days old, weighting 180-330g, were maintained under pathogen-free conditions in a temperature-controlled (23 ± 1^0C, 50–70% relative humidity) and light-controlled (illuminated from 0600–1800 h) room. None of the animals died unexpectedly.

Dietary iodine intake

Four groups of 40 animals, each group consisting in 10 rats, were investigated: group 1:control- normal iodine supply equivalent to a daily intake of 7000 ng iodine/100 g body weight, using a standard chow, group 2: high iodine diet by adding a defined admixture of potassium iodine to drinking water (1 μg/100g body weight /daily, the equivalent of approximate 500 μg iodide in man), group 3: high iodine diet, similarly to group 2, and Selenium (0.25 ml/body weight/day, subcutaneous); 1ml contains 0. 5 mg inorganic Selenium in stabilized water, and group 4: high iodine diet, similarly to group 2, and Carbimasole, an inhibitor of thyroid-peroxidase (0.1 mg/100 g/daily) added in drinking water. Distilled water

was available to all animals *ad libitum*. Experiments were performed for 10 days.

All animal studies were done according to the local guidelines for animal research and principals of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other (published in the Official Daily N.L. 358/1-358/6, 18, December 1986).

Experimental procedures

Blood was taken from the retro orbital sinus then animals were killed by decapitation under ether anaesthesia. Thyroid gland and the liver were rapidly excised and placed into Petri dishes containing ice-cold isolation medium. Tissues homogenates were used for analytical procedures. Lipid peroxides were assessed from the thyroid gland, hepatic tissue and blood. Catalase activity was determined from the thyroid gland and hepatic tissue. In serum, hydrogen donor ability was assessed too. Lipid peroxides were analysed measuring the production of thiobarbituric reactive substances (TBARS) according to the method Buege and Aust. Results were expressed in μmoles malondialdehyde (MDA) per protein milligram in the thyroid gland and hepatic tissue and in nanomoles MDA per sera millilitre (8). Catalase activity was determined using a classic permanganometric method. Results were expressed as catalasic number, which represents the amount of hydrogen peroxide,

expressed in mg, neutralized by 0.1 ml tissue homogenate. (6). Hydrogen donor ability (% inhibition) was assessed using Janasewska method (20). All results were expressed as the mean ± SEM (standard error of the mean). Data were analysed by Student "t" test. Differences were considered significant when $p<0.05$.

Results

In the thyroid gland, a high iodine diet determined a significant increase ($p<0.01$) in lipid peroxides concentration (24.81± 1.66 μmoles MDA/mg protein) as compared to control group (4.98± 2.32 μmoles MDA/mg protein)-fig.1, and a significant ($p<0.01$) higher **c**atalase activity (11.52 ± 2.66 N cat/mg protein) than animals fed a standard chow (2.23± 1.66 N cat/mg protein)-fig.2.

In the hepatic tissue, lipid peroxides level increased more then twice time due to the high iodine diet (38.54± 3.66 μmoles MDA/mg protein) as compared to animals from the control group (18.3± 1.66 μmoles MDA/mg protein)-fig.3. Also, catalase activity was found increased in animals fed a diet riche in iodide (40.58 ± 3.33 N cat/mg protein) as compared to control group (12.73± 2.33 N cat/mg protein)-fig.4.

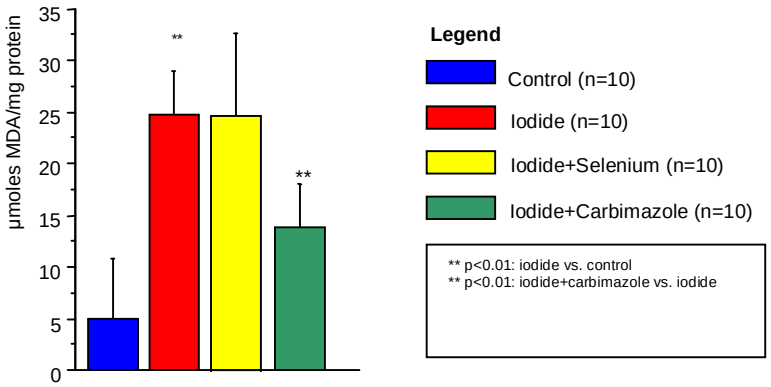

Fig. 1 – Lipid peroxides level in thyroid gland of Wistar rats

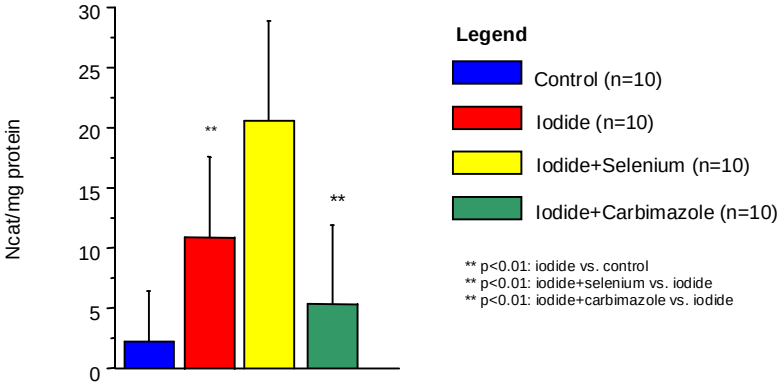

Fig. 2 – Catalase activity in thyroid gland of Wistar rats

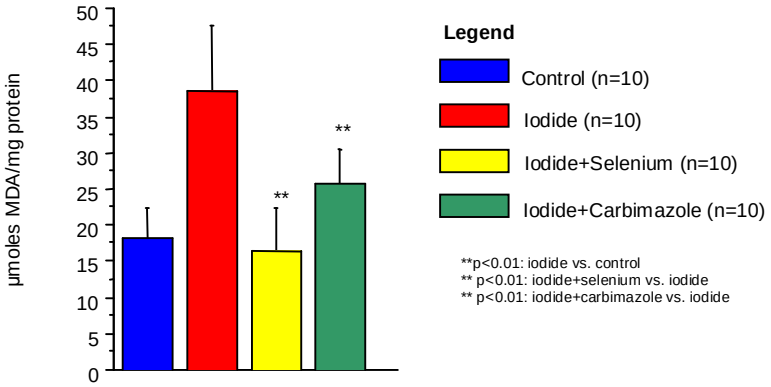

Fig. 3 – Lipid peroxides level in hepatic tissue of Wistar rats

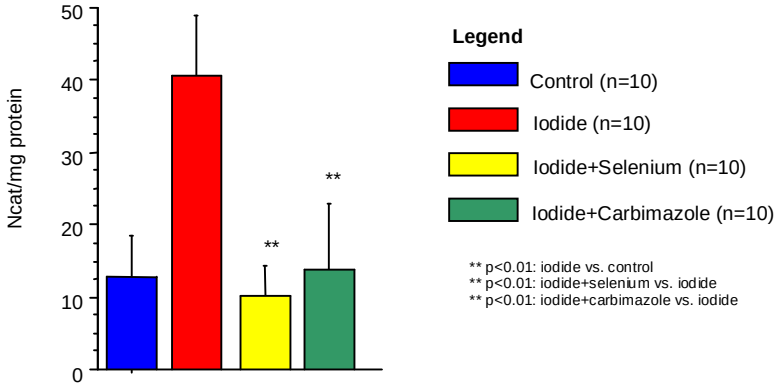

Fig. 4 – Catalase activity in hepatic tissue of Wistar rats

In the blood of the animals belonged to group 2, it was noticed a significant higher level of lipid peroxides (6.46± 1.33 nanomoles MDA/ml sera) compared to control group (3.6± 2.66 nanomoles MDA/ml sera)-fig. 5, and no significant decrease in antioxidant defence, appreciated through the hydrogen donor ability, in animals fed a high iodine diet (34.31± 3.66 %) as compared to control group (43.8± 2.66 %)-fig. 6.

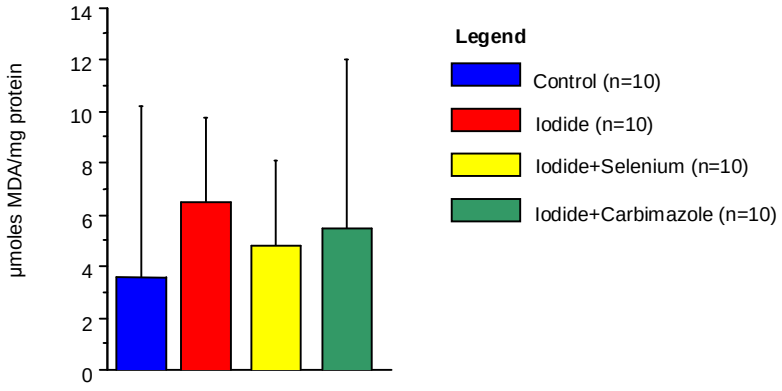

Fig. 5 – Lipid peroxides level in blood of Wistar rats

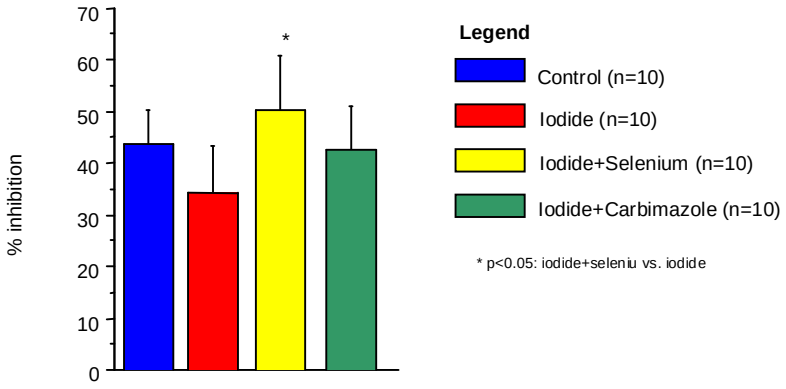

Fig. 6 – Hydrogen donor ability in blood of Wistar rats

Selenium administration to animals fed a high iodine diet had no measurable effect regarding lipid peroxides level in the thyroid gland comparing to animals from group 2-fig1. On the other hand, Selenium determined a significant increase ($p<0.01$) in catalase activity (20.58± 3.33 N cat/mg protein) as compared to iodide treated group, in the same tissue-fig. 2. In hepatic tissue, lipid peroxides level decreased more then twice time in iodide group co-treated with Selenium (16.53 ± 2.33 μmoles MDA/mg protein) as compared to group 2 –fig3, and catalase activity registered a significant diminution ($p<0.01$) when Selenium was added to iodide (10.08± 1.66 N cat/mg protein) comparing to animals fed a high iodide diet-fig.4. In serum of the animals from iodide group co-treated with Selenium it was noticed a decreased in lipid peroxides level (4.8 ± 1.33 nanomoles MDA/mg protein), as compared to iodide treated group, but not in a significant way–fig.5. The hydrogen donor ability of the serum increased significantly ($p<0.05$) when the animals fed a diet reach in iodide received Selenium (50.14 ±

3.33 %) as compared to animals fed only a high iodide diet-fig.6.

Carbimasole co-administration with iodide significantly diminished ($p<0.01$) the lipid peroxides level in the thyroid gland (13.91± 1.66 μmoles MDA/mg protein) fig. 1, and the catalase activity (5.28± 2.66 N cat/mg protein)-fig.2 as compared to iodide treated rats. Regarding hepatic tissue, the same alterations were noticed in lipid peroxides level that decreased to 25.73 ± 1.86 μmoles MDA/mg protein-fig.3 and in catalase activity (13.81±3.66 N cat/mg protein, fig. 4) due to Carbimasole co-administration with iodide as compared to high iodide treated rats. In serum, the lipid peroxides level did not significantly decreased (5.45± 2.66 nanomoles MDA/ml sera) and the hydrogen donor ability did not significantly increased (42.34 ± 3.33 %) when Carbimasole was added in the drinking water of the animals fed a high iodide diet-fig.5, 6.

Discussions

Thyroid hormones synthesis requires iodide, thyroglobulin and an oxidation system to oxidize iodide, to iodinate tyrosyl groups in thyroglobulin and couple them into iodothyronines. This oxidation system is constituted by a thyroperoxidase that oxidizes iodide in the presence of hydrogen peroxide and an ill-defined hydrogen peroxide generating system-using NADPH as coenzyme (10). Iodination mechanism consists in several steps,

having iodinium (I+) and hypoiodite (IO-) as intermediate products, extremely reactive (16).

The metabolism of iodide in the thyroid gland makes the most efficient use of an iodine supply that is often scarce and intermittent. But the thyroid also, has adaptation mechanisms that reduce iodide metabolism when the supply is abundant, thus avoiding thyrotoxicosis. These include direct inhibitory effect of iodide in the thyroid itself and inhibition by iodide of its own organification (Wolff-Chaikoff effect), its transport, thyroid hormones secretion, camp formation in response to thyroid-stimulating hormone (TSH) and several other metabolic steps (42).

Excess iodide displays different effects depending on the intake amount and on the thyroid status at that time. The physiologic requirement in adult is about 150-200 μg daily. In the acute inhibitory response (the well-known Wolff-Chaikoff effect), inhibition of its own organification is the fundamental phenomenon. Iodide oxidation requires thyroperoxidase and hydrogen peroxide generation that is stimulated by thyrotropin through Calcium-phosphatidyl-inositol cascade. Recent studies suggest that excess iodide inhibits Calcium-phosphatidyl-inositol cascade and hydrogen peroxide production doesn't occur. On the other hand, the acute inhibitory effect is temporary and escape occurs despite iodide continuous administration (16). Recent findings proposed that iodopeptides

are formed that temporarily inhibits thyroid peroxidase (TPO) mRNA and protein synthesis and, therefore, thyroglobulin iodinations. The Wolff-Chaikoff effect is an effective means of rejecting the large quantities of iodide and therefore preventing the thyroid from synthesizing large quantities of thyroid hormones. The acute Wolff-Chaikoff effect lasts for few days and then, through the so-called “escape" phenomenon, the organification of intrathyroidal iodide resumes and the normal synthesis of thyroxin and triiodothyronine returns. This is achieved by decreasing the intrathyroidal inorganic iodine concentration by down regulation of the sodium iodine symporter (NIS) and therefore permits the TPO-H202 system to resume normal activity (27).

In our experiment it was noticed that a high iodine diet induced oxidative stress in the thyroid gland, leading to an increased lipid peroxides level in this tissue. We propose, at least, two hypotheses that could explain the mechanism of the oxidative attack: excess iodide has an indirect effect, by altering the thyroid hormones synthesis, and a direct effect exerted on the thyroid gland. Regarding the first hypothesis, a high iodide diet determined an inhibition of thyroid hormones synthesis for a few days, explained through the acute Wolff-Chaikoff effect. After the escape from this phenomenon, a sudden increase in hydrogen peroxide production and thyroid hormones synthesis occurred. Hydrogen peroxide reacted with the polyunsaturated

acids from the follicular cell membrane leading to a high level of malondialdehyde in thyroid gland. Because the hydrogen peroxide represents the specific substrate for catalase, an antioxidant enzyme, an increase in hydrogen peroxide production led to an increase in catalase activity in order to neutralize this reactive oxygen specie, as it was noticed in our experiment. It is well known that the mitochondria contains specific receptors for the thyroid hormone and it is, also, the place were much of the reactive oxygen species production occurs, via oxidative phosphorilation (39). After the escape from the Wolff-Chaikoff effect, thyroid hormone stimulated hydrogen peroxide production acting on mitochondria. In this way the oxidative attack in the thyroid gland is emphasized. Furthermore, the thyroid hormone, having prooxidant effect on liver (12, 22), determined an increase in lipid peroxides level and in catalase activity in this tissue. Also, in the first days, when the Wolff-Chaikoff effect is present and thyroid hormone production is low, it could be possible a rise in thyroid-stimulating hormone (TSH) level, which directly stimulates the hydrogen peroxide production in the thyroid gland.

The second hypothesis that we proposed, regarding the direct oxidative effect of a high iodine diet on the thyroid gland, is sustained by other studies. As already proposed (28), the production of free radicals occurring after administration of a high dose of iodide could overwhelm the normal cellular

defences against free radicals (e.g. glutathione peroxidase, superoxiddismutase, catalase). This could be explain as follows: when iodide is in excess as compared to tyrosine residues, it reacts with the iodinium cation formed by iodide oxidation to give molecular iodine, which could in turn react with the peroxide to form oxygen-derived free radicals. These radicals would then induce not only lipid peroxidation and thus membrane damage, but also protein and even DNA alterations. All these events could be finally responsible for the cell necrosis by a mechanism dependent on the peroxidase activity and peroxide generation (24, 25).

The acute effects of increasing doses of sodium iodide were studied on human thyroid follicles isolated from normal paranodular tissue. The follicular function and morphology were strongly modified by high doses of iodide. The inhibition of iodide organification could be compared to the Wolff-Chaikoff effect, which was demonstrated in the rat for plasma iodide concentrations ranging between 10^{-6} and 10^{-5} M. In vitro, the inhibition of iodide organification and of thyroid hormones synthesis was obtained with10^{-4} M Na I (2), whereas inhibition of thyroid hormones secretion and of cAMP formation was demonstrated with 10^{-5} M Na I (38). At this concentration, in other in vitro studies, it was noticed a significant necrotic effect, which was further increased with 10^{-3} M Na I (10µ Ci/ml). These concentrations from 10^{-5} - 10^{-3} M

were thus, from 100-10,000 times higher than the normal iodine plasma levels estimated to be 10^{-7} M, in euthyroid human beings, with an optimal daily iodine intake of 100-200 µg (17). The necrotizing effect could result from the synthesis of an organic iodocompound. The nature of this compound is still unknown, but some iodinated derivatives of arachidonic acid mimic the action of iodide on thyroid growth (15, 33) and on cAMP production in vivo (33). A major thyroid iodolipid has been identified as an iodoaldehyde: 2-iodohexadecanal (32). However, the necroting effect could result from lipid peroxidation initiated by free radical attack. The ultrastructural changes induced by a high dose of iodide in human follicles: formation of blebs, membrane shedding, endoplasmic reticulum vesiculation, lipofuscin inclusions (28) are suggestive for a free radical attack as observed in many other cell types (7, 31).

Other in vitro studies showed that excess iodide displayed a dose dependent cytotoxicity, thyroid tissue specific. Thyroid cells treated with iodide excess underwent apoptosis as evidenced by morphological changes, plasma membrane phosphatidylserine exposure and DNA fragmentation. Also, it has been noticed that the apoptosis in the thyroid cells was mediated through a mechanism involving generation of free radicals (18, 40).

Administration of pharmacological quantities of iodide (180 mg, daily) for a few months enhanced the immunogenicity of

thyroglobulin reached in iodide resulting lymphocyte infiltration (Hashimoto thyroiditis) (11). It is also possible that in vivo, the increase in hydrogen peroxide synthesis induced by iodide in iodine-depleted thyroid may have a toxic role in the cell (40). A necrosis of follicular cells was already described after administration of iodide to iodine-deficient dogs but not to control dogs. A necrotizing effect of iodide was also described in iodine-deficient rats and mice. The toxicity of iodide was aggravated in cases of selenium deficiency, a circumstance in which defences against hydrogen peroxide are reduced due to a decreased activity of glutathione peroxidase (9).

The hypothesis that prooxidant effect of the iodide excess is mediated through an increase in thyroid hormone production is based on our results from the experimental group 4, in which Carbimasole, an inhibitor of thyroperoxidase, co-administrated with iodide determined a significant decrease in lipid peroxides level and in catalase activity in both thyroid gland and hepatic tissue. Also, this evidences suggests that ionic iodide is not directly toxic for the follicular cell, whereas its molecular form, produced by thyroperoxidase oxidation, mediates lipid peroxidation in the thyroid gland. Selenium administration in animals from group 3 had different effects on the lipid peroxides level. In the thyroid gland, Selenium, a well-known antioxidant in disorders caused by an excess in thyroid hormones (23), had almost no effect as compared to excess

iodide treated animals, despite of the fact that the thyroid gland contains the highest Selenium level in the whole body (10). Selenium prevented lipoperoxidation in hepatic tissue and in blood and increased antioxidant capacity in both thyroid gland and blood.

Besides the experimental data, there are some clinical evidences, which emphasized the toxic effect of excess iodide. Chronic administration of high doses of iodide produces in man three major thyroidal complications: iodide-induced thyrotoxicosis, iodide goiter and iodide induced thyroiditis (26). 150 micrograms iodine are daily required for thyroid hormone synthesis. Large quantities of iodide are presented in drugs, antiseptics, contrast media and food preservatives. Iodine induced hyperthyroidism is frequently observed in patients affected by euthyroid iodine deficient goitre when suddenly exposed to excess iodine. Possibly the presence of autonomous thyroid function permits the synthesis and release of excess quantities of thyroid hormones. The presence of thyroid autoimmunity in patients residing in iodine-insufficient areas who develop iodine-induced hyperthyroidism has not been unanimously observed. In iodine-sufficient areas, iodine-induced hyperthyroidism has been reported in euthyroid patients with previous thyroid diseases. Euthyroid patients previously treated with antithyroid drugs for Graves' disease are prone to develop iodine-induced hyperthyroidism. As well, excess iodine

in hyperthyroid Graves' disease patients may reduce the effectiveness of the antithyroid drugs. Occasionally iodine-induced hyperthyroidism has been observed in euthyroid patients with a previous episode of post-partum thyroiditis (34).

Also, drugs containing iodine can impair thyroid function. Amiodarone is a highly effective agent used for the treatment of various cardiac arrhythmias, ranging from paroxysmal atrial fibrillation to life-threatening ventricular tachyarrhythmias. However, the use of amiodarone is associated with several side effects, including photosensitivity, corneal micro deposits, pulmonary toxicity, hepatotoxicity, peripheral neuropathy, hyperthyroidism and hypothyroidism (19, 41). Amiodarone is a benzofuran derivative containing two atoms of iodine per molecule. This amounts to 37.5% of organic iodine by molecular weight, of which 10% is de-iodinated to yield free iodine. It has the potential to cause thyroid dysfunction because of this iodine-rich chemical structure. In the body, it is stored in adipose tissue, myocardium, liver, and lung and it has an elimination half-life of about 2-3 months (14). Hence, a normal daily maintenance dose of amiodarone (200-400 mg) generates about 6-12 mg of free iodine per day. This results in an iodine load that far exceeds the World Health Organisation's recommended optimal iodine intake of 0.15-0.3 mg per day. Amiodarone-induced thyrotoxicosis (AIT) occurs in 2-12% of patients on chronic amiodarone treatment. Some studies

indicate that the incidence varies according to the dietary iodine intake in the population; AIT prevails in areas with low iodine intake (e.g., central Europe) and is rather uncommon in iodine replete areas (e.g., North America and UK) (19, 30). However, in a Dutch study involving euthyroid subjects living in an area with a moderately sufficient intake of iodine, the incidence of AIT was twice that of Amiodarone-induced hypothyroidism (AIH) (36). Like hypothyroidism, there is no relation between the daily or cumulative dose of amiodarone and the incidence of thyrotoxicosis. In patients with an apparently normal thyroid gland, thyrotoxicosis results from glandular damage with consequent release of preformed thyroid hormones into the circulation (type II AIT). Studies *in vitro* had shown amiodarone to be cytotoxic to FRTL-5 thyroid cells; this effect was inhibited by treatment with dexamethasone or perchlorate. Similarly, moderate to severe follicular damage and disruption were demonstrated on histopathologic study of thyroid glands obtained from patients with type II AIT (4). The finding of markedly elevated serum levels of interleukin-6 (IL-6) in type II AIT patients further supports this destructive-cum-inflammatory process, whereas normal or slightly elevated levels of IL-6 are found in type I AIT patients (1).

Thyrotoxicosis in type II AIT patients is usually self-limiting, which may be explained by the dose-dependent cytotoxic effect of amiodarone. When intrathyroidal amiodarone concentrations

exceed a certain threshold, cell damage leads to thyrotoxicosis as the contents of the thyroid leak into the bloodstream. The intrathyroidal concentration of amiodarone would also decrease, allowing repair and the restoration of euthyroidism (37). Occasionally, hypothyroidism requiring levothyroxin substitution may result from extensive follicular damage (5).

Investigations with contrast media inevitably lead to the patient being exposed to large amounts of iodine. Under certain preconditions this gentails danger for the patient by causing either iodine-induced hyperthyroidism, which is difficult to treat, or even a thyrotoxic crisis. Patients with normal thyroid function and size have only minute changes of thyroid hormones and TSH within the normal range and are not at risk. Patients with unknown hyperthyroidism--independent of the etiological form--and patients with functional autonomy are at risk of exacerbation of pre-existing hyperthyroidism or development of iodine-induced hyperthyroidism. This development depends on two factors: the volume of autonomous tissue and the quantity of iodine exposure. Besides contrast media, other sources of iodine excess are possible, such as iodine-containing disinfectants, secretolytic agents, antiarrhythmics like amiodarone, eye drops and ointments, geriatrics, skin ointments, toothpaste etc. The development of hyperthyroidism can be prevented by combined treatment with antithyroid drugs and perchlorate in the case of preexisting

hyperthyroidism or the urgent clinical suspicion of thyrotoxicosis or with perchlorate alone, when the patients is euthyroid and does not have a large nodular goiter (20).

Iodine-induced hyperthyroidism can develop even in the presence of an otherwise normal gland. One of the less common sources of iodine is the tablet of seaweed, sold over the counter without prescription. It was reported the case of a 72-year-old female who developed clinical and laboratory evidence of hyperthyroidism while ingesting sea-kelp (Vitalia) tablets. Six months after stopping the tablets, the symptoms and laboratory evidence of hyperthyroidism had disappeared. No evidence of pre-existing thyroid disease was found (35).

Conclusions

Present experimental data allow us to conclude that a high iodide diet, more than three times over the daily physiological intake in man, administered for 10 days to animals with a normal thyroid function, induces alterations in prooxidant/antioxidant status of several target tissues of the thyroid hormones. We hypothesized that iodide excess has an indirect prooxidant effect through increasing thyroid hormones production but, also, a direct one, regarding its action in the thyroid gland. It is still questionable if iodide excess, during a short period of time, determined an enhancement in thyroid hormone production because the thyroid status was not

investigated in this experiment. Screening of the thyroid function and the assessment of prooxidant/antioxidant status in subjects treated with drugs containing iodine and after investigations with contrast media are recommended.

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