Pharmacokinetics of 3,5,3'-triiodothyroacetic acid and its effects on serum TSH levels

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Abstract. 3,5,3'-triiodothyroacetic acid is an effective inhibitor of TSH secretion in central hyperthyroidism. Serum 3,5,3'-triiodothyroacetic acid was measured with an RIA preceded by immunoprecipitation. An anti-3,5,3'-triiodothyroacetic acid antibody was obtained in rabbits, using 3,5,3'-triiodothyroacetic acid coupled to hemocyanin and diazotized benzidine as antigen (cross-reactivity with T3, T4, triiodothyroacetic acid was 0.2, 1.1, and 5%, respectively). Endogenous 3,5,3'-triiodothyroacetic acid levels could not be detected in 14 euthyroid, 10 hypothyroid and 10 hyperthyroid sera (detection limit 0.055 nmol/l). Kinetic studies were performed in 6 healthy male subjects who received an oral and an iv dose of 1050 µg of 3,5,3'-triiodothyroacetic acid. The serum measurements were analysed according to a non-compartmental method. The half-life of 3,5,3'-triiodothyroacetic acid was 6 h 22 min ± 29 min, the volume of distribution was 114 ± 9 l/70 kg, and the plasma clearance rate was 298 ± 14 l · (70 kg)−1 · day−1. Highest 3,5,3'-triiodothyroacetic acid levels were measured after 40 min (for T3 2–3 h) and its absorption was 67 ± 6%. The nadir of the mean TSH levels was 0.72 ± 0.09 mU/l 6 h after 3,5,3'-triiodothyroacetic acid administration. However, the time course of serum TSH response did not differ from that obtained after administration of 37.5 µg T3. The dose-response effect for TSH was studied using oral doses of 350, 700, 1400 and 2800 µg 3,5,3'-triiodothyroacetic acid and TSH was measured 9 h after 3,5,3'-triiodothyroacetic acid administration at 17.00 h, and compared with control serum TSH levels obtained at 08.00 h (1.53 ± 0.11) and at 17.00 h the day before the test (1.87 ± 0.11). They were 1.05 ± 0.15 (N = 9, mean ± SEM), 0.83 ± 0.08 (N = 24), 0.66 ± 0.06 (N = 24), and 0.43 ± 0.02 mU/l (N = 6), respectively. In conclusion, TSH inhibition by 3,5,3'-triiodothyroacetic acid is similar to T3, with a potency ratio of 1 to 18.

The acetic acid analogues were described as occurring in vivo in 1952, when Gross & Pitt-Rivers discovered the appearance of labelled 3,5,3'-triiodothyroacetic acid (TRIAC) after T3 administration to rats (1). The role of this pathway is not clearly established even though studies in man demonstrated that a large fraction of C14-labelled T4 was eliminated in the urine as the acetic acid analogue of thyronine (2). Serum TRIAC levels have been reported to be very low and its biological activity weak compared with T3 (3). The poor in vivo biological activity contrasts with the high affinity of TRIAC for the nuclear T3 receptor and its biological potency in cell cultures (4) and has been explained by its rapid in vivo metabolism (5).

Recently, however, it has been reported that TRIAC is a good inhibitor of TSH secretion. There has been no explanation for this particular effect. In an attempt to understand this observation, we have investigated the potency of single doses of TRIAC on serum TSH. We have also studied its pharmacokinetics.

Material and Methods

Tablets of TRIAC (Triacana®) were obtained from ANA Company, Paris, France; T3 tablets (Cynomel®) from Smith, Kline & French SA. Pure TRIAC, 3,5-diiodothyroacetic acid, and iodothyronines were purchased from Henning Company. For iv injection, 10 mg of pure TRIAC was dissolved in 1 ml 0.1 mol/l NaOH and diluted in 0.9% NaCl containing 1% human serum albumin. This
solution was filtered through a sterile 0.2 micron filter (Millex-GS, Millipore SA, Molsheim, France) and tested for sterility and pyrogenicity. \[^{125}I\]TRIAC was obtained by labelling TRIAC or 3,5-diiodothyroacetic acid (DlAC) by means of the chloramine T method. The separation of \[^{125}I\]TRIAC from DlAC and iodides was obtained by affinity chromatography using a polyclonal T\(_3\) antibody coupled to agarose (Affi Gel 10, Biorad Laboratories, Richmond, CA) according to the method described by the manufacturer. The TRIAC bound to the antibody was eluted with 50% ethanol in 0.1 mol/l acetic acid at 0°C. The specific activities were 50 to 80 \(\mu\)Ci/ug or, when using 3,5-DlAC as starting material 1500 \(\mu\)Ci/ug.

**Antisera**

The coupling of TRIAC to hemocyanin (Calbiochem, Lucerne, Switzerland) has been previously described (6). Briefly, 400 \(\mu\)g TRIAC and 2 mg of hemocyanin in 0.2 mol/l borate buffer (pH 9.3) were mixed with 150 \(\mu\)l of propylene-glycol and cooled on ice to 0°C. The reaction was started by adding 200 \(\mu\)l of freshly prepared dinitrobenzidine (0.23 g in 45 ml 0.2 mol/l HCl), diluted 1:10 with distilled water. After 15 min the solution was dialysed over 2 days against 0.1 mol/l borate buffer (pH 9.3) and the buffer was changed 3 times. Coupling was repeated if the material precipitated. Only non-precipitated TRIAC-hemocyanin complex was used for immunization. Three rabbits were immunized with 100 \(\mu\)g antigen in complete Freund's adjuvant (Calbiochem); the first booster injection was performed 6 weeks later with incomplete Freund's adjuvant, and thereafter injections were given at intervals of 3 to 5 weeks. Testing of the antisera was performed one week after the booster injections. Two animals responded. Rabbit non-immune gamma globulins and the globulins of the antisera to TRIAC were precipitated with 45% ammoniumsulphate. The precipitate was redissolved in 0.9% NaCl.

**Radioimmunoassay for serum TRIAC**

The standard curves were prepared in three different conditions: a. in untreated euthyroid serum; b. in charcoal-treated serum (Norit, Fischer Scientific Inc), and c. in serum stripped by an affinity column. For these purposes a 1-ml agarose column, to which T\(_3\) antibody was coupled, was used; 4 ml of serum was passed through the column and 85% of \[^{125}I\]TRIAC was retained.

**Immunoprecipitation.** 500 \(\mu\)l serum containing 0.7 mg of Na-salicylate and 2000 cpm \[^{125}I\]TRIAC were incubated with 50 \(\mu\)l, containing the immunoglobulines of T\(_3\) antisemur (diluted 1:25 in TRIS buffer, 0.1 mol/l, pH 7.7). The tubes were left for 2 h at 37°C and then overnight at 4°C. Separation of bound and free hormone were achieved with a goat anti-rabbit gamma globulin antibody. The immunoprecipitate was washed once with 500 \(\mu\)l 0.9% NaCl. The antibody binding capacity was destroyed by adding 50 \(\mu\)l 0.1 mol/l NaOH and heating the tubes for 10 min at 65°C; 200 \(\mu\)l of TRIS buffer was added. After counting, 2 aliquots of 100 \(\mu\)l were pipetted into 1.5 ml RIA tubes containing 10 000 cpm of \[^{125}I\]TRIAC; 20 \(\mu\)l of 0.02 mol/l NaOH with the standards, or NaOH alone, was added, followed by 50 \(\mu\)l TRIAC antisemur diluted 1:250 with TRIS buffer. Two tubes were incubated with normal rabbit serum. The reaction was allowed to proceed overnight at room temperature and the second antibody technique was again used for separation of bound and free fractions of TRIAC.

**Serum T\(_1\), T\(_3\) determinations** were performed according to previously published methods. Serum TSH was measured by an immunoenzymatic assay (Serozyme, Serono, Switzerland). The intra-assay variation was 5.4% and the inter-assay for a serum level of 2.4 mU/l was 2.8%.

**In vivo experiments**

The studies were approved by the ethics committee of the University Hospital of Geneva and written informed consent was obtained from the volunteers.

**Kinetic studies**

Six healthy male subjects (23 to 26 years) received a single oral dose of 1050 \(\mu\)g of TRIAC at 08.00 h, after an overnight fast. Blood samples were taken every 10 min for the first 2 h, every 30 min up to the 4th h, every hour up to 12 h and at 24, 36 and 48 h. The same subjects were studied, using the same sampling protocol, one week later, after an iv bolus injection of 1050 \(\mu\)g TRIAC. Non-compartmental analysis was used to evaluate the serum clearance rate of TRIAC (7, 8). In order to determine the half-life, we estimated visually from semi-log plots the beginning of the terminal log-linear phase of the decrease in TRIAC concentration. The slope (k) was then calculated by log-linear regression analysis. The half-life was defined as log 2/k.

The effect of single doses of TRIAC was evaluated by blood sampling 9 h after oral administration. Twenty-eight subjects (10 females and 18 males) were studied. Of these all except 4 subjects received 2 or 3 doses at intervals of at least 2 weeks. For each subject and for each experiment, control samples were obtained at 17.00 h the evening before and at 08.00 h just prior to TRIAC administration. Each subject was used as his own control. Nine subjects received 350 \(\mu\)g TRIAC, 24 subjects 700 and 1400 \(\mu\)g, and 6 subjects 2800 \(\mu\)g TRIAC. Six other subjects received a single oral dose of 75 \(\mu\)g T\(_3\) and blood was sampled during the following 4 days.

Measurement of endogenous concentrations of serum TRIAC was attempted in 14 healthy euthyroid subjects, 10 hypothyroid (serum TSH > 50 mU/l), 10 hyperthyroid subjects (range of serum free T\(_3\) levels: 29—85 pmol/l), and 4 subjects with cholestase (range of alkaline phosphatase levels: 114—832 U/l, normal values: 30—125 U/l).

**Statistical analysis**

The effects of TRIAC on serum TSH levels were analysed
by analysis of variance for repeated measurements and non-parametric tests (Wilcoxon Signed Ranks Test for paired observations and Wilcoxon’s Rank Sum Test for two independent samples).

Results

Radioimmunoassay for serum TRIAC

The cross-reactivity of the antiserum was for T3: 1.1%; T4: 0.2%; DIAC: 0.5%, and tetraiodothyroacetic acid (TETRAC): 5%. With immunoprecipitation, 88% of TRIAC, 80% of T3, 4% of T4, and 6% of TETRAC was extracted. According to Scatchard analysis, the affinity constant of the TRIAC antiserum was $3.6 \times 10^9$ mol/l. Using $^{125}$I TRIAC of low specific activity (50–80 μCi/μg), the lower limit of detection was 0.5 nmol/l whereas it was 0.055 nmol/l with TRIAC of high specific activity. The intra- and inter-assay variabilities were 9 and 18%. Standard curves obtained in untreated euthyroid serum, charcoal-extracted serum, and affinity-purified serum were identical (Fig. 1). For all further assays charcoal-extracted serum was used.

Endogenous TRIAC levels in euthyroid (N = 14), hypothyroid (N = 10) and hyperthyroid (N = 10) sera were not detectable. When the extracts of these sera were measured by RIA, the percent binding of $^{125}$I TRIAC was within 91 and 112% of the binding of charcoal-extracted serum. As TRIAC is known to be conjugated in the liver (9) we also tested sera from patients with obstructive jaundice. Here too, serum TRIAC levels were not detectable.

Kinetic studies

The kinetic data are shown in Fig. 2 and Table 1. TRIAC absorption was very rapid, reaching maximum after only 40 min. The peak was narrow and the serum TRIAC levels subsequently fell in a manner similar to iv administered TRIAC. The absorption of TRIAC was different from that of L-T3 (Fig. 3) which reached its maximal serum concentration 2 to 3 h after administration. Volume of distribution, plasma clearance rate and half-life were identical for both routes of administration. The plasma clearance rate (iv and po) was $298 \pm 14 \text{ l} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, a value slightly higher than reported by others (5). The volume of distribution was $114 \pm 9 \text{l/70 kg}$, and the half-life 6 h 22 min ± 29 min.

Serum TSH levels following TRIAC administration

Control serum TSH values were measured in 6 euthyroid subjects every 2 h from 08.00 to 18.00 h. They showed no significant variation (min: 1.6 ± 0.2, max: 1.9 ± 0.5 mU/l).

![Graph](image)

**Fig. 1.**

Serum 3,5,3'-triiodothyroacetic acid (TRIAC) levels following po and iv administration of 1050 µg. Close squares: After po administration. Open squares: after iv administration.

**Table 1.** Kinetic parameters of TRIAC.

| Subjects No. | Weight kg | Half-life h; min | VD 1/70 kg | PCR 1 · (70 kg)⁻¹ · day⁻¹ | AUC | Absorption % *
|--------------|-----------|-----------------|------------|----------------------------|-----|----------------
| Oral administration of 1050 µg of TRIAC | | | | | | |
| 1 | 79 | 3 h 23 | 53 | 284 | 4244 | 52 |
| 2 | 62 | 6 h 25 | 104 | 269 | 6338 | 63 |
| 3 | 72 | 9 h 52 | 153 | 258 | 5138 | 55 |
| 4 | 60 | 6 h 29 | 108 | 277 | 7168 | 71 |
| 5 | 83 | 5 h 28 | 98 | 297 | 4508 | 67 |
| 6 | 60 | 6 h 42 | 162 | 435 | 6521 | 93 |
| Mean | 69 | 6 h 24 | 113 | 303 | 5653* | 67 |
| SD | 10 | 2 h 06 | 40 | 66 | 1190 | 15 |
| SEM | 4 | 0 h 51 | 16 | 27 | 486 | 6 |
| IV administration of 1050 µg TRIAC | | | | | | |
| 1 | 79 | 4 h 16 | 88 | 262 | 8391 | |
| 2 | 62 | 6 h 04 | 104 | 284 | 9862 | |
| 3 | 72 | 7 h 51 | 131 | 277 | 8490 | |
| 4 | 60 | 6 h 47 | 119 | 291 | 9947 | |
| 5 | 83 | 5 h 19 | 98 | 305 | 6892 | |
| 6 | 60 | 7 h 42 | 154 | 332 | 8560 | |
| Mean | 69 | 6 h 20 | 116 | 292 | 8690* | |
| SD | 10 | 1 h 24 | 24 | 24 | 1125 | |
| SEM | 4 | 0 h 34 | 10 | 10 | 459 | |

* p < 0.05 between AUC po and iv; VD: Volume of distribution; PCR: Plasma clearance rate.
** Calculated from the ratio of AUC po to AUC iv.
Comparison between serum $T_3$ and 3,5,3'-triiodothyroacetic acid (TRIAC) levels following oral administration of either 75 $\mu$g $T_3$ (closed squares) or 1050 $\mu$g TRIAC (open squares).

Fig. 4.
Individual serum TSH levels during 48 h, following oral administration of 1050 $\mu$g 3,5,3'-triiodothyroacetic acid (TRIAC).
The serum TSH levels following oral administration of 1050 μg TRIAC are shown in Fig. 4. The nadir of the mean levels over time was 0.72 ± 0.09 mU/l, 6 h after administration. Serum TSH levels increased thereafter. TSH values at 24 h were not different from controls. With 37.5 μg of T₃ (Fig. 5), the nadir of the mean levels was identical (0.72 ± 0.14 mU/l) 8 h after administration. At 24 h after TRIAC and 36 h after T₃, serum TSH levels were not different from the control levels. By analysis of variance for repeated measurements, there was no difference over time between the effect on TSH of TRIAC or T₃ (p = 0.57). Assuming a 100% absorption of T₃, the molar potency ratio for T₃ to TRIAC is 18 to 1.

The dose-dependency of TSH inhibition by TRIAC was evaluated 9 h after administration, at 17.00 h (Fig. 6). As the control values showed a slightly but significantly lower TSH concentration

**Fig. 5.**
Individual serum TSH levels during 48 h, following oral administration of 37.5 μg T₃.

**Fig. 6.**
Dose-dependency of TSH inhibition by increasing doses of 3,5,3'-triiodothyroacetic acid (TRIAC). * p < 0.05, ** p < 0.01.
at 17.00 than at 08.00 h (p=0.016), the 17.00 h values were used for comparison. Already 350 µg resulted in significantly decreased serum TSH levels when compared with the control values at 17.00 h (p < 0.01). With higher doses, serum TSH concentrations decreased progressively; they were with 700 µg 0.83 ± 0.08 mU/l, with 1400 µg 0.66 ± 0.06 mU/l, and reached 0.43 ± 0.02 mU/l with 2800 µg TRIAC. This represented a 68 ± 3% or 78 ± 3% decrease when compared with the 08.00 or 17.00 h TSH levels, respectively.

Discussion

Several laboratories have tried to obtain an antiserum for TRIAC with little cross-reactivity for T₃ and T₄. Our earlier attempts resulted in antisera of low titre and low affinity. The cross-reactivity with TETRAC was always considerable. Although the technique was not changed, we carefully avoided using a solution of antigen which contained precipitated material this time, and succeeded in producing an antiserum suitable for RIA measurements. The specificity of the assay was further improved by an initial immunoprecipitation which eliminated interference by TETRAC. The assay was, therefore, specific for serum TRIAC. Yet, even in hyperthyroid sera, TRIAC levels were below the detection limits of 50 pmol/l. In euthyroid subjects, serum levels of TRIAC have been reported to be in the range of 37 to 61 pmol/l by Gavin et al. (10) and in the range of 88 to 245 pmol/l by Nakamura et al. (11). The levels reported by the second authors should have been easily detected by our assay, whereas the levels of Gavin et al. were at our lower detection limit.

The kinetic studies cannot be directly compared with previous studies in man where [¹²⁵I]TRIAC was infused continuously. It should be noted that TRIAC is rapidly absorbed. The peak of absorption was only delayed by 40 min, whereas with T₃ the highest serum levels were obtained between 2 and 3 h. The difference of absorption between T₃ and TRIAC can be explained by their state of dissociation at acid pH. In the stomach, TRIAC is a non-dissociated carboxylic acid and is therefore rapidly absorbed. In contrast, the absorption of T₃ is delayed as it is a cation at acid pH.

The disappearance of TRIAC was very rapid, as expected. The half-life was 6 h 22 min ± 29 min, whereas for T₃ it is reported to be 23 h (7, 8). The plasma clearance rate was 298 l/day which is slightly higher than the 222 l/day previously reported (10). On the basis of our serum concentrations and plasma clearance rate, one can conclude that in healthy euthyroid, hyperthyroid, hypothyroid or cholestatic subjects, serum production rate of TRIAC is minimal.

In cell cultures, the nuclear thyroid receptor has been shown to have an equal or greater affinity for TRIAC than for T₃ and recently it has been shown that the product of the r-Erb Aβ-2 gene a T₃-receptor protein, has a greater affinity for TRIAC than for T₃. This gene is expressed at high levels in the rat anterior pituitary (12).

In central hyperthyroidism, TRIAC has been reported to be an effective inhibitor of serum TSH with little peripheral action (13—15), but the effects on TSH levels in patients with central hyperthyroidism and in normal subjects have not been studied in detail. In the present article, we have investigated the acute effects of a single dose of TRIAC on serum TSH levels of euthyroid subjects. There was no difference in the serum TSH levels with 1050 µg of TRIAC and 37.5 µg of T₃. The nadir of serum TSH levels was obtained almost at a similar time. Even though serum TSH levels returned to control values 24 h after TRIAC and 36 h after T₃ administration, there was no significant difference between the two groups during the 48 h study period. Therefore, we conclude that there is no direct correlation between TRIAC half-life and its inhibition of TSH secretion.

Concerning the dose-response curve, a decrease in serum TSH levels was observed with administration of the lowest dose of 350 µg TRIAC. The TSH levels decreased further with higher doses of TRIAC, but were still measurable (0.43 mU/l ± 0.02). In contrast, serum TSH levels were unmeasurable (0.1 mU/l or less) in euthyroid goitrous subjects treated chronically with 1 to 2 mg TRIAC/day (16).

Two mechanisms may explain these observations: TRIAC may have a cumulative effect. However, this could not be the sole cause of the depressed TSH values in chronically treated subjects, as the highest single dose was 2 to 3 times higher than the dose which reduces serum TSH to 0.1 mU/l or less when given chronically. Alternatively, it is likely that the initial effect of TRIAC administration is primarily to inhibit active TSH secretion, whereas the pituitary stores remain unaffected.
Chronic treatment may result in complete deple-
tion of pituitary TSH stores. This would explain
the difference in serum TSH levels following single
or chronic administration of TRIAC.

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