

Review

Role of iodine in antioxidant defence in thyroid and breast disease

Peter P.A. Smyth*

Iodine Study Unit, Department of Medicine and Therapeutics, and Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Ireland

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Abstract. The role played in thyroid hormonogenesis by iodide oxidation to iodine (organification) is well established. Iodine deficiency may produce conditions of oxidative stress with high TSH producing a level of H_2O_2 , which because of lack of iodide is not being used to form thyroid hormones. The cytotoxic actions of excess iodide in thyroid cells may depend on the formation of free radicals and can be attributed to both necrotic and apoptotic mechanisms with necrosis predominating in goiter development and apoptosis during iodide induced involution. These cytotoxic effects appear to depend on the status of antioxidative enzymes and may only be evident in conditions of selenium deficiency where the activity of selenium containing antioxidative enzymes is impaired. Less compelling evidence exists of a role for iodide as an antioxidant in the breast. However the Japanese experience may indicate a protective effect against breast cancer for an iodine rich seaweed containing diet. Similarly thyroid autoimmunity may also be associated with improved prognosis. Whether this phenomenon is breast specific and its possible relationship to iodine or selenium status awaits resolution.

Keywords: Antioxidant, thyroid, breast, iodine, thyroid antibodies

1. Introduction

Iodine was first described as a constituent of burned seaweed [1]. Although its major role in the human thyroid was identified by Baumann [2], it was not until 1927 that Sir Charles Harrington [3] reported that the major part of the thyroxine (T4) molecule (65.3% by weight) was made up of iodine. Most of the investigations of iodine status in humans and animals have been focused on the role of iodine in thyroid function. Relatively little attention has been devoted to its extra thyroidal roles, one of the most important of which is its function as an antioxidant in human systems including the eye, thyroid and the breast [4–8]. The antioxidant properties of dietary iodide depend on a series of redox reactions underlying the iodination of tyrosine leading to the formation of thyroid hormones [9–11].

*Address for correspondence: Dr Peter P.A. Smyth, Iodine Study Unit, Department of Medicine and Therapeutics, University College Dublin, Woodview, Belfield, Dublin 4, Ireland. Tel.: +353 1 7162049; Fax: +353 1 7161136; E-mail: ppa.smyth@ucd.ie.

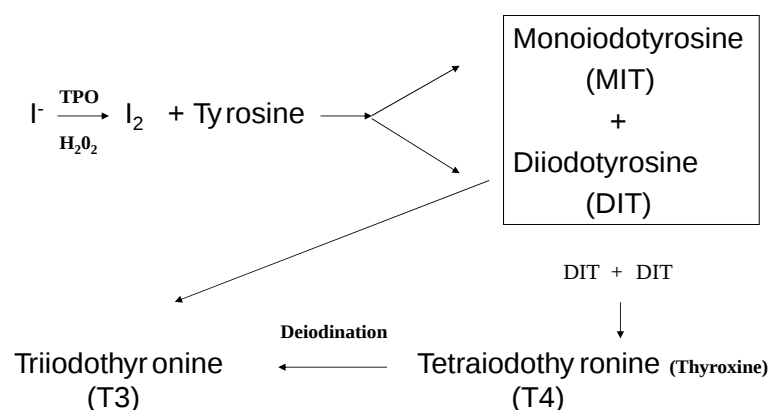


Fig. 1. Iodination of tyrosine and formation of thyroid hormones. TPO = Thyroid peroxidase.

2. Thyroid

As shown in Fig. 1, organification (oxidation) of iodide (I^-) to iodine (I_2) is accomplished by H_2O_2 catalysed by the enzyme thyroid peroxidase (TPO) and leads to the iodination of iodotyrosines and eventual formation of thyroid hormones triiodothyronine (T3) and thyroxine (T4). Since H_2O_2 is a major oxidant in the body, the more that is used up in the oxidation of I^- , the less is available for other potentially damaging oxidative processes and hence the role of I^- as an antioxidant. Provision of substrate H_2O_2 in the thyroid occurs via TSH mediated NADPH oxidase induction [12] and through the dismutation of superoxide (O_2^-) radicals thus formed, by the enzyme superoxide dismutase (SOD). While the antioxidant enzyme SOD enhances the rate of iodination in the thyroid [13], H_2O_2 is removed by antioxidative enzymes, principally those of the glutathione peroxidase (GPx) family [14] but also catalase. TSH exerts two principal effects on the thyroid gland. The best understood is a stimulus to produce thyroid hormones with a second function being the promotion of thyroid cellular growth [15]. The action of TSH on the thyroid follicular cell is mediated via intracellular signalling systems including the G protein linked cAMP and phosphoinositide cascades [15,16]. These signalling systems lead to TPO induction which, as previously stated, catalyses the oxidation of I^- to I_2 . This oxidation requires the presence of an oxidizing agent H_2O_2 which is generated as a result of NADPH oxidation to NADP [17,18]. The oxidation of NADPH and therefore the generation of H_2O_2 is mediated by the recently described thyroid oxidases (ThOX1 and ThOX2) [19].

It is the action of H_2O_2 and other reactive oxygen species (ROS) that produce oxidative damage in the thyroid which in normal circumstances is protected through the action of the selenium (Se) containing antioxidative enzymes, the GPx family [14]. Oxidative damage to the thyroid is more severe in iodine deficiency where the gland is under increased stimulation by TSH resulting in excessive H_2O_2 production within the cells with relatively little substrate I^- to be oxidised. Se deficiency causes a deficit in GPx with a failure to remove H_2O_2 , increased oxidative stress and thyroidal damage [17]. A combination of Se and I^- deficiency can lead to brain damage in the fetus or oxidative damage to DNA with the possibility of an increased incidence in thyroid malignancies [21,22].

NADPH oxidation resulting in H_2O_2 production is the rate limiting step for both iodination and the supply of NADP⁺ for the pentose phosphate pathway [17,18]. Interestingly inhibition of NADPH dependent H_2O_2 generation and thus of thyroid hormonogenesis forms a central feature of the mode of action of the antithyroid drugs propylthiouracil (PTU) and methimazole (MMI). PTU is a highly

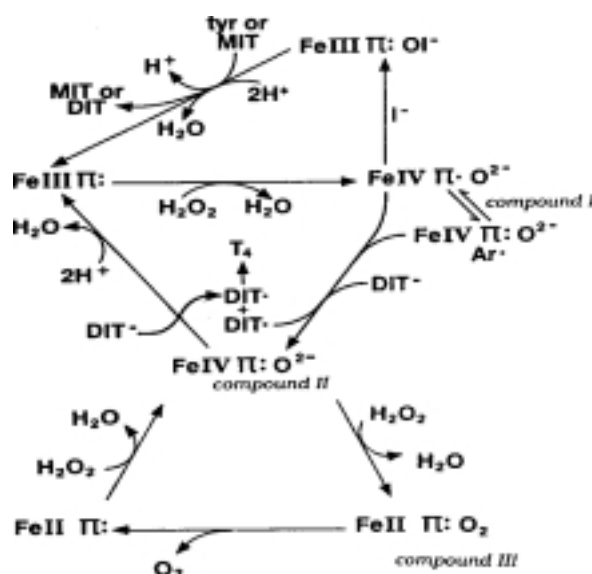


Fig. 2. Free radical involvement in thyroid hormonogenesis. Tyr = Tyrosine; MIT = Monoiodotyrosine; DIT = Diiodotyrosine; T4 = Thyroxine; (Fe III $\Pi:$) = porphyrin Π cation radical residing on the heme group of the thyroid peroxidase (TPO) molecule. Source: Bikker, H., Dissertation for PhD thesis, University of Amsterdam, 1996.

efficient scavenger of hydroxyl radicals [23] while both drugs inhibit TPO and NADPH driven H_2O_2 production [24]. Therefore both PTU and MMI can be termed antioxidant agents at least *in vitro* [23].

Although the greatly simplified reactions shown in Fig. 1 reflect what occurs in thyroid hormonogenesis, the actual pathways are more complex. A number of possible mechanisms through which iodination could take place have been postulated, of which the favoured was free radical formation [10]. A series of steps through which free radical mediated iodination might proceed are summarized in Fig. 2 for whose design I am grateful to J.J.M. de Vijlder, Amsterdam. This diagram is based on published findings [10,25,27]. TPO is a large (~ 105 kDa) heme containing glycoprotein that catalyses both iodination and iodotyrosine coupling in the thyroid gland [10]. The heme groups on the TPO molecule represented as a porphyrin Π cation radical (Fe III $\Pi:$) are oxidised by H_2O_2 formed principally through the action of the NADPH oxidase system at the thyroid follicular cell apical membrane to the Fe IV form giving up an electron to form superoxide ($\text{O}_2^{\cdot -}$) radicals (Fe IV $\Pi: \text{O}_2^{\cdot -}$). Oxidation of native TPO by a slight excess of H_2O_2 in the presence of both I^- and Tg leads to the formation of Compound I, containing a porphyrin Π cation radical [11]. Compound I is isomerized to a protein radical form in which an electron is transferred from a nearby aromatic amino acid to the porphyrin ring. This compound is reduced by one electron to compound II. In this reaction diiodotyrosine (DIT^-), present as anion at physiological pH because the pK of DIT is 6.5, can provide this electron, and a DIT radical is formed [27]. Compound II, containing a Fe(IV) heme group, is further reduced by one electron to native Fe(III) TPO, also producing a DIT radical. Two DIT radicals (in Tg) are able to couple, if they are located in the right position, and form T4. The structure of Tg is important in the successful catalysis of this reaction. An alternative iodinating mechanism suggested by Taurog [10] would involve the oxidized Fe IV $\Pi: \text{O}_2^{\cdot -}$ giving up another electron to react with I^- to yield a complex with the OI^- (hypoiodite) ion, which in turn breaks down to yield I^+ (iodinium ion), which acts as the iodinating intermediate by attaching itself to a tyrosine molecule yielding monoiodotyrosine (MIT) and subsequently diiodotyrosine (DIT). The actual iodination species

potentially involved in formation of iodotyrosines are many and varied including, I^- , I^+ (iodinium), I_2 , I^0 (iodine free radical), IO^- (hypoiodite), H_2IO^+ (hypoiodous acidinium ion), IO_3^- (iodate) [11].

A role for iodine in the evolutionary process has been postulated on the basis that in moving from the relatively iodine rich sea to the more iodine deficient land organisms had to develop systems such as the thyroid gland to provide a reservoir to permit survival in the iodine poor terrestrial environment [8]. While organisms moving from the iodine rich sea to the iodine poor land need mechanisms to trap iodide, they also face the hazard of having to deal with higher amounts of atmospheric O_2 which in its various forms can be cytotoxic. The so called stress reaction to O_2 can result from the presence of reactive oxygen species (ROS) such as superoxide radicals (O_2^-) and H_2O_2 . ROS can inactivate many enzymes and are a feature of lipid peroxidation and DNA damage and have been shown to be associated with carcinogenesis [28].

Early work on the stress effects of O_2 showed that removal of the thyroid gland decreased the toxic effects of O_2 while the administration of T4, cortisone or adrenaline made them worse [29]. Thyroid hormones are known to play a major part in the regulation of mitochondrial oxidative metabolism with overt hyperthyroidism causing enhanced oxidative metabolism with consequently reduced lipid and lipoprotein plasma levels while overt hypothyroidism results in reduced oxidative metabolism and markedly increased lipid and lipoprotein levels [30,31]. Iodine can react with double bonds on lipids such as polyunsaturated fatty acids rendering them less reactive to ROS. Polyunsaturated fatty acids such as arachidonic acid which is known to play a role in intracellular signalling in the thyroid contains four double bonds and can be easily oxidised and thus contribute to increased lipid peroxidation [32]. It has been postulated that formation of iodolipids such as iodolactones or iodoaldehydes represents a form of thyroidal autoregulation [33] which may be the mode of action of iodide inhibition of thyroidal function in the Wolff-Chaikoff effect [6,34,35]. While lower doses of iodide are necessary substrates for TPO mediated conversion into I_2 , iodinated compounds (so called XI) at high doses may exert inhibitory effects on adenylate-cyclase, NADPH-oxidase and TPO activities [6,35]. This effect seems to require oxidation of I^- to I_2 as inhibitors of TPO or I^- trapping can reverse the inhibitory effect [35]. It is interesting to speculate if such compounds are involved in the reported down regulation of the sodium iodide symporter (NIS) by high doses of iodide which is also a feature of the Wolff-Chaikoff effect [36].

Iodide may act as an antioxidant by reducing the sensitivity of the thyroid gland to TSH as suggested by Bray [37] thus diminishing both T4 and H_2O_2 production. It has been demonstrated that even low to moderate (1–10 μM) doses of iodide can inhibit a series of TSH mediated thyroidal events including cAMP production, TPO and NIS expression in both human and dog thyroids [36,38]. Excess iodide can interfere with iodination of thyroglobulin within the thyroid gland thus inhibiting thyroid hormone formation. This forms the basis for the Wolff-Chaikoff effect [34]. Such inhibition is usually of a transient nature with normal thyroid hormone production being resumed, the so called “escape” from the Wolff-Chaikoff effect. It has also been postulated that production of iodinated compounds could form the basis for the reduction of the hypervascularity and hyperplasia produced by administration of high doses of iodide [39]. A pro-oxidant effect of excess iodide on thyroid cells leading to apoptosis was however demonstrated by the characteristic DNA fragmentation pattern and as described above seems to require oxidation of I^- to I_2 as its cytotoxic effects could be blocked by the TPO inhibitor propylthiouracil (PTU) [35]. The probability that I^- induced thyroid cellular apoptosis was related to free radical formation was supported by the demonstration of a significant increase in lipid peroxidation following I^- treatment [35]. This is in agreement with one of the possible mechanisms for free radical induced cytotoxicity in the thyroid suggested by Denef et al. [6]. Apoptosis is not the only pathway through which I^- induced cytotoxicity can be mediated as both thyroidal apoptosis and necrosis were implicated

with necrosis predominating in goiter development and apoptosis during iodide induced involution [40]. These workers attributed the necrotic effect to free radical formation which was potentiated in vitamin E deficient rats having reduced antioxidative protection (increased malondealdehyde ; MDA) reflecting increased lipid peroxidation and decreased Se containing GPx. The cytotoxic effect of excess iodide may depend on the relative abundance of Se and Fe in the thyroid , at least in conditions of iodine deficiency where TSH stimulated H_2O_2 is abundant, as it has been shown that necrosis produced in selenium deficient rats by H_2O_2 and infiltration by mononuclear cells was not observed in selenium replete animals [41]. This effect is presumably due to the failure to produce selenium containing antioxidative enzymes such as GPx [14,42]. Similarly Fe, known to be an integral part of the heme portion of the TPO molecule [43], could limit removal of potentially cytotoxic H_2O_2 although this has not been established.

The iodine rich cardiac antiarrhythmic drug amiodarone which has been reported to induce either hypothyroidism or hyperthyroidism in humans can also produce ultrastructural features of necrosis or apoptosis in rat thyroids [44]. As with the Wolff-Chaikoff effect such cytotoxicity can be prevented by inhibition of TPO, suggesting the requirement for iodinated compounds [35]. Indeed oxidative stress could explain an increase in malondealdehyde (MDA), a product of lipid peroxidation in iodine deficient glands [45] and the absence of toxicity of an identical iodine dose reaching iodine/thyroglobulin-replete glands. Oxidative stress also provides an explanation for the hypothyroidism (myxedematous cretinism) described in combined iodide and selenium deficiency, a situation characterised by low H_2O_2 detoxification reflecting the absence of selenium containing glutathione peroxidase (GPx) activity [46]. Administration of Se before correction of the iodine deficiency can disimprove the hypothyroidism by increasing T4 breakdown by Se containing deiodinase enzymes. It is therefore important that iodine supplementation precede that of Se. Deficiency of Se is associated with autoimmune thyroid disease (AITD) perhaps as a result of increased inflammatory activity arising from decreased activity of Se containing antioxidative enzymes. Selenium supplementation or selenomethionine treatment in patients with AITD has been reported to decrease thyroid peroxidase antibody (TPO Ab) concentrations [47,48] thus enhancing immunocompetence without affecting thyroid hormone levels.

The postulate that I^- itself exerts a significant antioxidant effect has been advanced for many years [8]. Indeed the antioxidant effect of NaI levels as low as $15 \mu M$ have been shown to be equivalent to that of the established antioxidant ascorbic acid ($50 \mu M$) [49]. The use of iodine rich brines or seaweeds as thalassotherapy or balneotherapy in health spas has been well established for many centuries [7]. Beneficial effects of such treatments in the area of cardiac and respiratory disease, thyroid function, arteriosclerosis, diabetes mellitus and eye diseases have received considerable attention [5,49,50].

3. Breast

The antioxidant properties of iodide described for the thyroid also apply to other tissues having the ability to concentrate iodide. These include the salivary glands, gastric mucosa and mammary glands [51,52]. Although TSH has no known role in promoting I^- uptake into mammary cells, these cells have been shown to possess the sodium iodide symporter (NIS) [53–54]. Uptake of I^- into mammary cells can be promoted by prolactin and combinations of hormones (prolactin, oxytocin, estrogens) known to be involved in lactation [54,55]. As well as promoting NIS expression, prolactin enhances expression of a second I^- transporter Pendrin which in the thyroid facilitates I^- transport from the apex of the follicular cell into the follicular lumen and facilitates I^- accumulation in milk in the lactating mammary gland [56]. Although iodoprotein has been detected in breast tissue [57], it is not known if prolactin has a role in facilitating such iodinations. Free radicals have been associated with carcinogenesis in many

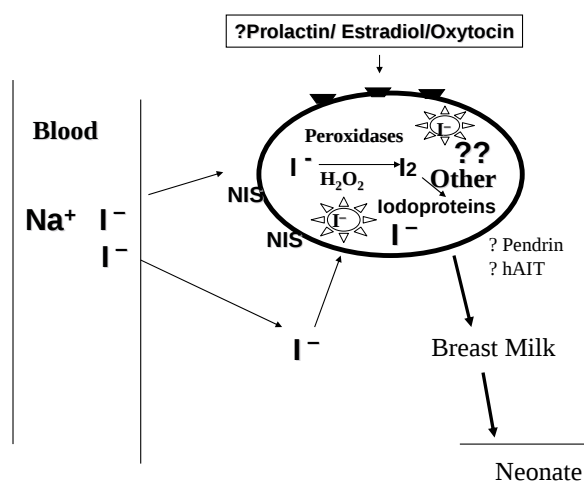


Fig. 3. Uptake and efflux of I^- in the mammary gland. NIS = Sodium iodide symporter; hAIT = Human apical iodide transporter.

organs including the human breast [58–60]. Increased serum levels of antioxidants have been associated with reductions in breast cancer risk [61] while increases in the activity of the antioxidant enzymes SOD, catalase and GPx have also been reported in both malignant breast tumours and benign breast disease, which has been suggested to represent a compensatory response to increased lipid peroxidation as evidenced by parallel increases in MDA levels [62].

A role for iodide as an antioxidant possibly through a protective action of iodolipids as described for the thyroid has been suggested [64–66]. This is on the basis of a shared iodide concentrating mechanism in both thyroid and breast as well as a requirement for an iodide oxidation system to provide for the formation of iodoamino acids leading to thyroid hormone formation in the thyroid and to iodinated milk proteins by the breast necessary for neonatal nutrition [50–52]. Figure 3 shows in cartoon form the uptake of I^- by the breast and its incorporation into iodoproteins. When taken into the breast I^- is incorporated into lactoproteins presumably as a result of organification into I_2 by lactoperoxidases [57,66]. These iodoproteins together with free I^- are secreted in breast milk. As mentioned elsewhere in this communication, I^- may also be incorporated into iodolipids such as iodolactones or iodoaldehydes which in the thyroid have been shown to possess antiproliferative properties. To date there is no evidence that a similar effect is produced in the breast.

Although there is no direct evidence that I^- acts as an antioxidant in the breast, increased rates of breast cancer have been reported in iodine deficient populations [67]. Thyroid enlargement as measured by ultrasound has been reported in a significant number of patients with breast cancer compared to controls from the same area [68]. Iodine deficiency has also been linked to increased fibrosis and adenosis of the mammary gland and administration of iodine has been used in the treatment of breast pain [65,69]. It has also been suggested that a combined I^- /Se deficiency may facilitate the development of breast cancer [63].

An anticarcinogenic role for iodine in experimental animals has been suggested by the work of Funahashi et al. [70] who found that administration of Lugol's iodine or iodine rich Wakame seaweed to rats treated with the carcinogen dimethyl benzantracene (DMBA) suppressed the development of mammary tumours. In further studies the same group [71] demonstrated that seaweed induced apoptosis in human breast cancer cells had a stronger effect than fluorouracil, a chemotherapeutic agent used

to treat breast cancer. This finding led the authors to speculate that “seaweed may be applicable for prevention of breast cancer ?” [72]. This hypothesis is in accord with the relatively low breast cancer rate reported in Japan [73] where the normal diet is seaweed rich and with increasing breast cancer rates in Japanese women who emigrate [74] or consume a western style diet [75]. Interestingly this finding applies to rates of breast cancer in both males and females [76]. This evidence favours the low rate of breast cancer being environmental rather than genetic in origin. One of the main dietary differences between Japanese and Western women is the consumption of large amounts of iodine rich seaweeds, the former yielding a dietary iodine intake of several mg/day in Japanese women compared to μg quantities in western women [63]. As with the thyroid, the antioxidant potential of I^- may require its oxidation to I_2 . Indeed Eskin et al. [77] have postulated that normal physiological function of mammary tissue requires I_2 .

Some support for a role for iodine in the human breast is provided by our own findings [53] which showed that tissue iodine levels were relatively low in patients with breast cancer compared to normal tissues or benign breast tumours by fibroadenomata. These findings coincided with decreased expression of the human sodium iodised symporter (NIS) in breast cancer patient tissues [78]. An association between various thyroid disorders and breast cancer has been suggested in many publications [79,80]. However, there is considerable disagreement concerning this linkage. A number of reports have demonstrated an association of autoimmune thyroid disease with breast cancer [81–84]. Despite these reports a recent meta analysis [85] showed a lack of any association between Hashimoto’s thyroiditis and breast cancer. In common with reports from our own laboratory [83] showing that TPOAb positivity in patients with breast cancer was associated with better disease free and overall outcomes, an increased prevalence of TPOAb and better diseases outcome has also been reported both basally and following interferon gamma treatment of renal cell carcinoma [86]. Preliminary findings indicated that the prevalence of TPOAb as measured by sensitive radioimmunoassay was significantly greater in breast cancer patients than in controls or those with diverse malignancies [87]. While this evidence points to an involvement of thyroid autoimmunity in the natural history of breast cancer, its exact role, breast specificity or possible association with reactive oxygen species remains unclear. Also awaiting elucidation is a possible role for I or Se prophylaxis and the consequences of impaired transport and incorporation of iodine into mammary tissues.

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