This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Thyroid gland: intrathyroidal metabolism, oxidative stress, and free radicals

by

Oksana Zagorodna

Biosciences Program
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2005

5. May 2005

Abbreviations

APE/Ref-1	Apurinic apyrimidinic endonuclease/redox factor 1
CD	Colloid droplets
EPR	Electron paramagnetic resonance
GC	Guanylyl cyclase
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LDL	Low density lipoprotein
NIS	Sodium iodide symporter
NOS	Nitric oxide species
ROS	Reactive oxygen species
T3	Thyroxine
T4	Triiodothyronine
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TTF-1, 2	Thyroid transcription factor 1, 2

Outline

Abstract

Thyroid problems can be characterized as the production of either too much or too little of the hormone. The main risk factors for thyroid diseases are style of life, diet, age, genetics of the family, and radiation exposure. Various types of treatments exist to address thyroid problems, but there is no cure. The main function of the thyroid gland is to make its hormones: thyroxine, triiodothyronine require iodine for complete development. Iodine accumulation is performed by key players in transporting and concentrating iodide: a sodium iodide symporter and thyroglobulin. There has been evidence that NIS promoter is regulated by apurinic apyrimidinic endonuclease/redox factor 1 (APE/Ref-1) and Pax-8 transcription factor that are known to be involved in redox activity. Similarly, in thyroglobulin, TTF-1 was demonstrated to respond to redox regulation. Experiments are proposed to verify redox regulation dependence. Another important player in thyroid gland iodide uptake is the thyroperoxidase enzyme; it is also proposed to be redox regulated. Reactive oxygen species (hydrogen peroxide) and reactive nitrogen species (nitric oxide) may cause abnormalities in thyroid diseases. Possible experiments are proposed for further explorations. Formation of thyroid diseases, such as goiter condition and autoimmune disease, is accompanied by oxidative stress. Radioiodine therapy can treat thyroid disorders, but at the same time can cause cancer. The molecule melatonin is proposed to prevent thyroid diseases. Experiments are proposed for verifying the hypothesis.

1. Introduction

Approximately 200 million people in the world have some form of thyroid disease. Thyroid problems can be characterized as the production of either too much or too little of the hormone that regulates the body's metabolism. Producing too much hormone is known as hyperthyroidism while producing too little is called hypothyroidism. During thyroiditis, an autoimmune destruction of thyroid gland takes place. In spite of its relatively low incidence, thyroid cancer is a prominent concern in the endocrinology field because it accounts for the majority of deaths from endocrine cancers [1].

The main risk factors for thyroid diseases are style of life, diet, age, genetics of the family, and radiation exposure. Some of these factors can be controlled by a human and some are independent of human control. Individuals with several risk factors can live and never develop the disease while persons without known risk factors will become ill. There have been various types of treatments created to address thyroid problems, but none of them has been able to become a cure. Untreated thyroid disease can cause general muscular weakness, hypersensitivity, restlessness, weight loss, and poor memory[1]. Thus, it is essential to understand the mechanisms of thyroid diseases in order to find efficient treatment, cure, and prevention of these diseases.

Free radicals and reactive oxygen species are known to interact with cell organelles and mimic biological responses through redox regulation. The idea that free radicals and oxidative stress can trigger thyroid diseases has not been fully explored. This paper will focus on intrathyroidal metabolism and redox regulation in thyroid diseases; the role of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) in thyroid gland will be explored; and oxidative stress in thyroid diseases will be examined. Aspects of the radioiodine therapy will be reviewed.

[1] Thyroid Cancer Overview. From www.oncology.com ; visited on 04/23/05

2. Insight into thyroid gland biology

Thyroid gland is a butterfly-shaped endocrine gland located in the front part of the neck on both sides of the trachea windpipe. In healthy individuals thyroid gland cannot be seen. It consists of two parts (lobes) that are connected by the isthmus (**Figure. 1**).

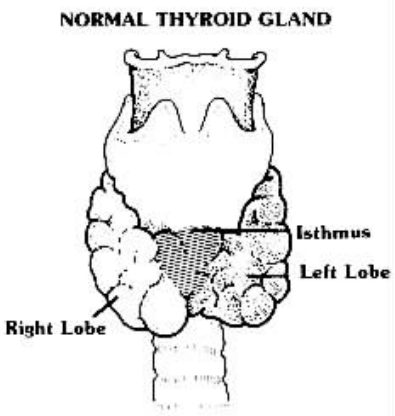

Figure. 1. Thyroid Gland[2].

The gland is capable of accumulating and efficient transferring iodide into its organic bound form. Regularly, the concentration of absorbed iodide in thyroid gland is at least twenty five times greater than in the cytoplasm[2].

There are mainly two types of cells in the thyroid gland: follicle cells and C-cells. The main function of thyroid gland is to make its hormones. Under normal conditions, the thyroid gland secrets three main hormones: thyroxine (T3) and triiodothyronine (T4) that are made and stored in the follicle cells; and calcitonin which is produced in C-cells (**Figure 2**)[3].

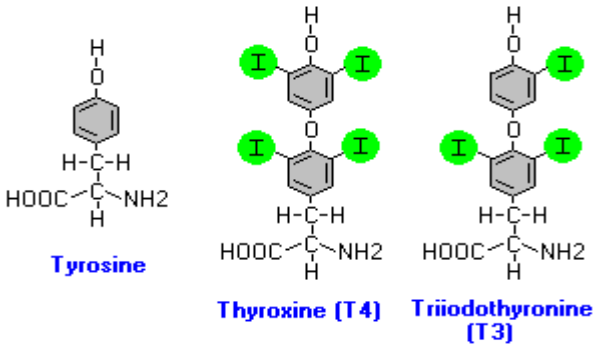

Figure 2. Synthesized thyroid hormones[3].

Thyroxine contains iodide* which is important for the body's normal growth and metabolism. This hormone helps in controlling body size, regulating growth, differentiation, and/or specialization of the tissues. Triiodothyronine has similar functions to thyroxine.

---

[2] Thyroid Gland. From http://www.carolinaent.org/broch_thyroid.asp ; visited on 04/20/05
* Iodide – ionic form; iodine – atomic form.
[3] About the Thyroid Gland. From www.cancerindex.org ; visited on 4/20/05

Calcitonin causes a decrease in the concentration of calcium in the blood. Calcitonin works with secretions from the parathyroid glands to maintain the balance of calcium necessary for the body functioning[3]. The thyroid gland is an organ with high vascularization [2]. It contains a broad network of blood capillaries associated with the thyroid follicles. The abundance in blood supply allows for efficient exposure of all thyroid cells to iodide. This exposure is very important in forming thyroid hormones. When the uptake of iodide is performed by thyroid cells, oxidation and coupling to thyroglobulin occurs. The oxidation is performed by the heme-containing enzyme thyroid peroxidase (TPO). The process of oxidation is held in a series of reactions with the involvement of hydrogen peroxide [3,4,5].

3. Intrathyroidal iodine metabolism

3.1. Overview of iodine metabolism.

Figure 3 summarizes the processes in the thyroid gland: iodination of thyroglobulin, formation and release of hormones, and the dynamics of these processes. General aspects of thyroid hormone production have been known for some time; however, new details are discovered regularly, which allows for a better understanding of why the system fails when it does.

Coming from the blood stream and before entering the thyroid cell, iodide concentrates at the basal cell membrane. This accumulation is performed by a sodium iodide symporter (NIS) [6]. Through the electrochemical gradient, NIS transports iodide to the apical cell surface. At the same time, thyroglobulin (Tg) polypeptide chain is synthesized on the surface of the endoplasmic reticulum to be transclocated into its lumen. After a series of conformational changes, Tg forms a stable dimer and moves to the Golgi apparatus to undergo its sulfation and completion of carbohydrate units. The apical vessels transport a nascent Tg protein to their surface. At the apical surface, Tg iodination and iodotyrosyl

precursor coupling leads to T3 and T4 formation. Micropinocytosis retrieves Tg and passes it through the endosomes and lysosomes to initiate proteolysis and hormone release. If Tg is retrieved by colloid droplets (CD), the endosomal step is skipped. T3 and T4 enter the blood stream, and the released iodide is re-circulated (**Figure 3**) [7,8].

The disruption of either of the steps for hormone synthesis and secretion will lead to thyroid diseases, such as thyroid cancer, hyperthyroidism, and hypothyroidism.

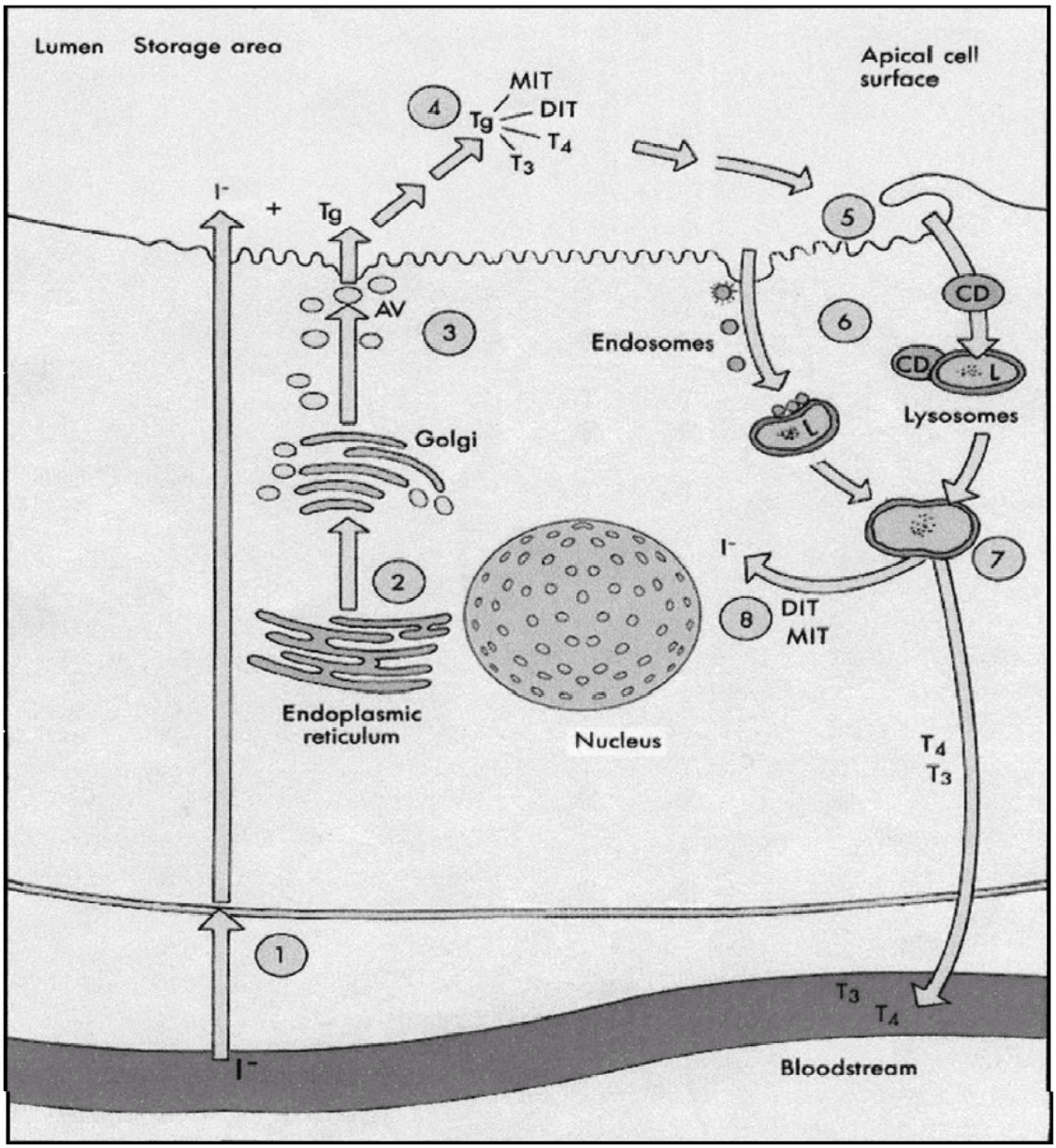

Figure 3. Synthesis and secretion of the hormones in thyroid gland [8].

3.2. Sodium iodide symporter (NIS) activity.

The NIS protein is the key player in transporting and concentrating iodide in thyroid gland from the blood stream. It is an integral membrane protein located in the basolateral membrane of thyroid epithelial cells. As its name indicates, the sodium-iodide symporter transports both Na^+ and I^- ions from extracellular fluid (i.e. blood) into the thyroid cell. Energy for this process is provided by the electrochemical gradient of sodium across the cell membrane; the low intracellular concentration of sodium is maintained by sodium pumps [9].

It was shown that NIS is capable of performing iodide uptake in normal and well differentiated neoplastic thyroid cells [3]. Taking to consideration the importance of iodide uptake in thyroid function, it is only logical to conclude that NIS abnormalities that prevent its regular function – iodide uptake – will trigger thyroid diseases. One of the reasons of NIS misfunction is an inactivating mutation in the gene that encodes the protein [9]. As a result, iodide uptake is lower than required by the organism, which leads to hypothyroidism.

3.2.1. Transcriptional regulation of NIS and redox regulation.

There has been evidence that NIS promoter is controlled by several transcriptional regulators, and Pax-8 is the most important [10]. Pax-8, in its turn, is regulated by apurinic apyrimidinic endonuclease/redox factor 1 (APE/Ref-1) that is known to be involved in redox activity [mentioned in 6]. Thus, APE/Ref-1 must be important in activating NIS pathway, and so is redox regulation [6]. In fact, there is evidence that a number of thyroid cancer cells have a defective NIS [4], which can be the reason of unsuccessful radioiodine therapy: due to a lack of iodide uptake ability, thyroid cancer cells do not respond to radioiodine treatment. Repairing NIS apparatus will restore the ability of cancer cells to be eligible for radioiodine therapy. The approaches currently explored for repairing NIS include: using viral vectors to deliver NIS-activated gene to thyroid tumor cells; and stimulating NIS expression through manipulating transcriptional regulators (Pax-8). The latter approach was based on

transfecting APE/Ref-1 and/or Pax-8 into thyroid cancer cells [6]. Perhaps another method can be suggested for activating NIS pathway in cancer cells. There is evidence that APE/Ref-1 stimulates the activity of NIS promoter, both by itself and through influencing Pax-8 activity [6]. It was also shown that reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), can activate APE/Ref-1 in human gastric epithelial cells [6a]. Based on this evidence, one can hypothesize that APE/Ref-1 in thyroid cells can be overexpressed by H_2O_2.

3.2.2. Proposed experiment 1: repairing activation of NIS pathway.

Analogous to the paper written by Ding et al., *Helicobacter pylori* could be used as the generator of ROS and the modulator of APE/Ref-1 activity. Examining the effect from bacterial strain, oxidative stress, and antioxidants on APE/Ref-1 expression can be performed with Western blot assay. Since oxidative stress was shown to increase APE/Ref-1 activity, it would be predicted that excessive H_2O_2 would cause the same effect of increased activity. Once activated, APE/Ref-1 will restore NIS pathway which will help with preventing hypothyroidism in normal tissues, or will enable NIS-deficient cells to respond to radioiodide therapy.

3.3. Thyroglobulin activity

Thyroglobulin is an important player in iodide uptake. Tyrosyls in the protein chains serve as a substrate for iodine attachment when producing thyroid hormones. The synthesis of Tg occurs in the endoplasmic reticulum and is regulated by transcription factors, such as thyroid transcription factor-1 (TTF-1), thyroid transcription factor-2 (TTF-2), and Pax-8 [4,6]. In order for the transcription to occur, transcription factors are required to bind to DNA. Similarly to the APE/Ref-1 and Pax-8 regulation in NIS pathway, TTF-1 was demonstrated to respond to redox regulation [3].

3.3.1. Regulation of Tg via redox regulation of TTF-1

There has been a large number of transcription factors found that are inactivated *in vitro* by oxidation of their cysteine residues and often by alkylating reagents. Primarily, the targets of the redox regulation are cysteine residues that are either directly involved in recognizing DNA or located close to it [3]. This does not seem to be the case for TTF-1 in which alkylation protects against oxidative damage rather than inactivating TTF-1.

TTF-1 is a homeodomain-containing protein that performs the DNA-binding activity. It was shown to be implicated in the transcription processes of thyroid gland. Arnone *et al*. demonstrated that oxidation of the homeodomain-containing protein TTF-1 causes the formation of both inter- and intramolecular disulfide bonds. These bonds lead to decrease in DNA-binding activity of TTF-1. Out of four cysteine residues that are present in the TTF-1 structure, only two undergo redox modification. The mediation is direct when the residues affected by oxidation are cysteines located in the homeodomain of the DNA protein. The indirect redox mediation takes place when no cysteines are present in the protein homeodomain [mentioned in 3]. Interestingly, neither of the cysteine residues is located inside the TTF-1 homeodomain. Even more, when the two residues that undergo redox regulation are replaced with serine or their sulfhydryl groups are alkylated, DNA binding activity of TTF-1 is not affected. These facts indicated that cysteine residues involved in redox do not have a direct involvement in DNA recognition. Nonetheless, their oxidation to cysteine reduces DNA-binding ability of TTF-1 via the formation of inter- or intrachain disulfide bonds. Thus, redox regulation appears to take an indirect route for regulating Tg. When cysteines are replaced with serines, TTF-1 is transformed to an oxidation-insensitive DNA-binding protein [3]. All these data have been shown *in vitro*, and the next step would be to check them *in vivo*.

3.3.2. Proposed experiment 2: repairing activation of Tg.

It has been shown that failure to uptake iodide leads to thyroid diseases, such as hypothyroidism, Goiter's disease, and thyroid cancer [5]. Thyroglobin pathway failure could very well be at fault for these diseases [4]. If examining the reexpression of Tg hormone shows poor activity, one can propose an experiment where the binding activity of TTF-1 is examined using glutathione modulation and measuring DNA binding activity of TTF-1 in a gel shift assay. Hypothetically, thyroid tissues, similarly to cell cultures, would show a decrease of DNA binding activity in TTF-1 under oxidative stress. The method used for attempting to determine TTF-1 binding activity could be gel mobility shift assay where the reduction of TTF-1 binding activity to DNA would be reflected by the appearance of a faster migrating protein-DNA complex due to oxidized sulfhydryl groups that form disulfides. To create oxidative environment, an oxidizing agent, such as glutathione (oxidized form, GSSG) or diamide, can be added to the assay.

3.4. Thyroperoxidase activity.

Thyroperoxidase (TPO) is an enzyme that is synthesized on polysomes and glycosylated in the Golgi apparatus. Its proper folding is extremely important for catalyzing iodide oxidation in the apical membrane, which is important for hormone production. TPO couples oxidized iodide to thyroglobulin after the uptake of iodide by thyrocytes. As a result of further TPO catalysis, T3 and T4 are formed by coupling iodotyrosines (**Figure 4**)[4].

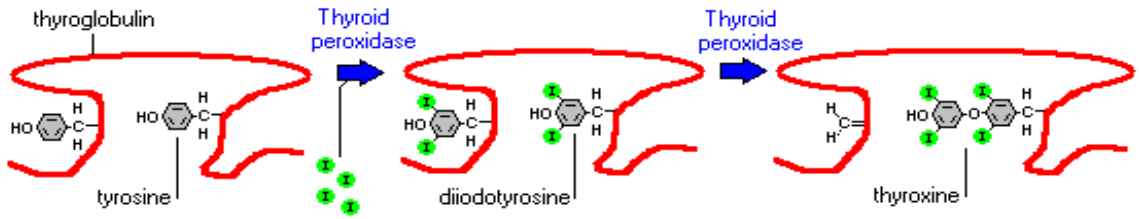

Figure 4. Thyroperoxidase catalyzes iodination of tyrosines on thyroglobulin and synthesis of thyroxine or triiodothyronine from two iodotyrosines[4].

[4] About the Thyroid Gland. From www.cancerindex.org ; visited on 4/20/05

TPO gene expression is mediated by the same thyroid specific factors as in NIS and Tg expression – TTF-1, TTF-2, and Pax-8 [5]. Thus, from the previously stated data on redox regulation of thyroid specific factors, one can conclude that TPO is redox dependent through its thyroid specific factors. The methods to experimentally confirm the hypothesis would be similar to proposed experiments for NIS and Tg cases – adding the oxidizing agent and determining whether there is an effect. Additionally, TPO is a heme-dependent enzyme: iron depletion impairs its function of biosynthesizing thyroid hormones [11]. On the other hand, iron can be a source of free radicals in the thyroid. One of the most basic reactions of oxidative stress that consumes iron is the Fenton reaction:

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + HO^-$

Zimmerman *et al*. showed that iron supplementation improves iodine supplementation [11]. Thus, TPO activity can be modified with oxidative stress. Recently, it has been shown that H_2O_2 positively affects TPO activity by stabilizing its binding with heme and decreasing TPO level [12]. Perhaps heme-linked histidine residue of TPO potentially creates a substrate for the Fenton reaction.

4. Role of free radicals, ROS, and RNS in thyroid gland

4.1. H_2O_2 and its role in the thyroid gland

Hydrogen peroxide is an important factor for thyroid hormone synthesis – it acts as an acceptor of electrons that are generated during oxidative reactions of the hormone synthesis. The production of H_2O_2 is performed in the thyroid gland by the NADPH oxidase system in the apical membrane (see **Figure 1**) of the thyroid cell [13]. Raspe and his group studied the role of thyroid-stimulating hormone (TSH, secreted by anterior pituitary gland) in the production of thyroid hormones [14]. In their study, Raspe *et al*. observed that TSH exerted toxicity in the cell through oxidative damage to DNA, lipids, and proteins. Using dog thyroid cells, they determined that TSH increased H_2O_2 levels through the cyclic adenosine 3',5'-

monophosphate (cAMP) cascade. The meaning of this discovery is that increased H_2O_2 levels enhance free radicals (such as HO•), which is present in hypothyroidism and in chronic iodine deficiency (goiter) in the thyroid gland. Another study showed that H_2O_2 is involved in Chaikoff's disease where iodide, when in excessive amounts, can inhibit its own organification. In the study, thyrotropin and carbamylcholine were used to stimulate H_2O_2 generation. Regardless of activation, H_2O_2 levels were significantly decreased by iodide due to the fact that thyroid slices were from animals with Chaikoff's disease [15].

4.1.1. H_2O_2 and cell death.

Riou *et al*. found that H_2O_2 influences thyrocyte cell death: even when used in small concentrations (50 μM), H_2O_2 caused signs of apoptosis in cells. A greater cell death effect was observed with increasing ROS concentration. When the dose of H_2O_2 increased to 500 uM, necrosis or "accidental" cell death were observed [16]. One may conclude from the evidence that ROS may participate in the thyroid cell death processes both in physiological and pathological conditions.

Vitale *et al*., using iodide, confirmed that ROS are involved in apoptotic processes. In the study, thyroid cells treated with iodide excess underwent apoptosis, which was verified by morphological changes, plasma membrane phosphatidylserine exposure, and DNA fragmentation. When the protein synthesis was inhibited, apoptosis was unaffected; however, inhibition of peroxidase enzymatic activity by propylthiouracil completely blocked iodide cytotoxicity. Western blot analysis demonstrated that p53, Bcl-2, Bcl-XL, and Bax protein expression did not change when cells were treated with iodide. The results of this experiment allowed one to exclude the possibility of these proteins contributing to apoptosis. More than likely, excess molecular iodide induces apoptosis in thyroid cells through a mechanism involving generation of free radicals. This type of apoptosis does not depend on p53, does not

require protein synthesis, and is not induced by modulation of Bcl-2, Bcl-XL, or Bax protein expression [17].

4.2. Reactive nitrogen species and thyroid hormone synthesis.

Nitric oxide (NO•), an endogenous free radical, is catalyzed by nitric oxide synthase (NOS). There are three distinct isoforms of NOS identified: type I (brain NOS), type II (inducible NOS), and type III (endothelial NOS). All forms were found in the thyroid gland [2].

4.2.1. Contradictory results of RNS influence in the thyroid gland

There is evidence that NO• helps in inhibiting TSH-stimulated iodide uptake via stimulating guanylyl cyclase (GC) activity and the production of cGMP (cGMP inhibits iodide uptake) [mentioned in 2]. On the other hand, Millatt *et al*. demonstrated that NO• stimulates TPO activity in primary human thyrocytes [2]. Thus, NO• is capable of regulating TSH in various ways, and the course of action will depend on the other factors of regulation. An interesting observation is that the NO• effect on TPO activity was immediate, while the inhibition of TSH took place after incubating for 2-4 hours [2]. Does this mean that NO• effect in thyroid gland depends on the length of its exposure? Comparing the results with different incubation periods still would not explain why the *stimulation* of TPO occurs instantaneously while the *inhibition* of TSH is observed even after a several-hour incubation time. Perhaps different forms of NOS are the cause of such variety of effects. Using immunohistochemistry, it has been determined that type III NOS is co-localized with TPO in the apical layer of thyroid tissue [18].

4.2.2. Proposed experiment 3: investigating the reasons of contradictory NO• effect in thyroid gland .

One can hypothesize that changes in the amount of NO• will reflect on TPO activity immediately. The experiments using various NO• levels can help in determining the

hypothesis. Another interesting aspect to investigate would be the inhibitory effect of NO• on TSH levels. The predictions would be: first, a different NOS type (other than type III) is involved in the mechanism of TSH regulation compared to TPO regulation; second, NOS and TSH are not co-localized, which causes the delay in observing the result. To support the first prediction, one would have to inactivate each NOS (e.g., by deletion of a corresponding NOS gene) and determine the absence of which type of NOS prevents TSH regulation. For the second prediction, perhaps the newfound key NOS protein could be tagged and its route could be tracked to determine NOS proximity in relation to TSH. The co-localization would not be expected as a result of observations. However, if it did occur, further research would have do be done to determine the presence of the intermediates that affect the delay in the TSH regulation. The intermediates could be proteins, and in that case the expression levels of proteins would have to be measured and compared to controls that do not express an obligatory NOS protein (Western blot). Or perhaps the intermediates could be free radicals (other than NO•). To check for the presence of the intermediate free radical, a method of electron paramagnetic resonance (EPR) could be used.

From the results known today, NO• is more than likely involved in thyroid hormone synthesis. It can affect the production and release of thyroid hormones, which makes it eligible for consideration in treating thyroid diseases, such as goiter, hypothyroidism, and hypothyroidism [2]. Perhaps combining the necessary type of NOS with NO• could activate TPO in thyroid cancer cells which would increase radioiodine therapy and help in treating thyroid cancer.

5. Oxidative stress in thyroid diseases

5.1. Oxidative stress in goiter formation.

In the areas where nutrition is low on iodine, goiter is highly vascularized and induced in size. There is a study that shows an induction of genes that encode for types I and III NOS

occurs during goiter formation [18]. This allows to conclude that NO• participates in controlling vasculature during goiter formation and is accumulated in the absence of iodide. It was determined that, in erythrocytes, the activity of antioxidants, such as GSH, CAT, and SOD, is lower in children with goiter disease than in children with non-goiter or even non-iodine deficient subjects [19]. The same is true for selenium levels in plasma and erythrocytes. Perhaps lack of iodine in diet leads first to the goiter condition, and later to oxidative DNA damage. This idea supports the relationship between iodine deficiency and increased incidence of thyroid cancer. There has been a mechanism of goiter formation, involving oxidative stress, proposed (**Figure 5**) [20]. If the mechanism is true, the explanation for a drastic increase of thyroid cancer incidents after radiation explosions, such as the Chornobyl atomic explosion, is obvious: a high rate of pre-existing goiter disease, due to iodine deficiency in diet, caused a greater than expected increase in thyroid diseases, and especially thyroid cancer, as a result of excessive radioiodine uptake.

5.1.1. Proposed experiment 4: determining what causes goiter disease.

It has been shown that increased amount of free radicals, decreased levels of antioxidants, excessive vascularization, and/or H_2O_2 and TSH levels contribute to developing a goiter disease. However it has not been clarified how exactly these conditions occur. The information known at this point is that iodine deficiency creates a pre-environment for the goiter form of thyroid abnormality. What exactly does the lack of iodine do? It retards the formation of thyroid hormones. That means tyrosine does not bind iodide, and perhaps tyrosine becomes a radical. If EPR can detect tyrosine radicals, that would be one experiment to plan on doing. Another effect that the lack of iodine causes is leaving TPO without being able to oxidize iodide. If for any reason disengagement of TPO causes its iron part to be loose, and if H_2O_2 is around, the Fenton reaction will occur. Adding a metal chelator (*i.e*. transferring) to the iodide-deprived thyroid cells and comparing the results with

no chelator in the cells would provide an evidence for possible iron engagement into free radical formation via Fenton reaction. If the data does not differ, that means the heme part of TPO does not 'contribute' to free radical formation, at least via Fenton reaction. Or, no difference in data can also mean that iron is loose, but for some reason not reactive (*i.e.*, absence of H_2O_2, but this is unlikely).

Antioxidants, such as vitamin E (TOH), were previously shown to reduce all of the negative changes caused by deficiency in iodine. Perhaps, this was due to its ability to neutralize free radicals:

$$R\bullet + TOH \rightarrow ROH + TO\bullet$$

Hypothetically, R• is a tyrosyl radical that is unengaged due to a system deficiency in iodine. If we confirmed its presence via EPR method, then adding vitamin E antioxidant and observing a reduction of goiter disease changes would suggest that tyrosyl radical is the radical that causes the disease.

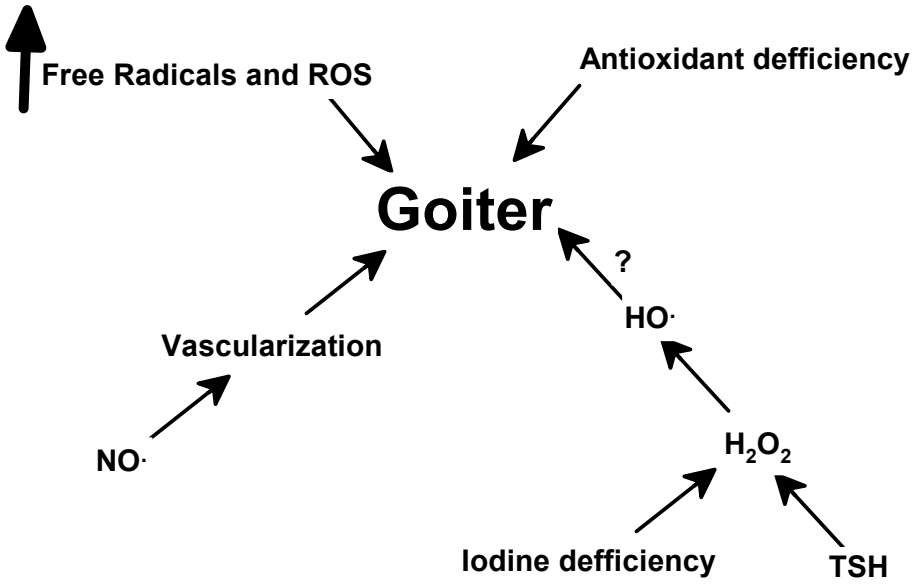

Figure 5. The proposed mechanism of goiter formation that involves oxidative stress.

5.2. Oxidative stress in thyroid autoimmunity

Thyroid gland can be attacked by several autoimmune diseases. The most common are Hashimoto's thyroiditis (a form of hypothyroidism) and Grave's disease (a form of

hyperthyroidism). As mentioned before, H_2O_2 was proposed to induce thyroid autoimmunity. During the experimental conditions in the presence of Fe^{3+}, oxidative stress caused the fragmentation of the Tg molecule into several peptides [21]. The peptides were recognized by Tg monoclonal autoantibodies, and the smallest immunoreactive Tg peptide had a molecular mass of 40 kDa. A small size of this peptide allowed for a more efficient entrance into human thyrocytes. The process of the protein fragmentation was accompanied by thyroid hormone formation, which suggests that Tg cleavage may take place during the synthesis of thyroid hormones [21]. The important fact is that immunoreactive Tg pieces were found only in dead cells and not in living cells, which means the fragments could very well start the autoimmune response.

5.2.1. Proposed experiment 5: researching NO• involvement in thyroid autoimmunity.

One of the studies has shown that NO• is a part of interleukin-1α-induced cytotoxicity in human thyrocytes. It is possible that this free radical may assist in exposing autoantigens to the immune system [22]. To research whether such assistance takes place, the experiment could be performed with exposing thyroid cells to various NO• levels and using antibodies for recognizing the presence of autoantigens.

6. Treating thyroid diseases with radioiodine therapy can cause thyroid cancer.

The main methods to treat thyroid diseases are through surgery, pharmaceutics, and radioiodine treatment. The latter one is controversial because in addition to treating thyroid diseases, it can cause them.

6.1. Radioiodine treats thyroid abnormalities.

Radioiodine therapy is used to treat hyperthyroidism and differentiated thyroid cancer. In this therapy, Iodine-131 (^{131}I) isotope is used. It is a beta-emitter with a half-life of 8.06 days; its maximum beta energy is 0.81 MeV; it emits gamma rays of 0.36 and 0.64 MeV and other energies [23]. Once ingested, the isotope is absorbed into the bloodstream and is taken

up by the overactive thyroid cells. In the next several weeks the thyroid gland usually shrinks in size. Also, blood levels of thyroid hormone and thyroid TSH return to normal. Sometimes a second radioactive iodine treatment may be needed to achieve the desired results [23].

6.2. Radioiodine causes thyroid diseases.

In addition to treating thyroid diseases, radioiodine therapy was shown to cause negative effects [24]. It was shown that after radioiodine therapy, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) undergo oxidative alteration [25]. This process involves free radicals that modify the scavenger receptors and by this enhance the uptake of LDLs. Such uptake can be excessive since the pathway does not have a feedback control. As a result, LDLs accumulate in macrophages or monocytes and promote the development of foam cells [26]. Foam cell accretion plays a fundamental role in the initiation of atherosclerosis [27].

During LDL oxidation, free radicals catalyze peroxidation of arachidonic acid. It was discovered that a series of bioactive prostaglandin F_2-like compounds (F_2-isoprostanes) are produced as a result of the reaction. The F_2-isoprostanes are derived from arachidonic acid (top), which undergoes peroxidation catalyzed by free radicals to yield arachidonyl radical intermediates, which are then transformed to a series of prostaglandin F_2-like compounds composed of four regioisomers (I through IV). When the organism undergoes oxidative stress, levels of F_2-isoprostanes increase dramatically [28]. Such increase allows for using F_2-isoprostane level as a biomarker for detecting oxidative injury. In fact, their level is far superior compared to measuring thiobarbituric-acid reacting substances as a marker of lipid peroxidation *in vivo* [mentioned in 28].

Wolfram *et al*. used isoprostane 8-epi-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to investigate whether the therapeutic use of Iodine-131 (^{131}I) alters isoprostane levels in plasma, serum, and urine.

The findings showed a dose-dependent increase of $PGF_{2\alpha}$ after radioiodine therapy which uttered a significant oxidative injury after radioiodine therapy [28].

6.3. Proposed experiment 6: revealing the mechanism of preventing oxidative damages caused by radiation.

When applying radiation to the body is a matter of choice, it is much easier to handle than unexpected radiation dose. If the antioxidant naturally belongs to the organism, negative effects of radiation would be easier to handle. There has been recently a molecule described that is shown to possess free radical scavenging and antioxidant ability - melatonin [20]. It is a hormone derived from serotonin and produced by the pineal gland that plays a role in many vitally important functions, such as sleep, aging, and reproduction in mammals. Although functions of melatonin have been explored [20], the mechanism of its protection is not clearly understood. To hypothesize the mechanism, one would have to state that in protection against lipid peroxidation, melatonin more than likely involves an antioxidant and free scavenging activities of such toxic species as hydroxyl radical and peroxynitrite anion. To test the hypothesis, in control thyroid cells production of melatonin would have to be stopped through blocking serotonin hormone production. After irradiating the samples, using EPR, detection of toxic species could be performed. Comparing of toxic species types and levels in samples with and without melatonin could presumably uncover some of the intermediate players in the mechanism of radiation damage prevention using melatonin.

7. Summary

Oxidative stress has been shown to play important role in many disorders, including thyroid abnormalities. Although not clearly understood, the mechanisms of iodide uptake, making and regulating the hormones are multi-step processes that involve various regulations. One of the foundation-type regulation pathways is redox; it involves free

radicals, oxidants, and antioxidants to alter thyroid cells. More research needs to be done on oxidative stress and thyroid diseases.

References

1. Akslen LA, Haldorsen T, Thoresen SO, Glattre E. (1991) Survival and causes of death in thyroid cancer: a population-based study of 2479 cases from Norway. *Cancer Res*. **51**:1234-41.
2. Millatt LJ, Johnstone AP, Whitley GS. (1998) Nitric oxide enhances thyroid peroxidase activity in primary human thyrocytes. *Life Sci*. **63**:373-80.
3. Furuya F, Shimura H, Miyazaki A, Taki K, Kobayashi T. (2004) Adenovirus-mediated transfer of thyroid transcription factor-1 induces radioiodide organification and retention in thyroid cancer cells. *Endocinol*. **145**:5397-405.
4. Arnone MI, Zannini M, Di Lauro R. (1995) The DNA binding activity and the dimerization ability of the thyroid transcription factor I are redox regulated. *J Biol Chem*. **270(20)**:12048-55.
5. Lonigro R, Donnini D, Fabbro D. Perrella G, Damante G, Ambesi Impiombato FS, Curcio F. (2000) Thyroid-specific gene expression is differentially influenced by intracellular glutathione level in FRTL-5 cells. *Endocrinol*. **141**:901-09.
6. Puppin C, Arturi F, Ferretti E, Russo D, Saggo R, Tell G, Damante G, Filetti S. (2004) Transcriptional regulation of human sodium/iodide symporter gene: a role for redox factor-1. *Endocrinol*. **145(3)**:1290-93.

6.a Ding SZ, O'Hara AM, Denning TL, Dirden-Kramer B, Mifflin RC, Reyes VE, Ryan KA, Elliott SN, Izumi T, Boldogh I, Mitra S, Ernst PB, Crowe SE. (2004) Helicobacter pylori and H_2O_2 increase AP endonuclease-1/redox factor-1 expression in human gastric epithelial cells. *Gastroenterology*. **127(3)**:845-58.

7. Brody TM, Larner J, Minneman KP. (1998) Human pharmacology: Molecular to clinical. 3rd ed.; *Mosby, St. Louis*. **535**.
8. Dunn JT, Dunn AD. (2001) Update on intrathyroidaliodine metabolism. *Thyroid*. **11(5)**:407-14.
9. Fujiwara H, Tatsumi K, Miki K, Harada T, Miyai K, Takai S, Amino N. (1997) Congenital hypothyroidism caused by a mutation in the Na+/I- symporter. *Nature Genetics* **16**:124, 1997.
10. Schmitt TL, Espinoza CR, Loos U. (2000) Cloning and characterization of repressory and stimulatory DNA sequences upstream the Na+/I- symporter gene promoter. *Horm Metab Res*. **32**:1-5.
11. Zimmerman M, Kohrle J. (2002) The impact of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. *Thyroid*. **12**:867-78.
12. Fayadat L, Niccoli-Sire P, Lanet J, Franc JL. (1999) Role of heme in intracellular trafficking of thyroperoxidase and involvement of H_2O_2 generated at the apical surface of thyroid cells in autocatalytic covalent heme binding. *J Biol Chem*. **274**:10533-38.

13. Dupuy C, Ohayon R, Valent A, Noel-Hudson MS, Deme D, Virion A. (1999) Purification of a novel flavoprotein involved in the thyroid NADPH oxidase: cloning of the porcine and human cDNAs. *J Biol Chem*. **273**:37265-69.
14. Raspe E, Dumond JE. (1995) Tonic modulation of dog thyrocyte H_2O_2 generation and I^- uptake by thyrotropin through the cyclic adenosine 3'5'-monophosphate cascade. *Endocrinol*. **136**:965-73.
15. Corvilain B, Van Sande J, Dumont JE. (1988) Inhibition by iodide of iodide binding to proteins: the "Wolff-Chaikoff" effect is caused by inhibition of H_2O_2 generation. *Biochem Biophys Res Commun*. **154(3)**:1287-92.
16. Riou C, Remy C, Rabilloud R, Rousset B, Fonlupt P. (1998) H_2O_2 induces apoptosis of pig thyrocytes in culture. *J Endocrinol*. **156**:315-22.
17. Vitale M, Di Matola T, D'Ascoli F, Salzano S, Bogazzi F, Fenzi G, Martino E, Rossi G. (2000) Iodide excess induces apoptosis in thyroid cells through a p53-independent mechanism involving oxidative stree. *Endocrinol*. **141**:598-605.
18. Colin IM, Nava E, Toussaint D, Maiter DM, VanDenhove MF, Luscher TF, Ketelslegers JM, Denef JF, Jameson JL. (1995) Expression of nitric oxide synthase isoforms in the thyroid gland: evidence for a role of nitric oxide in vasculature control during goiter formation. *Endocrinol*. **136**:5283-90.
19. Giray B, Hincal F, Tezic T, Okten A, Gedik Y. Status of selenium and antioxidant enzymes of goitorous children I s lower than healthy controls and nongoitorous children with high iodine deficiency. *Biol Trace Elem*. *Res*. **82**:35-52.
20. Karbownik M, Lewinski A. (2003) Melatonin reduces Fenton reaction-induced lipid peroxidation in porcine thyroid tissue. *J Cell Biochem*. 90(4):806-11.
21. Duthoit C, Estienee V, Giraud A, Durand-Gorde JM, Rasmissen AK, Feldt-Rasmussen U, Carayon P, Ruf J. (2001) Hydrogen peroxide-induced production of a 40 kDa immunoreactive thyroglobulin fragment in human thyroid cells: the onset of thyroid autoimmunity? *Biochem J*. **360**:557-62.
22. Van den Hove MF, Stenoiu MS, Croizet K, Couvreur M, Courtoy PJ, Devuyst O, Colin IM. (2002) Nitric oxide is involved in interleukin-1 alpha–induced cytotoxicity in polarized human thyrocytes. J Endocrinol. 173:177-85.
23. Dillman LT, Von der Lage FC. (1975) Radionuclide decay schemes and nuclear parameters for use in radiation-dose estimation. NM/MIRD Pamphlet No.10. *Soc Nucl Med*. 1975.
24. Poverennyy AM, Ryabuhin YC, Tsyb AF. (1994) Probable cause of thyroid diseases in people suffered from Chornobyl accident. *Radiaz biol. Radioekol*. **34(1)**:8-15.
25. Haba T, Sakai Y, Koizumi J, Miyamoto S, Mabuchi H, Takeda R. (1983) A case of heterozygous familial hypercholesterolemia associated with hyperthyroidism: effects of triiodothyronine on low-density lipoprotein receptor and cholesterol synthesis. *Metabolism*. **32(12)**:1129-32.
26. Morel DW, Docorleto PE, Chisolm GM. (1984) Endothelial and smooth muscle cells after low-density lipoprotein in vitro by free radical oxidation. *Artheriosclerosis*. **4**:357-64.
27. Carpenter KL, Wilkins GM, Fussell B, Ballantine JA, Taylor SE, Mitchinson MJ, Leak DS. (1994) Production of oxidized lipids during modification of low-density lipoproteions. *Biochem J*. **304**:625-33.
28. Morrow JD, Fyei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ. (1995) Increase in circulating products of lipid peroxidation (F2-Isoprostanes) in smokers — smoking as a cause of oxidative damage. *New Eng J Med*. **332**:1198-1203.

Ciampi R, Knauf JA, Roswitha K, Gandhi M, Zhu Z, Nikiforova MN, Rabes HM, Fagin JA, Nikiforov YE. (2005) Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. *J Clin Invest*. **115**:95-101.

Colin IM, Kopp P, Zbaren J, Haberli A, Grizzle WE, Jameson JL. (1997) Expression of nitric oxide synthase III in human thyroid follicular cells: evidence for increased expression in hyperthyroidism. *Eur J Endocrinol*. **136**:649-55.

Doniach I. (1963) Effects including carcinogenesis of I-131 and x-rays on the thyroid of experimental animials – a review. *Health Phys*. **9**:1357-62.

Gilliland FD, Hunt WC, Morris DM, Key CR. (1997) Prognostic factors for thyroid carcinoma. *Cancer 1997*. **79**:564-73.

Haigh PI. (2000) Anaplastic thyroid carcinoma. *Current Treat Opt Oncol*. **1**:353-57.

Komosinska-Vassey K, Olczyk K, Kucharz EJ, Marcisz C, Winsz-Szczotka K, Kotulska A. (2000) Free radical activity and antioxidant defense mechanisms in patients with hyperthyroidism due to Graves' disease during therapy. *Clin Chim Acta*. **300**:107-17.

McTiernan A, Weiss N, Daling J. (1984) incidence of thyroid cancer in women in relation to previous exposure to radiation therapy and history of thyroid disease. *J Natl Cancer Institute* (Bethesda). **73**:575-81.

Modigliani E, Franc B, Niccoli-sire P. (2000) Diagnosis and treatment of medullary thyroid cancer. *Baillieres Best Pract Res Clin Endocrinol Metab*. **4**:631-49.

Passler C, Scheuba C, Prager G, Kaserer K, Flores JA, Vierhapper H, Niederle B. (1999) Anaplastic (undifferentiated) thyroid carcinoma (ATC). *Langenbeck's Arch Surg*. **384**:284-93.

Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM. (1995) Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiat Res. **141**:259-77.

Shore RE. (1992) Issues and epidemiological evidence regarding radiation-induced thyroid cancer. *Radiat Res*. **131**:98-111.

Wiseman SM, Loree TR, Rigual NR, Wesley HL, Douglas WG, Anderson GR, Stoler DL. (2003) Anaplastic transformation of thyroid cancer: review of clinical pathologic, and molecular evidence provides new insights into disease biology and future therapy. Head Neck. **25**:662-70.

Hall P, Hom LE. (1998) Radiation-associated thyroid cancer – facts and fiction. *Acta Oncologica*. **37(4)**:325-30.