PHYTOTHERAPEUTICAL ALTERNATIVES IN PREVENTING OXIDATIVE STRESS DISORDERS DUE TO HYPERTHYROIDISM- EXPERIMENTAL DATA

Adela Elena JOANTA[1]*, Viorel MICLĂUŞ[2], Stelian Vasile SARLEA MERCA[1], Vasile RUS[2], Carmen SOCACIU[2], Nicoleta DECEA[1], Remus MOLDOVAN[1]
[1]University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

*** Correspondence:** Adela Elena JOANTA, Physiology Department, University of Medicine andPharmacy, Cluj-Napoca, Romania, e-mail: adelaelena@yahoo.com
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ABSTRACT. Increased oxidative stress has been described previously in models of hyperthyroidism and in human subjects with Basedow disease. In this study, the influence of a diet enriched in soy on reactive oxygen species production and on antioxidant defence in hepatic tissue was explored. Soy products are attractive because of their beneficial effects on chronic diseases such as cardiovascular diseases, atherosclerosis, and type II diabetes

Keywords: oxidative stress, thyroid hormones, liver, histological alterations

INTRODUCTION

Thyroid disease is common, affecting around 2% of women and 0.2% of men in the world. Our understanding of the effects of thyroid hormones under physiological circumstances, as well as in pathological conditions, has increased dramatically during the last two centuries and it has become clear that overt thyroid dysfunction is associated with significant morbidity and mortality. Increased oxidative stress has been described previously in models of hyperthyroidism and in human subjects with Basedow disease. In this study, the influence of a diet enriched in soy on reactive oxygen species production and on antioxidant defence in hepatic tissue was explored.

MATERIAL AND METHODS

Animals and housing conditions

White, male, Wistar rats, 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.

Experimental groups

Three groups of animals, each group consisting in 10 rats, were investigated, as follows:

Group I: untreated animals (control) fed a standard diet.

Group II: L-Thyroxin injected rats L-thyroxin (10µg/100 g body weight/day), intraperitoneal administered, fed a standard diet.

Group III: L-Thyroxin injected rats (10µg/100 g body weight/day) fed a standard diet enriched in *soy*: 500 mg/100 g body mass/day.

Distilled water was available to all animals *ad libitum*.

Experiments were performed for 14 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) and according to *The UFAW Handbook on the Care and Management of Laboratory Animals* published by Blackwell Science (www.tiny.cc/9y7Sa).

Experimental procedures

Animals were sacrificed by decapitation under ether anesthesia in the 8 th day of the experiment. The liver was quickly excised and placed into Petri dishes containing ice-cold isolation medium.

Analytical procedures

Level of lipid peroxides was evaluated fluorometrically as thiobarbituric reactive substances (TBARS), according to the Satoh method (Satoh *et al.* 1978). Fluorescent reaction products were estimated spectrofluorometrically at 515 nm excitation and 553 nm emissions using a Kontron SFM25 spectrofluorometer. Results were expressed as µmoles malondialdehyde (MDA) per milligram of protein (Simon *et al.* 1990).

Superoxiddismutase (SOD) was evaluated using the Matkovics method with adrenaline. SOD has the capacity to prevent adrenaline oxidation to adrenocrom. One unites of SOD activity is the amount of enzyme which exhibits 50% inhibition of adrenaline oxidation to adrenocrom (Ciurdaru et al., 2001).

Histological study of the liver was performed using TRICROM MASON staining.

Statistical analysis

Data are reported as mean ± SEM (standard error of the mean). Data were analyzed by Student "t" test. Statistical significance was defined as $P<0.005$.

RESULTS AND DISCUSSION

Acceleration of the basal metabolic rate and the energy metabolism of tissues in several mammalian species represent one of the major functions of the thyroid hormones (Williams et al., 1998). The liver is the place where tetra-iodothyronine provides the metabolic thyroid hormone: tri-iodothyronine through deiodination by deiodase type I (Nagaoki et al., *2000*).

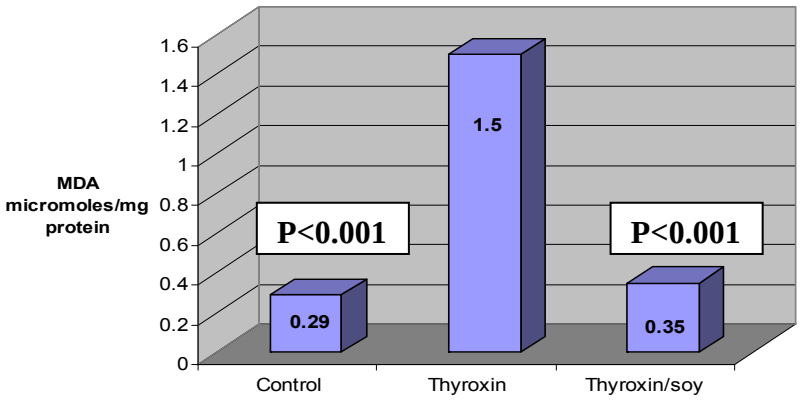
Oxidative stress is a misbalance between reactive oxygen species production and antioxidant defence. These free radicals of the oxygen are very harmful because they have an unpaired electron. For this reason, they have to steel another electron from other molecules, generating chain reactions (Das et al., 2001).

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 (Joanta et al., 2005, 2006). 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 (Corvilain et al., *2000*).

In the liver, T_3-induced acceleration of 0_2 consumption leads to elevation in superoxide radical and hydrogen peroxide generation at mitochondrial, microsomal, or cytosolic subcellular sites, as well as nitric oxide by nitric oxide synthase. T_3 also leads to hyperplasia and hypertrophy of Kupffer cells, with the resulting enhancement in the respiratory burst activity (Videla, 2000). This T_3-induced free radical activity diminishes the antioxidant defenses leading to oxidative stress (Fernández & Videla, 1996), a condition that may lead to a variety of responses depending on the cell type, the level of pro-oxidants achieved, and the duration of the exposure.

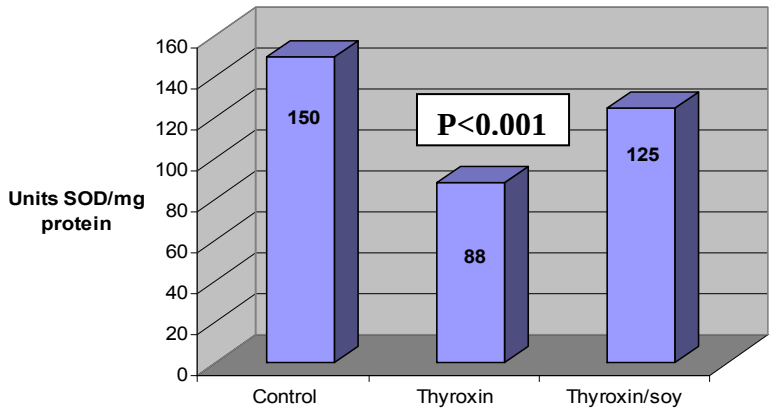
In our study, the oxidative stress in hepatic tissue was assessed by measuring the level of lipid peroxides, using as standard: malondialdehyde (MDA). In the hepatic tissue of the thyroxin treated rats, lipid peroxides were found significantly increased ($P<0.001$) as compared to control values. Administration of both thyroxin and soy led to a significant reduction ($P<0.001$) of the lipid peroxides as compared to control values (Graphic.1).

Graphic 1. Lipid peroxides in hepatic tissue



Regarding the antioxidant defense, in our study it was noticed a significant decrease in liver superoxiddismutase activity due to thyroxin administration, as compared to control. This evidence should be explained through the fact that thyroid hormones increase superoxide anion production in hepatic mitochondria and superoxiddismutase neutralized this extremely reactive free radical. On the other hand, the diet enriched in soy improved the antioxidant defense of the hepatic tissue, showing a significant high level of superoxiddismutase as compared to thyroxin treated rats (Graphic. 2.)

Graphic 2. SOD activity in hepatic tissue



The soybean has been implicated in diet-induced goiter by many studies. The extensive consumption of soy products in infant formulas and in vegetarian diets makes it essential to define the goitrogenic potential. It was observed that an acidic methanolic extract of soybeans contains compounds that inhibit thyroid peroxidase-catalyzed reactions essential to thyroid hormone synthesis. Because inhibition of thyroid hormone synthesis can induce goiter and thyroid neoplasia in rodents, delineation of anti-thyroid mechanisms for soy isoflavones may be important for extrapolating goitrogenic hazards identified in chronic rodent bioassays to humans consuming soy products (Divi et al., 1997).

In our experiment, biochemical determinations regarding the oxidative stress induced by an excess in thyroid hormone and the soy antioxidant effect were completed by the histological study of the liver. In the thyroxin treated rats, it was noticed a lot of apoptotic cells spreading all over the hepatic lobule surface. At the lobule periphery, there are a lot of damaged hepatocytes with different sort of injuries like necrosis and Remak strains disorganization (Fig 1). Our results could be explained using the evidences found by Malik et al., 2003: triiodothyronine is of particular interest as a primary mitogen, transcriptional factor which enhances hepatocytes proliferation, in view of its potential exploitation as a pharmaceutically available hormone that has been critically appraised in humans. On the other hand, the histological disorders induced by thyroid hormone administration are the results of oxidative stress which damages lipids, proteins and nucleic acids. ROS species occur at low levels under normal conditions, however, persistent production of large amounts of them may induce significant oxidation of biomolecules, persistent changes in gene expression and signal transduction, thus leading to cell death (Droge, 2002; Martindale & Holbrook, 2002).

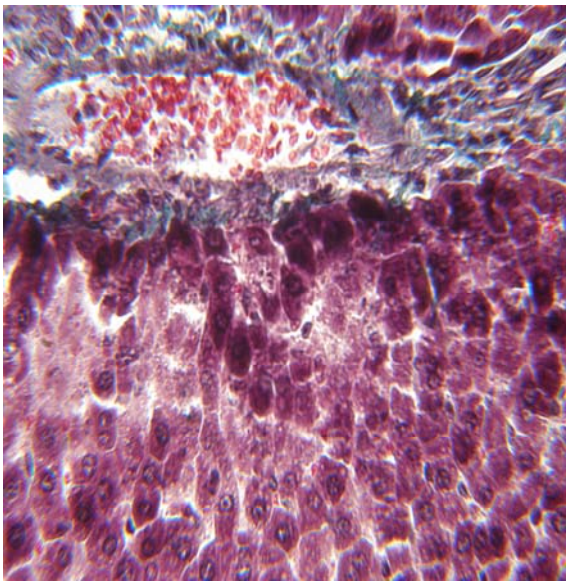


Fig. 1 Liver injuries in thyroxin treated rats (TRICROM MASON staining)

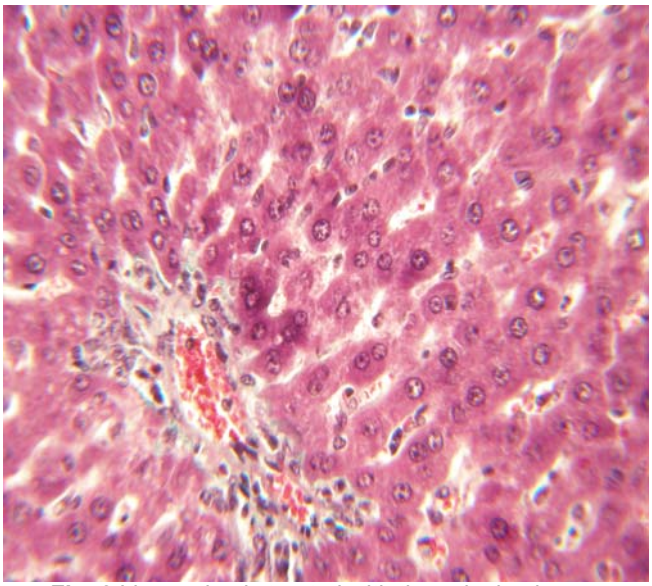


Fig. 2 Liver animals treated with thyroxin, having an enriched soy diet

Regarding the animals treated with thyroxin, having an enriched soy diet, it was noticed just a few damaged hepatocytes dispersed on the lobule surface, but with no necrosis and Remak strains disorganization (Fig. 2).

CONCLUSIONS

Our study demonstrates that thyroxin administration caused liver oxidative stress and hepatocytes injuries. The enriched soy diet attenuated the negative effects of thyroid hormones. This evidence allows us to recommend the screening of hepatic function and oxidative stress markers assessment in patients with thyroid disorders. Soy association to classic treatment of the hyperthyroidism could be recommended with prudence.

REFERENCES

Ciurdaru V, Andrei S, Pintea A et al, Biochimie medicala veterinara, Metode si tehnici de laborator, Academic Press, Cluj-Napoca, pp. 84- 89, 2001

Corvilain B., Collyn L. & van Sande J, Stimulation by iodide of H_2O_2 generation in thyroid slices from several species. The Journal of clinical endocrinology and metabolism, 278, pp. 692-699, 2000

Das K, Chainy G B: Modulation of rat liver mitochondrial antioxidant defence system by thyroid hormone. Biochimica et Biophysica acta- Molecular Basis of Disease, 1537: pp. 4439- 4447, 2001

Dróge W, Free radicals in the physiological control of cell function. Physiol Rev, 82: pp. 47-95, 2002

Fernandez V, Videla LA, Biochemical aspects of cellular antioxidant systems. Biol Res 29: pp. 177-182, 1996

Divi RL, Chang HC, Doerge DR, Anti-thyroid isoflavones from soybean: isolation, characterization, and mechanisms of action. Biochem Pharmacol., 54(10): pp. 1087-96, 1994

Joanta A., Clichici S, Filip A, Andrei S, Changes in prooxidant/antioxidant status of hyperthyroid rats treated with Selenium. Central European Journal of Occupational and Environmental Medicine, 11: pp. 123-129, 2005

Joanta A E, Filip A, Clichici S, Andrei S, Daicoviciu D, Iodide excess exerts oxidative stress in some target tissues of the thyroid hormones. Acta Physiologica Hungarica, 93: pp. 347-359, 2006

Martindale JL, Holbrook NJ, Cellular responses to oxidative stress: signaling for suicide and survival. J Cell Physiol 192: pp. 1-15, 2002

Malik R, Mellor N, Selden C, Hodgson A, Triiodothyronine enhances the regenerative capacity of the liver following partial hepatectomy. Hepatology, 1: pp. 79-86, 2003

Nagaoki T, Kaptein E & Berry MJ., Structure- Activity Relationships for Thyroid Hormones Deiodination by Mammalian Type I Iodothyronine Deiodinases. Endocrinology, 138: pp. 213- 219, 2000

Satoh K., Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica chimica acta, 90: pp. 37- 43, 1978

Simon BC, Cunningham LD, Cohen RA., Oxidized low density lipoproteins cause contraction and inhibit endothelium-dependent relaxation in the pig coronary artery. The journal of clinical investigation, 87: pp. 75–79, 1990

Videla, LA, Energy metabolism, thyroid calorigenesis, and oxidative stress: functional and cytotoxic consequences. Redox Rep, 5: pp. 265-275, 2000

Williams, Textbook of Endocrinology: edn 9, pp. 435-456.Eds WB Sounders Company, Philadelphia, 1998