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Studies on the goitrogenic mechanism of action of *N*,*N*,*N*′,*N*′-tetramethylthiourea

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Abstract

N,*N*,*N*′,*N*′-Tetramethylthiourea (TMTU) is a rat goitrogen inducing thyroid hyperplasia, hypertrophy, and tumor formation. Little is known about the exact underlying mechanism of action. As thyroid peroxidase (TPO) and type I iodothyronine deiodinase (ID-I) have been established as targets of goitrogenic thiourea derivatives, we investigated interactions of TMTU with target enzymes using a partially purified fraction from hog thyroids or solubilized hog thyroid microsomes and 10,000 g supernatant from rat liver homogenate, respectively, as enzyme sources. For comparison, comprehensively characterized goitrogenic thiourea derivatives were studied as well. In contrast to propylthiouracil (PTU), and like ethylenethiourea (ETU), TMTU only marginally affected TPO-catalyzed oxidation of guaiacol. TMTU, like ETU, concentration-dependently suppressed TPO-catalyzed iodine formation with concomitant oxidative metabolism. Suppression ceased upon consumption of thiourea derivatives, the rate of the reappearing iodine formation was similar to that of controls. TMTU, like ETU, also suppressed non-enzymatic and TPO-catalyzed monoiodination of L-tyrosine with a stoichiometry of 2:1, i.e., one molecule of thiourea derivative suppressed two times monoiodination. TMTU and ETU were unable to irreversibly inhibit TPO. In contrast to PTU, TMTU did not inhibit ID-I. These findings provide evidence that TMTU interferes with thyroid hormone synthesis at the level of iodination and demonstrate a metabolic route for the oxidative detoxification of TMTU in the thyroid suggesting that low-level or intermittent exposure to TMTU would have only minimal effects on thyroid hormone synthesis. Finally, it can be concluded that meaningful toxicological studies on TPO inhibition can be performed without a need for highly purified TPO.

Keywords: Thyroid toxicity; Thyroid peroxidase; Type 1 iodothyronine deiodinase; *N*,*N*,*N*′,*N*′-Tetramethylthiourea; Ethylenethiourea

1. Introduction

N,*N*,*N*′,*N*′-Tetramethylthiourea (TMTU) has been used as an accelerator for neoprene vulcanization and more recently as a scavenger of reactive oxygen species in biochemical and cell culture studies. TMTU has been shown to be a rat goitrogen decreasing blood L-thyroxine levels and inducing thyroid hyperplasia, hypertrophy, and tumor formation following prolonged exposure (Stula et al., 1979). Although this effect of TMTU is already known for long, little is known about the underlying mechanism. Goitrogenic thiourea derivatives have been demonstrated to interfere in various ways with thyroid peroxidase (TPO; Engler et al., 1983), but also – depending on their chemical structure – with type I iodothyronine deiodinase (ID-I, Oppenheimer

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et al., 1972). In order to characterize the underlying mechanism of action of TMTU, we studied interactions with these target enzymes. A partially purified fraction from hog thyroids or solubilized hog thyroid microsomes and 10,000 g supernatant from rat liver homogenate, respectively, served as enzyme sources. For comparison, other comprehensively characterized goitrogenic thiourea derivatives were investigated as well. The results of these studies are presented herein.

2. Materials and methods

2.1. Chemicals

Acetonitrile HPLC grade, methanol HPLC grade, trichloroacetic acid p.a., EDTA p.a., tris(hydroxymethyl)-aminomethane, and sucrose were supplied by E. Merck (Darmstadt, Germany). Hydrogen peroxide 30% p.a. was obtained from Ferak (Berlin, Germany). Amitrole 99%, propylthiouracil ≥ 99%, *N*,*N*,*N*′,*N*′-tetramethylthiourea 98%, 3-iodo-L-tyrosine 95%, 3,3′,5′-triiodothyronine (rT3) ≥ 97%, dithiothreitol 99%, guaiacol ≥ 98%, and bovine serum albumin 96–99% was purchased from Sigma (Taufkirchen, Germany). Ethylenethiourea (98%) and L-tyrosine > 99% were supplied by Aldrich (Taufkirchen, Germany) and Fluka (Taufkirchen, Germany), respectively. [5′-^{125}I]-3,3′-Triiodothyronine, specific activity 42.1 MBq/μg was obtained from Amersham Biosciences (Freiburg, Germany)

2.2. Preparation of partially purified thyroid peroxidase (TPO)

Partial purification of TPO and preparation of solubilized thyroid microsomes (only used for studies on irreversible TPO inhibition) was performed as described by Neary et al. (1984). Hog thyroids obtained from a nearby slaughterhouse and kept deep-frozen at −80 °C until use served as the starting material. TPO activity was characterized by means of the guaiacol assay. The amount of protein catalyzing an ΔE per min of 1.0 was defined as 1.0 "guaiacol unit" (see below).

2.3. Determination of TPO-catalyzed guaiacol oxidation

Guaiacol oxidation was used as a measure for peroxidative activity. Incubations were carried out at room temperature in 0.1 M potassium phosphate buffer, pH 7.4 in a total volume of 1.0 mL. Guaiacol, TPO and test compounds as indicated were preincubated for 1 min, then the reaction was initiated by addition of hydrogen peroxide in a small volume. Test compounds were added in 40 μL DMSO, likewise control incubations lacking test compounds contained the same amount of DMSO which did not affect TPO activity. Final concentrations were 5 mM guaiacol, 0.1 "guaiacol unit" TPO/mL, and 200 μM H_2O_2. Incubations containing test compound were performed in duplicate, control incubations were performed at least in triplicate. The initial linear increase of the absorption at 470 nm was measured in a spectrophotometer (Lambda 2, Perkin-Elmer, Rodgau-Jügesheim, Germany) and used to calculate the peroxidase activity.

2.4. Determination of TPO-catalyzed iodine formation

Incubations were carried out as described above, however, guaiacol was replaced by potassium iodide (final concentration 10 mM) and the H_2O_2 concentration used was 250 μM. The initial linear increase of the absorption at 350 nm was used to calculate the enzymatic activity.

2.5. Determination of TPO-catalyzed and non-enzymatic L-tyrosine monoiodination

TPO-catalyzed and non-enzymatic monoiodination of L-tyrosine was performed as follows: L-tyrosine, KI, and test compounds as indicated and dissolved in DMSO were preincubated for 1 min in the presence or absence of TPO as described above, then the reaction was initiated by addition of hydrogen peroxide in a small volume for 15 (enzymatic iodination) or 60 min in a total volume of 0.5 mL. Final concentrations were 500 μM L-tyrosine, 10 mM KI, H_2O_2 (250 μm), and 0.2 guaiacol units/mL TPO. Sodium thiosulfate (1 mM final concentration) and half a volume of methanol were then added to trap any residual iodine and to stop the reaction. Samples were centrifuged, and the supernatants were analyzed for monoiodo-L-tyrosine by HPLC (HP 1084 liquid chromatograph, Agilent, Böblingen, Germany). The analyte was eluted isocratically from a RP 8 column (7 μm, 250 mm × 4 mm, LiChrosorb, Merck, Darmstadt, FRG) at a flow rate of 1.2 mL/min using a mixture of acetonitrile and water (15/85, v/v) containing 1% acetic acid. Quantification was carried out by recording the UV signal (285 nm), determination of peak areas, and by comparison with a calibration curve. Under the conditions of the assay no di-iodotyrosine is formed.

2.6. Determination of TPO-catalyzed in vitro metabolism of thiourea derivatives

ETU and TMTU as indicated, TPO (0.1 guaiacol units/mL) and KI (10 mM) were incubated as described above for TPO-catalyzed iodine formation. After 4 min reactions were stopped by addition of sodium thiosulfate (1 mM final concentration). Then half a volume methanol (TMTU) or acetonitrile was added, samples were centrifuged, and the supernatants were analyzed for thiourea derivatives by HPLC (HP 1090 liquid chromatograph, Agilent, Böblingen, Germany). ETU was eluted isocratically from a RP 18 column (5 μm, 250 mm × 4 mm, LiChrosorb, Merck, Darmstadt, FRG) at a flow rate of 1 mL/min using a water/acetonitrile (85/15, v/v) mixture for ETU. TMTU was eluted isocratically from the same column with a mixture of 1% acetic acid and methanol