

IN VITRO NUCLEAR MEDICINE

Would I-123 Di-Iodotyrosine Provide a Harmless Deiodination Test?
Concise Communication

A. Aurengo, F. Savoie, A. F. Leger, and J. C. Savoie

Service Central de Médecine Nucléaire, Hôpital de la Pitié, Paris, France

Defective iodotyrosine deiodinase activity may benefit from a specific treatment, thus requiring an unequivocal diagnosis. In reported cases this diagnosis has been obtained from an in vivo deiodination test making use of di-iodotyrosine (DIT) labeled either with I-131 or I-125. Dosimetric calculation indicates that such tests may result in unacceptable irradiation of the thyroid of a child wrongly suspected of having defective iodotyrosine deiodinase activity; therefore other methods are needed. The use of I-123 DIT is shown to be feasible, but even a 1:30 reduction in the thyroid dose still remains too high. Suppression of thyroid I⁻ uptake by ClO₄⁻, together with I-125 DIT, eliminates almost all thyroid irradiation and provides a sensitive, harmless, and rapid test.

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The iodotyrosine deiodinase defect (IDD) is an exceptional and seldom diagnosed congenital disease (1,2) defined by the lack of this enzyme both in the thyroid gland and at the periphery (3). Undeiodinated iodotyrosines are excreted in the urine, resulting in a state of secondary iodine deficiency manifested as a nonspecific hypothyroidism with goiter. However rare the condition may be compared with other congenital defects in thyroid hormone synthesis, the detection of IDD is very important, since pharmacological doses of iodine can provide temporary remissions and lipiodol may be effective for years. IDD should be diagnosed by investigating peripheral iodotyrosine deiodination, which can be obtained by straightforward methods. These methods involve the i.v. injection of labeled DIT and the measurement of its recovery in urine, either undegraded or converted to iodide (I⁻). This deiodination test is usually performed with DIT labeled with I-131 or I-125. Adverse effects in the clinical use of those labels, however, should not be overlooked. When DIT labeled with either I-131 or I-125 is injected into a patient wrongly sus-

pected of IDD, the normal deiodination occurs and the label I⁻ can be concentrated by the thyroid, irradiating the gland. This hazard, not fully evaluated as yet, we found to be unacceptable.

Our aim, therefore, was to investigate two methods suitable for reducing thyroid irradiation, namely:

1. The use of a short-lived label (I-123 DIT) to lower the cumulated activity of the label.
2. The conjoint use of I-125 DIT together with a competitive perchlorate anion (ClO₄⁻) suppressing the thyroid uptake of iodide.

MATERIALS AND METHODS

Selection of patients and controls. Iodotyrosine deiodinase activity (IDA) was investigated in vivo in seven patients and eight controls using I-123 DIT, and at least 6 mo earlier or 6 wk later using I-125 DIT together with a competitive anion (ClO₄⁻). The seven patients were well-known cases of defective IDA, all with proven in vivo failure of DIT deiodination according to the usual test (4). All seven patients were being treated by iodine excess. The eight controls were healthy volunteer members of our department, free from any known disease and undergoing no treatment.

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For reprints contact: Pr. J. C. Savoie, Service Central de Médecine Nucléaire, Hôpital de la Pitié, 83 Boulevard de l'Hôpital 75651 Paris cedex 13, France.

Methods pertaining to I-123 DIT. *Preparation of I-123 labeled DIT.* The labeling of I-127 DIT was carried out by exchange reaction according to the chloramine-T method (5) with I-123 produced by the ^{127}I (p , $5n$) ^{123}Xe reaction. The labeling procedure began immediately after delivery of the tracer, whose radionuclidic purity at that time was over 98%, the only contaminant being I-125.

First, 100 μl of the I-123 solution provided (containing 0.5 mCi/ml) and 100 μl of chloramine-T solution dissolved to a concentration of 2.18 mg/ml in 0.1 M phosphate buffer (pH = 7.4) were stirred with 100 μl of the stable DIT solution dissolved to a concentration of 10 $\mu\text{g/ml}$ in 0.1 M phosphate buffer at pH = 7.4. After the mixture had been allowed to stand for 3 min, the reaction was stopped by adding 100 μl of 5% Na_2SO_3 , then 100 μl of 1% KI.

To purify the labeled DIT, the mixture was passed through a 3-ml ion-exchange resin column 6 cm high.* The column was washed with 15 ml of water, $^{123}\text{I}^-$ being retained on the resin. The yield of the labeling procedure (I-123 recovered as DIT) was between 80% and 90%. The specific activity obtained was close to 45 $\mu\text{Ci/g}$ DIT. The chromatographic effluent was evaporated to dryness under vacuum at 40°C, then dissolved in 6 ml of 0.155 M NaCl. The mixture was sterilized through a 0.22- μm Millipore filter and collected in a sterile vial. The radioactive concentration of the final solution ranged between 10 $\mu\text{Ci/ml}$ and 12 $\mu\text{Ci/ml}$. The free radioiodide contaminating the tagged DIT was found to be less than 2%.

These procedures and controls took approximately 3 hr and the I-123 DIT was used as soon as it has been prepared.

Quality control. The lack of bacteriological or pyrogenic contamination of the final preparation was checked first on a blank preparation and, retrospectively, on the preparation actually injected. Sterility tests were performed on two culture media (one for aerobic or anaerobic bacteria, the other for fungi). Apyrogenicity was checked for endotoxins by the *Limulus* lysate test (6). All these tests showed the bacteriological and pyrogenic safety of the injected solution. Radiochemical purity was checked according to methods previously described for I-125 labeling (7).

In vivo investigation of IDA with I-123 DIT. For each patient or control, 8 μCi to 10 μCi (3.5×10^5 Bq) of I-123 DIT solution was injected into an arm vein. The syringe was weighed before and after injection for an accurate determination of the injected volume, with activity referred to as A_0 . Urine was collected one hour after the injection. The total activity excreted in the first-hour urine (U1) was measured with correction for exponential decay. A sample of the first-hour urine was then analysed for the excretion of either intact labeled DIT or free radioiodide. This test used two independent and comple-

mentary methods. The first uses the iodide retention by the anionic AG 1X2 resin in the Cl^- form, to separate free I-123 from DIT as described in "Preparation of I-123 labeled DIT." The second separation method makes use of a cationic resin† which retains DIT but not iodide. The retained DIT is eluted with 2N NH_4OH . In both cases the total activity deposited on the column and the activity recovered as DIT were counted. The ratio of DIT activity to total excreted activity will be referred to as R1 (result obtained with the first method) or R2 (obtained with the second). For each patient and control, the thyroid uptake fraction, 24 hr after the injection, was determined by external counting with appropriate background subtraction and correction for radioactive decay.

Methods using I-125 DIT plus ClO_4^- . *Preparation of I-125-labeled DIT.* The labeling of stable DIT with I-125 has been described previously (7) and is similar to the labeling with I-123. I-125 DIT was stored at -20°C . It can be used as such for 2 mo and even longer after repurification from released I-125 $^-$ by anion exchange resin chromatography.

Quality control. The quality control was the same as in the previous case.

In vivo investigation of IDA with I-125 DIT plus ClO_4^- . Each patient or control received 400 mg KClO_4 , taken by mouth half an hour before the deiodination test. The test by itself was the same as with I-123 DIT. For dosimetric checking, the urines were collected for the 48 hr following the injection, and the excreted activity was determined.

Calculation of the absorbed dose. In the case of real IDD, no deiodination occurs and injected DIT was assumed to be uniformly distributed in the extracellular space. DIT is excreted in the urine with a clearance of about 3 l/hr per m^2 (8). Using a single-compartment model, we can determine the cumulated activity, A, of radiotracer in the whole body and especially in the thyroid. Conversely, in the case of wrongly suspected IDD, we have assumed that total and immediate deiodination of the injected DIT occurs. Two cases must be considered:

1. In the absence of competitive anion, we have assumed for a normal subject a maximum thyroid uptake of 25%. The cumulated activity, A, of the radiotracer in the thyroid is calculated from Berman's model (9,10). In the case of a hypothyroid subject with normal IDA, an estimate of the maximum dose to the thyroid was calculated, assuming the worst dosimetric conditions: immediate and complete iodine uptake, and no release of iodine from the thyroid.

2. With the use of a competitive anion, the thyroid uptake is completely inhibited and the liberated iodine is eliminated in the urine with a clearance of about 1 l/hr per m^2 . The cumulated activity is calculated as in the case of real IDD.

TABLE 1. RESULTS OF DEIODINATION TEST IN VIVO WITH I-123 DIT OR WITH I-125 DIT TOGETHER WITH ClO₄⁻

	Patient	Age	Sex	I-123 DIT					I-125 DIT + KClO ₄			
				U1/Ao*	R1†	R2†	R†	T24‡	U1/Ao	R1	R2	R
IDD	1	18	M	50.1	98.1	96.3	97.2	1.0	49.6	97.2	96.6	96.9
	2	40	F	50.9	98.8	98.0	98.4	0.7	47.2	98.5	96.3	97.4
	3	13	M	23.6	96.2	97.6	96.9	0.8	32.6	98.4	96.8	97.6
	4	25	M	11.0	97.5	97.3	97.4	0.6	23.5	95.8	97.2	96.5
	5	26	F	38.8	96.7	95.5	96.1	0.4	42.3	97.1	94.5	95.8
	6	30	M	29.7	96.6	97.4	97.0	0.3	25.6	95.3	97.5	96.4
	7	15	M	34.7	97.6	95.8	96.7	1.1	38.3	97.1	97.3	97.2
Mean		23.9		34.1	97.4	96.8	97.1	0.7	37.0	97.1	96.6	96.8
± s.d.		9.4		14.3	0.9	1.0	0.7	0.3	10.2	1.2	1.0	0.6
Control	1	30	M	16.1	19.9	17.1	18.5	28.1	15.1	16.0	15.8	15.9
	2	27	F	16.3	10.5	9.9	10.2	33.4	10.6	14.4	12.2	13.3
	3	57	M	14.6	9.8	10.2	10.0	20.5	16.0	12.2	11.0	11.6
	4	29	F	14.0	22.6	21.8	22.2	23.2	14.9	22.8	19.0	20.9
	5	25	M	10.1	11.0	11.8	11.4	31.6	13.4	13.4	12.2	12.8
	6	23	F	10.8	4.5	5.7	5.1	28.4	9.4	6.1	6.5	6.3
	7	31	M	12.5	4.8	6.0	5.4	22.0	17.2	6.8	7.6	7.2
	8	45	F	16.6	20.7	19.1	19.9	26.2	12.6	20.6	19.6	20.1
Mean		33.4		13.9	13.0	12.7	12.8	26.7	13.7	14.0	13.0	13.5
± s.d.		11.6		2.5	7.2	6.0	6.6	4.6	2.7	5.9	4.8	5.3

* U1/Ao = total activity in first-hour urine, as percentage of injected activity.

† R1, R2, R = first, second, and mean determination of proportion of urinary radioiodine recovered as intact DIT (%).

‡ T24 = thyroid uptake at 24 hr (%).

In every case by knowing the cumulated activity A and the thyroid mass m, the dose D to the thyroid was calculated by the usual method (11):

$$D = \frac{A}{m} \sum_i (\Phi_i \Delta_i)$$

[for every radiation emitted i, Δ_i is the equilibrium dose constant (10) and Φ_i the fraction absorbed in the target organ (12,13)].

The dose to the thyroid resulting from any extrathyroidal source was found to be negligible due to the very low values of the absorbed fraction Φ_i .

The thyroid was considered as a pair of ellipsoids with principal axes in the ratio 4:2:1. This method allows doses to be calculated for various thyroid masses (2–200 g), compatible with normal children's thyroids and with goiters. For a normal thyroid mass (19.6 g) our method gave dose calculations that are within 10% of the doses obtained by other authors (10) using a more accurate thyroid model. In the case of I-123 DIT, 2% contamination by I-125 DIT was assumed (14).

RESULTS

Comparative study of tests with I-123 DIT and with

I-125 DIT + KClO₄. The diagnostic values of the test are summarized in Table 1.

For both patients and controls:

No significant difference [with a Mann-Whitney test (15)] was found between the ratios R1 and R2 (proportion of label excreted as DIT as determined by the two methods). The mean value will be referred to as R = (R1 + R2)/2.

No significant difference was found between the values of R obtained with the "I-123 DIT" test or with the "I-125 DIT + ClO₄⁻" test. Therefore ClO₄⁻ administered in vivo modifies neither peripheral iodotyrosine deiodinase activity nor the ion-exchange separation of I⁻ from DIT used in the tests. This last point was verified in vitro. In the group of patients with IDD, almost all the urinary activity was recovered as intact DIT. In the control group, by contrast, 12.5% of the urinary activity was recovered as intact DIT.

Whatever the test, the difference in R between the two groups was obvious and highly significant (p < 0.001).

Calculation of absorbed doses. Absorbed doses from I-123 DIT and from I-125 DIT (without any competitive anion) are summarized in Fig. 1 and Table 2.

In Fig. 1, the thyroid dose is plotted against thyroid

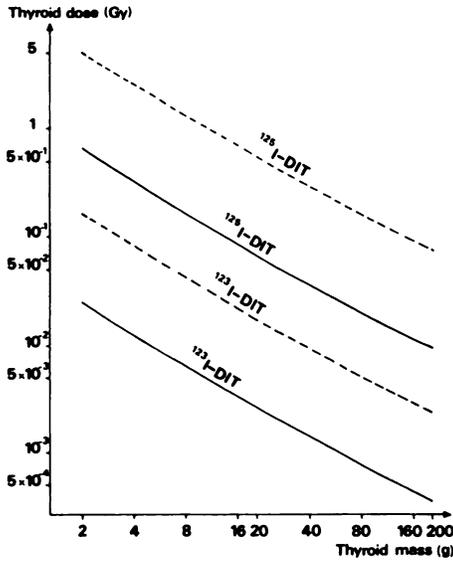


FIG. 1. Dose estimates to thyroid in patient with normal iodotyrosine-deiodinase activity, for normal iodine uptake (—), and maximal iodine uptake (---).

mass for the case of normal IDA. The doses obtained with I-123 DIT are about 1/30 of those obtained with I-125 DIT.

In the case of defective IDA, the calculations showed that the doses to the thyroid are very weak and approximately equal, with either I-125 or I-123 DIT.

In order to provide a clinically meaningful order of magnitude, the dose estimates were calculated for two extreme models: a child and an adult.

In both cases, the thyroid's absorbed dose is almost independent of the label's physical half-life, due to the high urinary clearance of DIT. The doses with I-123 DIT are somewhat greater than with I-125 DIT, since the equilibrium dose constant for nonpenetrating radiations is 50% higher for I-123 than for I-125. It was assumed in the preceding calculations that no thyroid uptake of the tracer occurs in IDD. This was all the more likely in

TABLE 2. DOSE ESTIMATES TO THE THYROID IN MODEL INFANT, CHILD, AND ADULT WITH DEFECTIVE IODOTYROSINE DEIODINASE ACTIVITY (TESTED WITHOUT COMPETITIVE ANION)

	Dose to thyroid with an I-123 DIT test	Dose to thyroid with an I-125 DIT test
Infant of 7 kg, thyroid 2 g	94 μ rad (0.94 μ Gy)	68 μ rad (0.68 μ Gy)
Child of 20 kg, goiter 20 g	47 μ rad (0.47 μ Gy)	36 μ rad (0.36 μ Gy)
Adult of 70 kg, goiter 200 g	25 μ rad (0.25 μ Gy)	20 μ rad (0.20 μ Gy)

TABLE 3. DOSE ESTIMATES TO THE THYROID WITH A I-125 DIT TEST AND A COMPETITIVE ANION (ClO₄⁻) (INFANT, CHILD, AND ADULT MODELS AS IN TABLE 2)

	Dose to thyroid in patient with IDD	Dose to thyroid with normal IDA
Infant of 7 kg, thyroid 2 g	68 μ rad (0.68 μ Gy)	200 μ rad (2.00 μ Gy)
Child of 20 kg, goiter 20 g	36 μ rad (0.36 μ Gy)	110 μ rad (1.10 μ Gy)
Adult of 70 kg, goiter 200 g	20 μ rad (0.20 μ Gy)	60 μ rad (0.60 μ Gy)

our group of seven IDD patients since they were already on high iodine intake before the tests.

Estimates of the thyroid's absorbed doses from a "I-125 DIT + ClO₄⁻" test are given in Table 3. The doses are very weak, and of the same order of magnitude whatever the peripheral iodotyrosine deiodinase activity may be. Evidence for a lack of thyroid uptake cannot be obtained from external counting with I-125 DIT. However:

(a) The complete inhibition of thyroid uptake by ClO₄⁻ has been reported by many authors (16);

(b) In every control the injected activity recovered in urine was over 90% in 24 hr and 98% in 48 hr; and moreover,

(c) Thyroid uptake inhibition by ClO₄⁻ was tested by using I-131 DIT in two of our eight control volunteers (400 mg KClO₄ taken by mouth half an hour before the i.v. injection of 10 μ Ci of I-131 DIT) and the 24-hr thyroid radioactivity could not be distinguished from background.

DISCUSSION

The diagnosis of defective iodotyrosine deiodinase activity has to be unequivocal, since a specific quasieuthyroidic treatment is available. Correction of the iodine deficiency—due to the urinary loss of mono- and diiodotyrosines—cures the hypothyroidism and in a patient with IDD restores normal hypothalamo-hypophyseal control of thyroid hormone secretion. This treatment avoids the frequent clinical and hormonal follow-up checks demanded (especially for children) by substitution treatment in other cases of hypothyroidism. IDD, however, is a nonspecific clinical state, and its diagnosis may only be suspected in the case of hypothyroidism with goiter and a very rapid turnover of the thyroidal radioiodine. In other cases the defect is suspected among the euthyroid relatives of a known case. The proof must be found in in vivo deiodination tests. Dose calculations, however, show that in a case of wrongly suspected IDD, irradiation of a child's thyroid (8 g) may reach 100 rad

if such a test is performed with I-125 DIT. Doses approximately 70% higher would occur with the use of I-131 DIT, since the higher equilibrium dose constant for nonpenetrating radiation overcompensates for the shorter half-life of the label. Such doses are obviously unacceptable, since in the case of a child they involve a definite risk of subsequent thyroid cancer (17).

The purpose of this work, therefore, was to analyze quantitatively the advantages and pitfalls of varieties of DIT deiodination tests in order to propose one that is sensitive, specific, dosimetrically harmless, and—if possible—rapid and easy to perform. The first possibility is to use stable I-127 DIT, but even with a large amount (we tried up to 500 mg) of DIT given per os, the urinary concentration of intact DIT is very low, even in a case of IDD.

In order to evaluate excreted intact DIT, this involves:

(a) a 48-hr collection of urine, much more troublesome than the rapid 1-hr test proposed in this paper; and

(b) either a chemical iodine measurement (which involves special equipment and troublesome manipulations), or a sophisticated purification of DIT from urinary pigments in order to assay DIT by optical density measurement. (Such a purification proved to be ineffective with ion-exchange chromatography such as described in Materials and Methods, "In vivo investigation of IDA with I-123 DIT.")

The second possibility, widely used in nuclear medicine, is to make use of a short-lived label. We have established the feasibility of I-123 DIT as a homemade radiopharmaceutical with satisfactory radiochemical purity and the highest isotopic purity available (contamination with I-125 lower than 2%). This preparation was free from bacteriological or chemical contamination, which made possible its administration in vivo. In vivo deiodination testing with this I-123 DIT discriminates between presence and deficiency of IDA with both specificity and sensitivity at 100%. These results are comparable to those obtained with I-125 DIT, which merely proves the lack of isotopic effect. This test involves, in the case of IDD, a very weak dose to the thyroid. Unfortunately, when the tracer is administered to a patient with a normal iodotyrosine deiodinase activity, the dose to the thyroid (though with I-123 it is ~3% of that with I-125 DIT) may in some cases result in unacceptable irradiation, especially the small thyroid gland of a child.

Since the overwhelming part of thyroid irradiation arises from thyroid uptake, another way of obtaining a reduction of this irradiation is to inhibit the active transport of iodide. Obviously this could be easily obtained by isotopic dilution (achieved by giving per os as few drops of Lugol's solution half an hour before the test). In IDD, however, such a blocking agent would

impair any further thyroid kinetic investigation. Moreover, sudden iodine repletion may be harmful in a case of iodine deficiency.

Thus one last method should be considered, which is to abolish the active transport of iodide by using competitive inhibitors such as anions related to iodide. This reasoning substantiates our present method, which consists of giving 400 mg of KClO_4 , taken by mouth half an hour before the I-125 DIT deiodination test.

In the case of thyroïdal deiodinase defect with a normal peripheral deiodination, the proposed test would fail to recognize the metabolic disorder. Such cases, however, are exceptional among IDD patients (18), and no test in vivo is available to confirm such a diagnosis (which must be proved by investigating dehalogenase activity on a sample of thyroid needle biopsy).

Several advantages of the proposed test must be emphasized:

1. Its 100% specificity and sensitivity in case of peripheral deiodination defect.

2. The dose to the thyroid remains in an acceptable range for an investigative test, whatever the IDA and the thyroid mass may be.

3. The test is rapid (1 hr) and requires no delayed return of the patient, since the labeled tracer can be prepared in advance with sufficient radioactivity to compensate for physical decay, and is stored at -20°C for months until needed.

The efficiency of this test enabled us to detect twenty cases of IDD, as will be published elsewhere.

FOOTNOTES

* Anionic Resin AG 1X2, Cl^- form, 200-400 Mesh, BIORAD.

† Cationic Resin AG 50W-X2, H^+ , 200-400 Mesh, BIORAD.

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