European Journal of Endocrinology (2002) **147** 293–298 ISSN 0804-4643

CLINICAL STUDY

Effect of iodine or iopanoic acid on thyroid Ca^{2+}/NADPH-dependent H_2O_2-generating activity and thyroperoxidase in toxic diffuse goiters

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Abstract

Objective: The aim of the present study was to compare the effects of iopanoic acid (IOP) or a saturated solution of potassium iodide (SSKI) administration to patients with toxic diffuse goiters (TDG).
Design: Patients with TDG are treated with thionamides and high doses of iodine preoperatively. In this study, two types of preoperative drug regimens were used: propylthiouracil or methimazole plus SSKI for 10–15 days ($n = 8$) or IOP for 7 days ($n = 6$).
Methods: Serum thyroid hormones (total and free thyroxine (T_4), total tri-iodothyronine (T_3) and reverse T_3 (rT_3), were evaluated after 7 days of either SSKI or IOP treatment, and after 10–15 days of SSKI administration. During thyroidectomy, samples of thyroid gland were obtained to evaluate thyroperoxidase and thyroid H_2O_2-generating activities.
Results: Serum total T_3 was significantly decreased after 7 days of either treatment, and serum rT_3 was significantly increased in IOP-treated patients. Serum total and free T_4 were unaffected by 7 days of IOP treatment, but decreased after 7 days of SSKI treatment, although significantly diminished levels were only reached after a further 3–8 days of SSKI administration. During both drug regimens, serum TSH remained low (SSKI: 0.159±0.122; IOP: 0.400±0.109 μU/ml). Thyroperoxidase activity was significantly lower in thyroid samples from patients treated with SSKI for 10–15 days than in the thyroid glands from IOP-treated patients. However, thyroid H_2O_2 generation was inhibited in samples from patients treated with either IOP or SSKI.
Conclusions: We show herein that IOP treatment can be effective in the management of hyperthyroidism and that this drug inhibits thyroid NADPH oxidase activity, just as previously described for SSKI, probably due to its iodine content.

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Introduction

Toxic diffuse goiter (TDG) is characterized by hypertrophy and hyperplasia of the thyroid gland and hyperthyroidism. In TDG patients, the management of hyperthyroidism can be achieved by treatment with thionamides and iodine preoperatively (1).

Acute iodine administration causes inhibition of thyroid hormone release and reduces thyroid gland vascularity (2, 3). Thus, patients with TDG are usually treated for 10–15 days with iodine preoperatively, in order to improve the control of thyrotoxicosis and decrease bleeding during surgery.

Apart from the inhibition of thyroid hormone release, thyroid auto-regulation by iodine involves the inhibition of adenylate cyclase activity, iodide transport into thyrocytes, and iodine organification (3–7). Thyroid iodine organification depends on thyroperoxidase (TPO) activity and thyroid H_2O_2 generation, a limiting step in thyroid hormone biosynthesis (8). Previous studies have shown that high iodide concentrations inhibit TPO activity *in vitro* (9). Furthermore, it has recently been demonstrated that the thyroid H_2O_2-generating system – NADPH oxidase – is inhibited in TDG patients who receive high doses of iodine preoperatively (10). In fact, Ohayon *et al.* (11) demonstrated that NADPH oxidase activity is inhibited by iodide *in vitro*.

Some iodinated radiographic contrast agents, like iopanoic acid (IOP) and sodium ipodate, when used for short periods, have been successful in the treatment of thyrotoxicosis (12–17). They seem to be safe, rapid

and efficacious drugs to use in hyperthyroid patients before surgery. IOP might release iodine (1 g IOP contains 650 mg iodine) and thus decrease thyroid hormone production and secretion (18–20). Nevertheless, this drug is also a potent inhibitor of thyroxine (T_4) 5′-monodeiodination, blocking the conversion of T_4 to tri-iodothyronine (T_3) (19, 21).

The aim of the present study was to compare the effects of either IOP or iodine administration on thyroid function in TDG patients programmed for thyroidectomy. The study was performed with two types of regimens: one conventional, propylthiouracil (PTU) or methimazole (MMI) plus a saturated solution of potassium iodide (SSKI) for 10–15 days, and another with PTU or MMI plus IOP for 7 days. We evaluated serum thyroid hormone (total and free T_4, total T_3 and reverse T_3 (rT_3)) levels after 7 days of either SSKI or IOP treatment, as well as 10–15 days after SSKI treatment. TPO iodide oxidation activity and thyroid H_2O_2-generating activity (NADPH oxidase) were evaluated in the thyroid samples obtained from these patients during thyroidectomy.

Materials and methods

Materials

NADPH, lyophilized horseradish peroxidase (HRP, grade 1) and glucose oxidase (25 000 U) were purchased from Boehringer (Mannheim, Germany); scopoletin, digitonin and FAD were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Patients

Fourteen patients with TDG were randomly selected to receive either SSKI or IOP prior to surgery (12 females and 2 males). Eight patients (one male, age median: 32.5 years, range: 16–43 years) received SSKI (5 drops three times per day) for 10–15 days before thyroidectomy. Six patients (one male, age median: 30.5 years, range: 21–41 years) received 1 g IOP (Telepaque, Merck do Brasil) daily for 6 days plus 3 g at 12 h before surgery, which took place on the 7th day of treatment. All TDG patients received either PTU (500–900 mg/day) or MMI (25–50 mg/day) until the day before surgery. Paranodular thyroid tissue samples were obtained from 13 female patients with cold nodules and normal serum T_4, T_3 and thyrotropin (TSH) levels who did not receive any treatment before surgery. All patients gave their informed consent. The study was approved by the Institutional Human Research Committee.

Thyroid tissue samples were obtained at thyroidectomy and either freshly processed for NADPH oxidase measurements or stored at − 20 °C for TPO extraction and activity determination.

Serum hormone levels

Blood samples were collected just before the start of treatment (basal), 7 days afterwards and on the day of surgery (day 7 for IOP-treated patients and day 10–15 for SSKI-treated patients). Total T_4, total T_3 and TSH (third generation) were measured using a solid-phase, chemiluminescent enzyme immunoassay (Immulite); free T_4 and rT_3 were measured by RIA and all kits were purchased from Diagnostic Products Corporation, Los Angeles, CA, USA.

Thyroid samples processing

For NADPH oxidase preparations, fresh human thyroid tissue samples (1 g) were cleaned from fibrous tissue or hemorrhagic areas, minced and homogenized in sodium phosphate buffer, pH 7.2, containing 0.25 mol/l sucrose, 0.5 mmol/l dithiothreitol and 1 mmol/l EGTA, using an Ultra-Turrax (IKA, Staufen, Germany). The homogenate was filtered through cheesecloth. The particulate fraction was collected by centrifugation at 3000 ***g*** for 15 min at 4 °C and resuspended in 3 ml 50 mmol/l sodium phosphate buffer, pH 7.2, containing 0.25 mol/l sucrose and 2 mmol/l $MgCl_2$ (buffer A). The pellet was washed twice with 3 ml buffer A and centrifuged at 3000 ***g*** for 15 min at 4 °C. The last pellet (P 3000 ***g***) was gently resuspended in 1 ml buffer A. The supernatant of the first centrifugation was centrifuged at 100 000 ***g*** for 1 h at 4 °C. The pellet (microsomal fraction, P 100 000 ***g***) was washed twice in 2 ml buffer A, and gently resuspended in 0.5 ml buffer A. Protein concentrations were measured by the method of Bradford (22), using BSA as standard. The particulate fractions (P 3000 ***g*** and P 100 000 ***g***) were incubated with 2.5 mol/l NaOH (30 min, 20 °C) to dissolve particulates before protein determination.

For TPO preparation, thyroid tissue samples (1 g) were cleaned from fibrous tissue or hemorrhagic areas, minced and homogenized in 50 mmol/l Tris–HCl buffer pH 7.2, containing 1 mmol/l KI, using an Ultra-Turrax. The homogenate was centrifuged at 100 000 ***g*** for 1 h at 4 °C and the pellet was resuspended in 2 ml digitonin (1%, w/v). The mixture was incubated at 4 °C for 24 h and then centrifuged at 100 000 ***g*** for 1 h at 4 °C. The supernatant containing solubilized TPO was used for the iodide oxidation assays, as previously described (23, 24).

Ca^{2+}- and NADPH-dependent H_2O_2-generating system: NADPH oxidase activity

H_2O_2 formation was measured by incubating samples of thyroid particulate fractions (P 3000 ***g*** and P 100 000 ***g***), at 30 °C, in 1 ml 170 mmol/l sodium phosphate buffer, pH 7.4, containing 1 mmol/l sodium azide, 1 mM EGTA, 1 μmol/l FAD, 1.5 mmol/l

$CaCl_2$, as previously described (10, 25). The reaction was started by adding 0.2 mmol/l NADPH; aliquots of 100 μl were collected at intervals up to 20 min, and mixed with 10 μl 3 mol/l HCl to stop the reaction and destroy the remaining NADPH. Initial rates of H_2O_2 formation were determined from eight aliquots of each assay by following the decrease in 0.4 μmol/l scopoletin fluorescence in the presence of HRP (0.5 μg/ml) in 200 mmol/l phosphate buffer, pH 7.8, in a spectrofluorimeter (Hitachi F 4000; Hitachi, Tokyo, Japan), as previously described (10, 25, 26). The excitation and emission wavelengths were 360 and 460 nm respectively. All measurements were performed at least in triplicate for each particulate preparation and expressed as nmol H_2O_2/h. Specific activities were expressed per mg protein (nmol H_2O_2/h per mg protein) in the thyroid P 3000 ***g*** and P 100 000 ***g*** fractions.

TPO iodide oxidation activity

Thyroid peroxidase iodide oxidation assays were performed using 12 mmol/l KI in 50 mmol/l phosphate buffer (pH 7.4), and glucose–glucose oxidase as the H_2O_2-generating system, as previously described (23, 24). The increase in absorbancy at 353 nm (Δabs_{353nm}) was followed for 4 min on a U-3300 double-beam spectrophotometer (Hitachi). TPO activity was estimated from the Δabs_{353nm}/min determined from the linear portion of the reaction curve. One unit of iodide oxidation activity is defined as Δabs_{353nm}/min (U) = 1.0, and activity was related to the protein concentration in the enzyme preparation (U/g protein).

Analysis of TPO levels by gel electrophoresis and immunoblot

TPO preparations (50 μg protein) from one SSKI-treated and one IOP-treated patient were submitted to SDS-PAGE using a 6.5% bis-acrylamide gel, for 4 h at 120 V. Electro-transfer of proteins from gel to nitrocellulose was performed for 2 h at 250 mA. The nitrocellulose blot was incubated with anti-rabbit TPO antibody diluted 1:1000 (a gift from Dr Rees Smith, Cardiff, UK) for 2 h. The blots were subsequently incubated with anti-rabbit IgG diluted 1:10 000, as second antibody, for 2 h. The immunoblots were revealed by an enhanced chemiluminescence technique (ECL; Amersham, Amersham, Bucks, UK).

Statistical analysis

Statistical analysis of serum hormone levels at different periods of iodine or IOP treatment was done using the two-way ANOVA (Super ANOVA; Abacus, Berkeley, CA, USA). Serum free T_4 levels in SSKI-treated patients were also analyzed by one-way ANOVA for repeated measures followed by Bonferroni multiple comparison

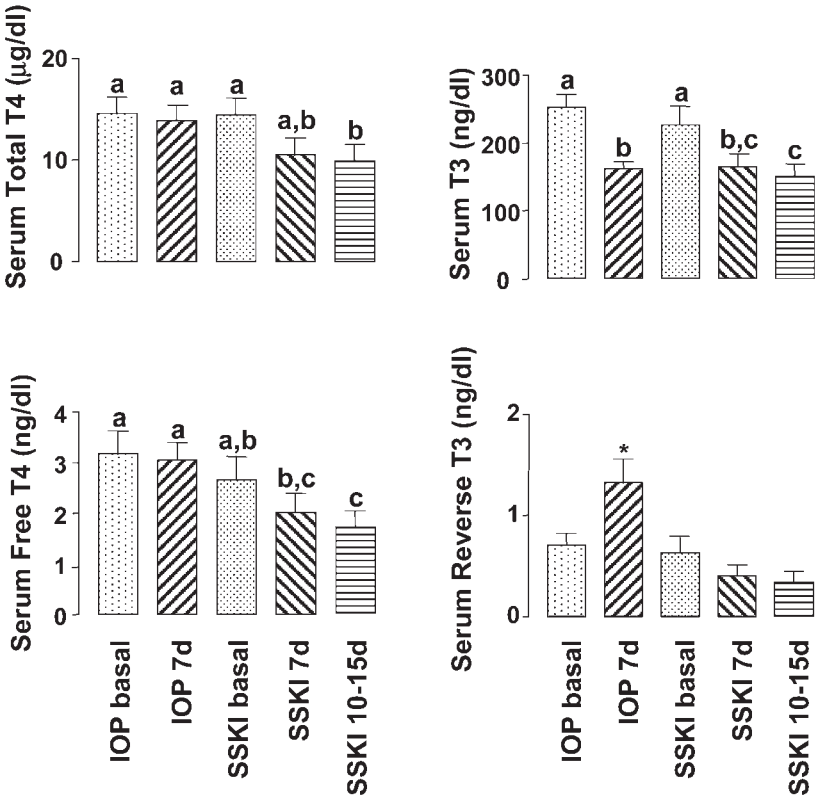

Figure 1 Serum total and free T_4, total T_3 and rT_3 levels in TDG patients treated with SSKI for 10–15 days before thyroidectomy or with 1 g IOP daily for 6 days plus 3 g 12 h before surgery. All TDG patients received either PTU (500–900 mg/day) or MMI (25–50 mg/day) until the day before surgery. Results are expressed as means±S.E.M. Normal range: total T_4: 4.0–13.0 μg/dl; free T_4: 0.80–2.0 ng/dl; T_3: 70–210 ng/dl; rT_3: 0.09–0.35 ng/dl. Different letters mean statistically different values (significance level: $P < 0.05$).

tests. TPO and NADPH oxidase activities were analyzed by ANOVA followed by a Newman–Keuls multiple comparison test. Results are expressed as means±S.E.M.

Results

Basal serum thyroid hormone levels did not differ significantly between the groups of patients studied (IOP or SSKI). Total and free T_4 did not decrease after 7 days of IOP treatment (total T_4 basal: 14.61±1.51, total T_4 7 day: 13.90±1.43 μg/dl; free T_4 basal: 3.19±0.45, free T_4 7 day: 3.05±0.36 ng/dl) but were diminished, although not significantly, after 7 days of SSKI treatment (total T_4 basal: 14.47±1.67, total T_4 7 day: 10.56±1.63 μg/dl; free T_4 basal: 2.66±0.46, free T_4 7 day: 2.02±0.37 ng/dl) and reached significantly lower levels after 10–15 days of SSKI administration (total T_4: 10.05±1.56 μg/dl; free T_4: 1.73±0.32 ng/dl), when compared with basal levels (Fig. 1).

T_3 decreased significantly after 7 days of IOP (basal: 252.40±19.06, 7 day: 162.10±10.21 ng/dl) or SSKI (basal: 226.10±25.57, 7 day: 164.20±19.77 ng/dl) treatment, and rT_3 significantly increased in IOP-treated patients (basal: 0.71±0.12, 7 day: 1.33±0.23 ng/dl) (Fig. 1). Serum TSH levels remained low in both groups studied (SSKI: 0.159±0.122; IOP: 0.400±0.109 μU/ml, normal range: 0.30–5.0 μU/ml).

TPO activity is increased in non-treated TDG patients, but in this study TPO activity in the thyroid of SSKI-treated patients did not differ significantly from the activity found in paranodular tissues, while TPO activity was higher in IOP-treated patients, indicating that SSKI administration for 10–15 days reduces TPO activity more efficiently (Fig. 2). A TPO immunoblotting also suggested a greater amount of TPO in the thyroid samples from IOP-treated patients than in those from SSKI-treated patients (Fig. 3).

Thyroid NADPH oxidase activity was not detected in the P 3000 ***g*** fraction from tissues of either SSKI- or IOP-treated patients; however, in the P 100 000 ***g*** fraction the enzymatic activity was within the normal range in both groups of patients (Fig. 4).

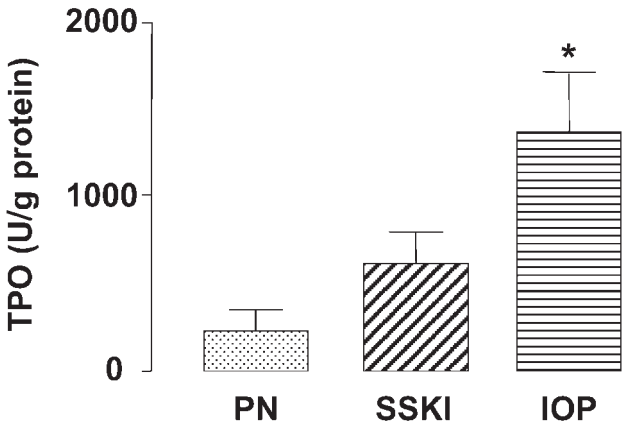

Figure 2 TPO iodide oxidation activity. TPO iodide oxidation activity was measured in 14 patients with DTG. Eight patients were treated with SSKI and six patients received 1 g IOP. Paranodular thyroid tissue samples (PN) were obtained from female patients with cold nodules. Results are expressed as means±S.E.M. *$P < 0.05$ when compared with PN and SSKI, parametric one-way ANOVA followed by a Newman–Keuls multiple comparison test.

Discussion

The use of oral radiographic contrast agents like IOP may be useful in the treatment of thyrotoxicosis before surgery, in neonatal hyperthyroidism and in massive L-T_4 ingestion, or when other antithyroid drugs are contraindicated (20). In the present study, the period of treatment with IOP or SSKI in association with anti-thyroid drugs was sufficient for all to achieve clinical euthyroidism before surgery.

Some authors reported that the use of these agents could induce a rapid decrease in serum T_3 levels, which reached normal levels after 2–5 days of treatment (13, 27). In the present study, both SSKI and IOP treatment significantly decreased serum T_3 levels to values within the normal range after 7 days of treatment. The finding that rT_3 was significantly increased by IOP treatment is in accord with the previously described action of this drug as a potent T_4 5′-deiodinase inhibitor. Thus, IOP might induce serum T_3 decrease due to T_4 deiodinase inhibition; however, SSKI treatment could lead to a direct inhibition of T_3 secretion by the thyroid gland. This hypothesis is in agreement with the fact that SSKI treatment was able to alter serum total and free T_4 levels, which were already decreased, although not significantly, after 7 days, and decreased further 10–15 days after the beginning of iodine administration.

The effect of excess iodide, accumulated in a short period of time, in the thyroid gland, is known to

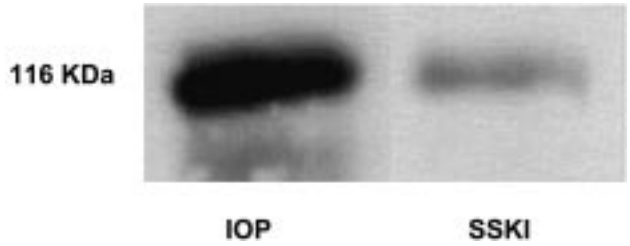

Figure 3 TPO immunoblotting. TPO preparations (50 μg protein) were submitted to SDS-PAGE using a 6.5% bis-acrylamide gel, for 4 h at 120 V. Electro-transfer of proteins from gel to nitrocellulose was performed for 2 h at 250 mA. The immunoblotting revealed a reasonable signal in a TPO sample from a patient treated with SSKI when compared with an IOP-treated patient.

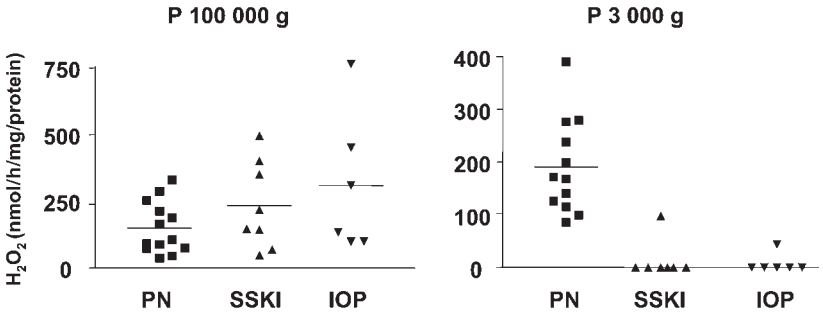

Figure 4 Thyroid NADPH oxidase activity. Ca^{2+}- and NADPH-dependent H_2O_2-generating activity were measured in 13 paranodular to cold nodule tissue samples (PN), from eight SSKI-treated and six IOP-treated patients with TDG. Particulate P 3000 ***g*** fraction or microsomal P 100 000 ***g*** fraction was evaluated.

promote an acute inhibition of the synthesis of iodotyrosines and iodothyronines (4). Increased amounts of iodide transported into the thyrocyte induce the formation of an iodinated compound (3), which could be the mediator for some effects of excess iodine. A decrease in the sodium/iodide symporter 24 h after excess iodide has also been demonstrated in rats, and could explain the escape from iodine administration (28).

We recently reported (10) that SSKI administration to TDG patients irreversibly inhibited thyroid NADPH oxidase activity in the P 3000 ***g*** fraction, even in one patient who received only iodine and propranolol prior to surgery. NADPH oxidase inhibition occurred in the present study in both SSKI- and IOP-treated patients. These findings suggest that IOP releases iodide *in vivo*, since iodide appears to be a potent inhibitor of this enzymatic system both *in vitro* (11, 29) and *in vivo* (10). NADPH oxidase synthesis is dependent on the cAMP cascade stimulated by TSH in thyrocytes (30, 31). Excess of iodide decreases cAMP in thyroid cells in response to TSH, but the strong inhibitory effect of iodide on thyroid NADPH oxidase, which was only found in the P 3000 ***g*** fraction of TDG, is probably not only due to decreased enzyme synthesis, as enzyme activity was within the normal range in the P 100 000 ***g*** from the same samples. Besides, TPO synthesis is also stimulated by cAMP, and this enzyme activity was not decreased after 7 days of IOP administration when NADPH oxidase has already been inhibited in the P 3000 ***g***.

Our present findings showing that SSKI administration, but not IOP, is able to significantly decrease TPO activity, maybe due to the longer period of treatment with SSKI than with IOP. These results indicate that iodine might inhibit TPO synthesis, probably due to decreased thyroid response to TSH. Nevertheless, we demonstrate that TPO seems not to be directly inhibited by iodide, at least irreversibly, as has previously been suggested (9). Furthermore, high doses of *in vivo* PTU or MMI administration did not irreversibly inhibit TPO, as both SSKI- and IOP-treated patients received these drugs during the study.

We conclude that SSKI (10–15 days) and IOP (7 days) have similar effects on thyroid function and that both are effective in the management of hyperthyroidism prior to surgery.

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Received 26 March 2002
Accepted 10 June 2002