ANTI-GOITROUS EFFECT OF LECITHIN-BOUND IODINE IN PROPYLTHIOURACIL TREATED RATS

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Objective. Excess iodine and some iodine-containing compounds are known to affect various parameters of thyroid function. Lecithin-bound iodine (LBI) is a compound which induces involution of an enlarged thyroid. LBI was tested for its ability to affect thyroid ornithine decarboxylase (ODC) activity and apoptosis.

Methods. LBI was given orally to propylthiouracil-pretreated rats and the changes in ODC activity and apoptosis were observed. The thyroid apoptosis was detected by DNA laddering and flow cytometry.

Results. LBI suppressed the thyroid ODC activity within one hour after its administration and lowered slightly but significantly the thyroid putrescine levels at 3 h. The DNA cleavage ladder was evident at 3-6 h after LBI treatment. Propylthiouracil induced thyroid enlargement was reduced significantly at 3 days after chronic treatment with LBI. The thyroidal content of putrescine was also decreased after chronic treatment. These effects of LBI were essentially the same as those of excess iodide, while other iodinated compounds including amiodarone, iopanoic acid and erythrosine had no effect on the thyroid ODC activity.

Conclusions. These results suggest that LBI may exert its anti-goitrous effects, consisting of the inhibition of ODC activity and apoptosis, in the form of inorganic iodide in vivo.

Key words: Lecithin bound iodine – Ornithine decarboxylase – Propylthiouracil – Anti-goitrous effect – Apoptosis

Lugol solution transiently decreases the thyroid weight of Graves’ patients and ameliorates the manifestations of thyrotoxicosis (PLUMMER 1923). Because of its ability to block thyroid hormone release acutely, Lugol’s iodine is used in the management of severe thyrotoxicosis. Iodine has been shown to inhibit abruptly the release of thyroxine (T4) in patients with hyperthyroidism (WARTOFSKY et al. 1970). Excess iodide also exerts various inhibitory effects on thyroid functions in experimental animals as well as in humans (NAGATAKI 1991). For instance, excess iodide decreases the thyroid enlargement induced by antithyroid drugs without changing serum levels of TSH and thyroid hormone levels (MATSUZAKI et al. 1978; PISAREV 1986). Polyamines such as spermidine and spermine are known to play an important role in the regulation of cell proliferation and differentiation (PEGG 1986). We have already shown that polyamine biosynthesis is closely associated with goiter formation. Thyroid content of polyamines and putrescine, a precursor of polyamines fluctuates greatly during thyroid growth and involution (MATSUZAKI et al. 1978). Among the enzymes involved in polyamine biosynthesis, ornithine decarboxylase (ODC) is the rate-limiting enzyme and responds very rapidly and sensitively to TSH stimulation (MATSUZAKI et al. 1978). We have also shown that potassium iodide inhibits very rapidly the elevated thyroid ODC activity induced by chronic treatment with propylthiouracil (PTU) (MA et al. 1996). The apparent biological half-life of the thyroid ODC activity is 19 min after KI treatment. Recently we have shown that excess iodine induces
apoptosis in the thyroid of goitrogen-pretreated rats (Burikhanov and Matsuzaki 2000). Such inhibitory effect of iodide on thyroid ODC and apoptosis is quite different from that on other thyroid activities. This inhibitory effect is observed only in goitrogen-treated rats but not in intact animals. No evidence was obtained that cAMP is involved in iodide inhibition of ODC. Usually various inhibitory effects of iodide are blocked by goitrogens such as methimazole and PTU (Van Sande et al. 1973; Nagataki 1991). The inhibitory effects of excess iodide on thyroid ODC activity and apoptosis, however, were observed even in the presence of thionamides (Matsuzaki et al. 1978; Ma et al. 1996; Burikhanov and Matsuzaki 2000). Usually the iodide effect on thyroid is only transient (Nagataki 1991), but the effect on the rat thyroid ODC lasts at least for 1 week (Matsuzaki et al. 1978). Several iodocompounds are known to cause various effects on thyroid physiology (Germain 1988; Harjai and Licata 1997). However, iodinated compounds including L-diiodotyrosine (DIT) have little inhibitory effect on the thyroid ODC within 3 h even though DIT is rapidly deiodinated in vivo (Matsuzaki et al. 1968).

Lecithin-bound iodine (LBI) is a complex consisting of two molecules of lecithin and one molecule of iodine (Namba et al. 1993). Namba et al. (1955) first used this compound for the treatment of thyroid disorders. It was also used for the treatment of ocular hypersensitivity (Komoto et al. 1970). Iodide in this complex binds very loosely and is released immediately after intragastric administration (Namba et al. 1993). This compound was reported to be clinically effective for the treatment of simple goiter after the survey in a number of cases (Namba et al. 1955) and has been shown to be useful for the treatment of Graves’ disease (Ito et al. 1963; Noguchi et al. 1965; Kuribara et al. 1977; Miyagawa et al. 1987). LBI has no effect on type I iodothyronine 5’-deiodinase (Namba et al. 1993).

The precise mechanism whereby LBI ameliorates the manifestations of hyperthyroidism still remains unknown. Therefore, we thought it of interest to examine if this compound exerts any effect on stimulated thyroid ODC activity induced by PTU and on thyroid apoptosis. The present study deals with the inhibitory effects of LBI on the thyroid ODC, putrescine contents and goiter formation induced by PTU. Effect of LBI on thyroid apoptosis was also studied in PTU pretreated rats, because excess iodine has been shown to induce thyroid apoptosis in goitrogen pretreated rats (Burikhanov and Matsuzaki 2000) and in thyroid cells in vitro (Vitale et al. 2000).

**Materials and Methods**

**Chemicals.** LBI and purified soybean lecithin were kindly supplied by Daiichi Pharmaceutical Co. (Tokyo, Japan). LBI was prepared by the reaction of soybean lecithin with iodine in carbon tetrachloride. PTU, amiodarone, iopanoic acid and erythrosine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Protease K and deoxyribonuclease-free ribonuclease (RNAase A) were obtained from Pharmacia (Uppsala, Sweden). Apop Tag Plus kits were purchased from Oncor, Inc. (Gaithersburg, MD, USA).

**Animals and sample preparation.** Studies with the rats were approved by the Institutional Animal Care and Use Committee. Male Wistar rats weighing around 150 g were used for all experiments. The animals were housed under controlled temperature (24±0.5 °C) and illumination (light on 07.00-19.00 h). Food and water were available *ad libitum*. PTU at 0.03 % in the drinking water was given to rats for 10 days. LBI which contained 6.8% iodine and soybean lecithin were suspended in distilled water and given to rats intragastrically by gastric tube. KI, amiodarone, iopanoic acid and erythrosine were also given by gastric tube. The iodide content of all these compounds was adjusted so that each rat received 10 mg iodine per kg body weight. The volume of ingestion was 2 ml/kg body weight for each compound.

The rats were sacrificed by decapitation. Their thyroids were removed, weighed and homogenized in four volumes of cold 50 mM sodium phosphate buffer (pH 7.2) containing 5 mM dithiothreitol. The homogenates were centrifuged at 10,000 x g for 1 h. The supernatants were filtered through centrifuging filters, Ultrafree C3-GC (Millipore, Bedford, MA, USA). The precipitates were suspended in the same volumes of phosphate buffer (pH 7.2) containing 5 mM dithiothreitol and filtered again. The precipitates were resuspended in the same buffer and used for ODC assay. Polyamines were assayed by high-performance liquid chromatography (HPLC) as described elsewhere (Araki et al. 1991).

**Enzyme assay.** The thyroid ODC activity was determined as described previously (Ma et al. 1996).
Briefly, the enzyme preparations were incubated for 3 h in the presence of 0.5 mM L-ornithine, 0.05 mM pyridoxal 5-phosphate, 1 mM aminoguanidine, 0.1 mM EDTA and 5 mM dithiothreitol. The amounts of putrescine generated during incubation were determined by HPLC (ARAKI et al. 1991). ODC measurements were performed in triplicate.

DNA LADDER FORMATION. Rat thyroids were frozen on dry ice immediately after their removal, washed with PBS containing 0.25 M sucrose and then homogenized in DNA lysis buffer (10 mM Tris, pH 7.4, 10 mM EDTA, 0.5 % Triton x 100). The homogenates were incubated for 30 min at 4°C. The lysates were centrifuged at 10,000 x g for 20 min to separate low molecular weight DNA (supernatants) from high molecular weight DNA (pellets). The pellets were digested with proteinase K (0.2 mg/ml) in the lysis buffer containing 10 mM Tris, pH 8.0, 25 mM EDTA and 0.5 % sodium dodecylsulfate (SDS) for 12 h at 37 °C and then digested with deoxyribonuclease-free RNAase A (40 mg/ml) for 1 h at 37 °C. DNA was extracted twice with the mixture of phenol:chloroform:isoamylalcohol (25:24:1, v/v) and ethanol precipitated. The DNA was resuspended in the buffer containing 10 mM Tris (pH 7.4) and 1 mM EDTA. The DNA concentrations were determined spectrophotometrically at 260 nm. One to 2 mg of DNA were loaded onto each lane of 2.5 % agarose gel and then run over 1 h. DNA bands were visualized by UV illumination after ethidium bromide staining and photographed.

Detection of apoptosis by flow cytometry. The extent of apoptosis was quantified using Apop Tag Plus kits (Oncor). After obtaining cell suspensions from collagenase-EDTA (0.25 % in PBS) incubation and filtration through nylon mesh, cells were fixed for 15 min in ice-cold PBS (pH 7.4) containing 1 % paraformaldehyde. Cells were labelled by indirect immunofluorescence using digoxigenin nucleotide as first antibody which was catalytically added to the DNA by terminal deoxynucleotidyl transferase, and as second antibody antidigoxigenin fluorescein was used according to the manufacturer’s recommendation. Flow cytometry was carried out by analyzing 10,000 cells/test using a Facsscalibur flow cytometer (Becton-Dickinson, Mountain View, CA) equipped with an argon ion laser, 15 mW and multiparameter data acquisition system (LYSIS).

Fig.1 Effects of a single dose of lecithin bound iodine (LBI) on thyroid ornithine decarboxylase (ODC) activity (A) and putrescine levels (B). All rats received 0.03 % propylthiouracil (PTU) in drinking water for 10 days. KI (10 mg/kg body weight as I) and LBI (10 mg/kg as I) were given intragastrically 1 to 3 h before sacrifice. Control rats received only distilled water. Data were shown as means±SEM. Each assay was performed in triplicate. ** = P<0.01 vs. control.

Statistical evaluation was performed by Dunnett’s multiple comparison test to control and non parametric method by Scheffe’s multiple comparison test. When probability was less than 0.05, the results were considered to be significant. All data are presented as the mean±SEM.

Results

PTU treatment for 10 days resulted in a marked increase in thyroid ODC activity (about 15 fold) and in thyroid weight (3 to 5 fold). When iodide was given per os at a dose of 10 mg per kg body weight
(13.1 mg/kg as KI), the thyroid ODC activity was reduced to about 20 % of the initial PTU induced level within 1 h (Fig. 1). LBI (10 mg/kg as I) also suppressed the thyroid ODC activity to 31 % and 21 % at 1 and 3 h, respectively (Fig. 1A), while lecithin itself had no effect on the thyroid ODC activity.

Thyroid levels of putrescine, which is the product of ODC reaction were significantly decreased only at 3 h after LBI treatment (Fig. 1B). Neither KI nor LBI decreased the thyroid putrescine levels at 1 h after their administration.

Iodine containing compounds such as amiodarone, iopanoic acid and erythrosine showed no significant effect on the PTU-induced thyroid ODC activity at 3 h after treatment (Fig. 2). All the iodocompounds were given to rats at a dose of 10 mg/kg body weight as iodine.

When LBI was given orally once daily to PTU-treated rats, both thyroid ODC activity and thyroid weight were decreased at 3 and 5 d of its treatment (Fig. 3A and 3B).

**Induction of apoptosis by LBI.** An internucleosomal DNA fragmentation ladder was evident at 3 h after LBI treatment but restored to normal at 6 h in PTU-pretreated rats (data not shown). The percentage of DNA fragmentation was maximal at 3 h after LBI treatment (Fig. 4). When LBI or soybean lecithin (L) was given to normal rats, no internucleosomal DNA degradation was observed. Soybean lecithin rather lowered the rate of DNA fragmentation though it was not statistically significant. Apoptotic cells were increased in number after LBI treatment as shown by flow cytometry (Fig. 5) and by agarose gel electrophoresis (Fig. 6). The percentage of apoptotic cells was maximal at 6 h after LBI administration when analyzed by flow cytometry. The percentage of digoxigenin labelled cells for the control and LBI-treated group were 5.0 and 10.7 %, respectively.
Discussion

The results of the present study have shown that LBI inhibits rapidly the thyroid ODC activity and, at the same time, induces apoptosis in the thyroid of rats pretreated with PTU. When given chronically to PTU pretreated rats, LBI induces thyroid involution. These effects of LBI seem to be essentially the same as those of KI (MATSUZAKI et al. 1978; MA et al. 1996; BURIKHANOV and MATSUZAKI 2000). Immediately after ingestion, iodide is released from LBI and, therefore, the changes in serum inorganic iodide concentration following the administration of LBI are nearly the same as those occurring after the administration of inorganic iodide (NAMBA et al. 1955). Beneficial effects of LBI on patients with hyperthyroidism have been reported repeatedly (NAMBA et al. 1955; ITO et al. 1963; NOGUCHI et al. 1965; MIYAGAWA et al. 1987). Thus, both LBI and excess iodide can ameliorate signs and complications of thyrotoxic patients essentially in the same manner. LBI, however, is unique in that it has much less undesirable side effects than iodide that induces hypersensitivity or gastrointestinal disorders in some cases (KURIBARA et al. 1977). LBI liberates iodide soon after its ingestion and yet iodide is not easily excreted in the urine (NAMBA et al. 1955). Thus, this drug can serve as a good iodide-carrier in vivo.

Recently several iodinated compounds are found to affect thyroid physiology. For example, iopanoic acid inhibits iodothyronine 5'-deiodinases of type I and II (GERMAIN 1988). Thyroid dysfunction commonly occurs with amiodarone therapy. Amiodarone decreases the peripheral deiodination of T₄ to triiodothyronine by inhibiting type I iodothyronine 5'-deiodinase (HARJAI and LICATA 1997). Amiodarone can induce hypothyroidism either by itself or by iodide liberated in vivo. Our recent study (BURIKHANOV and MATSUZAKI 2000) suggests that amiodarone itself induces thyroid apoptosis before it is deiodinated to release iodide.
Iodine containing compounds such as amiodarone, iopanoic acid and erythrosine have no inhibitory effect on the thyroid ODC activity within 3 h either when given intraperitoneally (Ma et al. 1996) or given per os as shown by the present study. These compounds are thought to undergo deiodination rather slowly in vivo. When taken up by the thyroid, they may inhibit thyroid activity in the form of either iodide or iodocompounds. Probably the concentrations of iodide in the thyroid would not be high enough to suppress the thyroid ODC activity within a few hours after treatment with these iodocompounds.

LBI has been shown to have an antigoitrogenic effect but its action mechanism still remains unclarified. Possibly LBI induces thyroid involution at least partially by inhibiting polyamine biosynthesis, because ODC is the rate limiting enzyme in the polyamine biosynthetic pathway. There is a body of evidence to show that polyamines are involved in goiter formation. For example, diaminopropane which induces antizyme biosynthesis to suppress polyamine biosynthesis, inhibits goitrogenesis (Friedman et al. 1977). The decrease in thyroid polyamine content, in turn, may lead to thyroid apoptosis as shown in the thymus (Gaselli et al. 1995). It is well known that glucocorticoids induce not only thymic involution but also inhibition of polyamine biosynthesis (Scalabrino et al. 1979). The polyamine biosynthesis in the thyroid is reduced and apoptosis is induced by LBI in the same manner as KI (Burikhanov and Matsuzaki 2000). In conclusion, LBI induces thyroid involution partially by inhibiting polyamine biosynthesis and partially by triggering apoptosis.

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Fig. 6 Effect of LBI on DNA fragmentation in the thyroid of PTU-pretreated rats. LBI (10 mg/kg as I-) was given to PTU pretreated rats intragastrically. Lane 1: DNA size marker; Lane 2: DNA from thyroid cells of intact control rats; Lane 3: that of rats after LBI administration; Lane 4: that of PTU pretreated rats 3 h after LBI; Lane 5: that of PTU pretreated rats 6 h after LBI; Lane 6: that of LBI administered rats; Lane 7: that of PTU administered rats; Lane 8: that of control rats; Lane 9: that of PTU pretreated rat 6 h after LBI.


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BOOK REVIEW

THYROID CANCER
A COMPREHENSIVE GUIDE TO CLINICAL MANAGEMENT

EDITED BY LEONARD WARTOFSKY (WASHINGTON, DC)

HUMANA PRESS (TOTOWA, NEW JERSEY) 1999
E-MAIL: HUMANA@HUMANAPR.COM, 515 PAGES, HARD COVER US $ 175.00,

“It sometimes seems that thyroid carcinoma is a neglected orphan among human cancers, which is at the root of some important issues. Thyroid carcinomas comprise a diverse group of malignancies ranging from indolent microscopic papillary carcinomas that pose no treat to survival to anaplastic carcinomas that are the most vicious carcinomas afflicting humans. Yet, because of its low incidence, there have been no prospective randomized clinical trials of the treatment of thyroid carcinoma.” This is a fragment of the “Foreword” written by outstanding thyroid surgeon Ernest L. Mazaferri.

This comprehensive book brings an instructive review of present knowledge on the pathology, etiology, epidemiology, diagnostic methods and the methods of surgical, radioiodine and chemotherapeutic treatment of various forms of thyroid cancer. Written by outstanding experts and professionally edited by Leonard Wartofsky, it may be considered of substantial utility to all physicians dealing with thyroid diseases.

A total of 52 comprehensive chapters is divided into 9 sections dealing with the diagnostics and management of thyroid nodule (Part I), general considerations on the thyroid cancer (Part II), clinical aspects, pathology, treatment, follow-up, prognosis and special aspects in children of papillary carcinoma (Part III), the same aspects of follicular (Part IV) and anaplastic carcinoma (Part V) and lymphoma (part VI). Next sections are devoted to medullary carcinoma (Part VII), unusual thyroid cancers (Part VIII) and future directions (Part IX).

A number of up to date references and instructive tables, figures and photos are attached to each chapter. In addition to basal theoretical knowledge on each problem discussed in individual chapters, there is a number of practical and handy instructions such as a detailed description of fine needle aspiration technique, detailed descriptions of external irradiation techniques and doses, sonography, various imaging procedures and strategies of follow-up the patients after the surgical and radiation treatment. However, some actual questions on the extent of thyroidectomy such as lobectomy versus subtotal or neal-total thyroidectomy would perhaps deserve more detailed discussion.

Of special value may be considered the chapters on molecular pathology of thyroid cancer including significant recent achievements of molecular genetic analysis of inheritance pattern in families with medullary carcinoma.

Finally, again few words of Ernest L. Mazaferri: “I believe the knowledge contained in Thyroid Cancer will give the practicing clinicians the necessary information to provide patients the latest and best diagnostic and therapeutic techniques.”

Pavel Langer