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Effect of radiation on thyroid preoxidase activity in rabbit

[1]Alcaraz, M., [2]Solano, F., [3]Vicente,V and [4]Canteras M.

[1]Radiology and Physical Medicine Department; [2]Biochemistry and Molecular Biology Department, [3]Pathology Department and [4]Biostatistical Department. Faculty of Medicine. University of Murcia. Spain

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Abstract

This work studies the effect of different X-rays doses on thyroid peroxidase activity in three groups of rabbits: normofunctioning, propylthiouracil-treated and TRH-treated. The results show a significant decrease in peroxidase activity in all animals irradiated with a single or fractionated X-rays dose, produced without pathological ultrastructural alterations in their thyroid glands.

Key words: *radiation; thyroid peroxidase; tumour; animal model*

INTRODUCTION

Thyroid dysfunction is a recognized side effect after radiotherapy on head and neck tumours. The risk of hypothyroidism is 14-36% when radiotherapy alone is used in laryngeal cancer. When radiotherapy is combined with surgery, which may include hemithyroidectomy, the risk increases to 43-66%. Another potential risk factor includes the dose of radiotherapy delivered to the region of the thyroid gland, chemotherapy prior to radiation therapy, gender, age, and total vs. partial irradiation of the thyroid gland [5,6]. This paper presents the results on thyroid peroxidase activity in rabbit thyroid glands irradiated with different X-rays doses and highlights the significance of the findings.

MATERIALS AND METHODS

Sixty-nine male specimens of 3-month-old New Zealand rabbits, weighing approximately 2,700 g at the beginning of the study and kept at 18-22 °C, with an ambient humidity between 50-70% and in a 12-h day-night cycle, were fed with commercial fodder (U.A.R. 112 Panlab., Spain) and allowed water *ad libitum*.

Address for correspondence:

Dr. Miguel Alcaraz.
Department of Radiology and Physical Medicine. Faculty of Medicine. University of Murcia. 30100-MURCIA. SPAIN. Phone: 34 968 363601. Fax: 34 968 363602; 34 968 364150. E-mail: mab@um.es

The animals were divided into four groups: Group I: Twelve animals that had not received any type of treatment prior to irradiation (normofunctioning group). Group II: Twelve animals whose thyroid functional activity was reduced by the administration of 6n-propyl-2-thiouracil (PTU) (Sigma, USA) during the 4 weeks prior to irradiation. The PTU was administered to the drinking water at a concentration of 0.2% and supplemented with 1% sucrose. Group III: Twelve animals whose thyroid functional activity was stimulated by administration of protirelin (TRH) (Lab. Frumtost-Prem, Spain) during the week prior to irradiation. The TRH was administered intravenously, at doses of 200 µg/8 hours, through a polyethylene catheter inserted into the right external jugular vein. Group IV: A fourth group of 33 animals, denominated the "variable post-irradiation period group", was used to plot a response curve of thyroid peroxidase activity (TPO) at 0, 24 and 48 hours after irradiation.

The irradiation field was 5 cm in diameter upon the thyroid cartilage. The X-rays were delivered by a therapy unit (Securix 2612 Compact, CGR) with 120 kV, 12 mA, 1 mm Cu of HVL, filtration of 1 mm Cu and 0.5 mm Al, FSD 50 cm and absorbed dose rate of 1.15 Gy/min. In the first three groups, the total X-rays dose administered was 0 Gy (as control), 10 Gy and 20 Gy, fractionated at 2 Gy/day, on alternative days. In the fourth group, the doses administered were 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 Gy, in a single session. All the animals were immobilised and conscious during irradiation. In the first three groups, the animals were slaughtered 24 hours after irradiation by traumatic decerebration, and their thyroid glands were subsequently removed. In the variable post-irradiation period group, the animals were slaughtered 0, 24 and 48 hours after irradiation.

Preparation of thyroid peroxidase (TPO)

After thawing, the thyroid glands were weighed and divided into small pieces, which were homogenized in a 100 mM carbonate buffer pH 10.2, containing 0.1 mM KI to stabilize the enzyme. The ratio of weight to volume of homogenisation was 1:5 (g:ml). The homogenate was fractionated by centrifugation to obtain the post-nuclear fraction at 700 g during 10 min. This fraction was treated with 1% Triton X-100 for 2 h at 4 °C to solubilize the enzyme from the membrane fraction. Finally, the preparation was ultracentrifuged at 105,000 g for 1 h and the supernatant was used for TPO activity determination.

Assay for peroxidase activity

The peroxidase activity of the thyroid preparations was tested with guaiacol method as described by Chance and Maehly [3]. Briefly, the method consists in measuring the absorbance increase per minute, at 470 nm using a spectrophotometer and cuvettes of 1 ml total volume. The reaction mixture contained 0.9 ml of 20 mM guaiacol in 50 mM Tris-HCl buffer, pH 8.2, 50 µl of 10 mM H_2O_2 and 50 µl of rabbit thyroid extracts. The activity unit was defined as the amount of enzyme that produced an increase of an absorbance unit per minute under the assay conditions.

Protein determination

The protein content of the enzymatic extracts was determined by a modified Lowry method [4] using BSA as standard. The statistical treatment consisted of equality contrasts of the means and correlation.

Samples of the thyroid gland of the first three groups (three animals/group) was examined as previous described by Alcaraz et cols., [1] for the ultrastructural study.

RESULTS AND DISCUSSION

Non-irradiated animals

The values for TPO activity in the non-irradiated animals are shown in Figure 1. The results only demonstrate significance in the fall in the TPO activity of the PTU-treated animals when compared to the other two groups; the values of the latter two groups are similar. This relation may be expressed as follows: N = TRH > PTU ($p<0.01$).

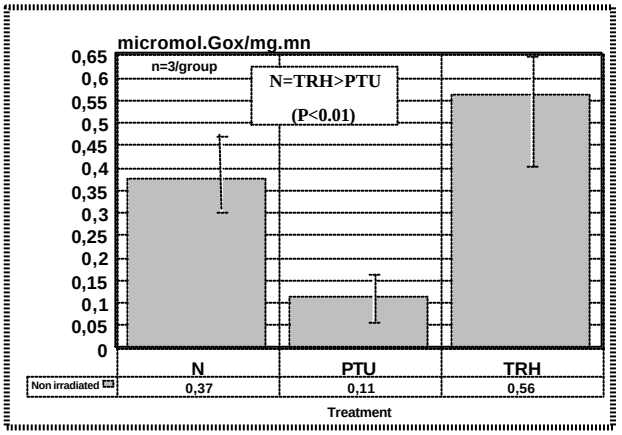

Fig. 1. Thyroid peroxidase activity in non-irradiated animals.

Normofunctioning variable post-irradiation period group

The values for TPO activity in the variable post-irradiation period group are shown in Figure 2. The irradiated animals presented a decrease in TPO activity, even with the lowest radiation doses (2 Gy), in the three study periods (0, 24 and 48 hours). This relation may be expressed as follows: N > N24 > N0 = N48 ($p<0.001$) (Fig. 3).

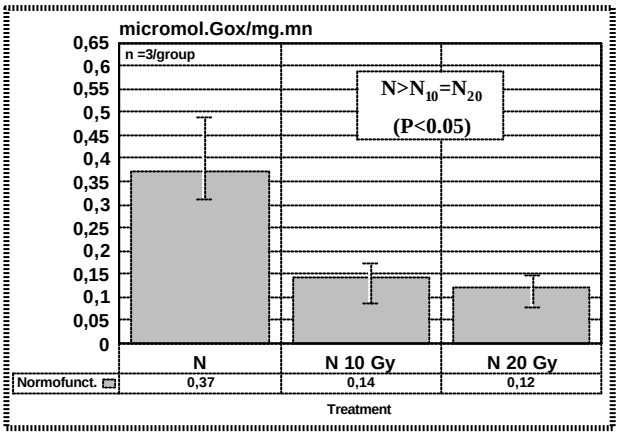

Fig. 2. Thyroid peroxidase activity in normofunctioning group.

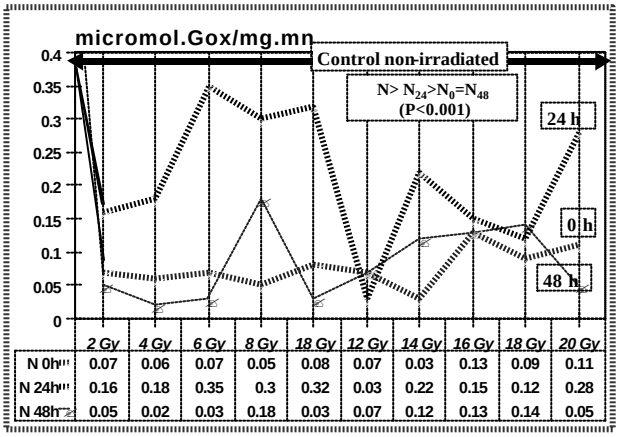

Fig. 3. Normofunctioning animals of the variable post-irradiation period: 0, 24 and 48 hours.

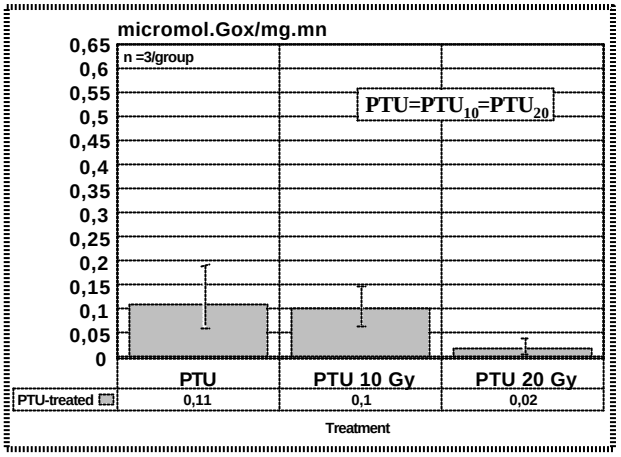

Fig. 4. Thyroid peroxidase activity in PTU-treated animal group.

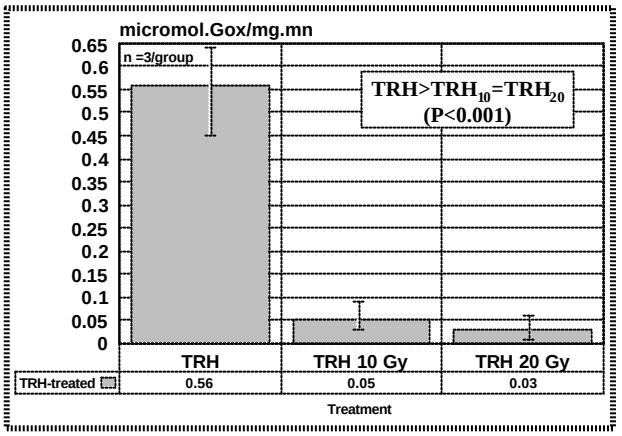

Fig. 5. Thyroid peroxidase activity in TRH-treated animal group.

Irradiated animals

The values for TPO activity in these animals are shown in Figure 1. The normofunctioning animals irradiated with 10 and 20 Gy show a sharp decrease in TPO activity compared to that presented by the non-irradiated normofunctioning animals. This relation shows statistical significance and can be expressed as follows: N0 > N10 = N20 ($p < 0.05$).

The PTU-treated animals irradiated with 10 and 20 Gy show no significant differences in TPO activity from that presented by the non-irradiated PTU-treated animals: PTU0 = PTU10 = PTU20 (Fig. 4).

The irradiated TRH-treated animals present a drastic fall in the TPO activity of their thyroid glands compared with the values of the non-irradiated TRH-treated animals: TRH0 > TRH10 = TRH20 ($p<0.001$) (Fig. 5).

The most distinctive ultrastructural features in the normofunctioning group are the cytoplasmic vacuolation, the sloughing of some follicular cells and cell debris into the follicular lumen, as well as the presence of interstitial epithelioid cells in rounded clusters similar to new-formed small follicles. A decrease in the size of the follicular cell and an increase in the follicular lumen were also observed and seemed to be related to the X-rays dose. The ultrastructural changes described were limited to numerous dispersed foci of the thyroid parenchyma, which were intermingled with thyroid follicles with a normal appearance. The TRH-treated group showed earlier and more intense ultrastructural modification induced by radiation than the PTU and untreated ones.

Our results showed a decrease in TPO activity in all the irradiated animals without evident ultrastructural alterations. Similar results have been described in cultured thymocytes after external gamma irradiation, which describe a significant

decrease in the expression of TPO without alteration in the cell viability [2]. Decrease of the activity/expression of TPO with a suppression of thyroid hormone synthesis could contribute to an early development of thyroid dysfunction following irradiation [2]. Thus, analysis of thyroid function, even early after external radiation therapy, should be routinely performer in the follow-up of head and neck cancer patients, especially if radiotherapy was part of the treatment [2,5,6].

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Resumen

Se estudia el efecto de diferentes dosis de rayos X sobre la actividad de la peroxidasa tiroidea (TPO) en tres grupos diferentes de animales: normofuncionantes, tratados con propylthiouracilo y tratados con TRH. Los resultados ponen de manifiesto un descenso estadísticamente significativo de la actividad enzimática de todos los animales irradiados, tanto cuando se utiliza una dosis única de rayos X como cuando se realiza un fraccionamiento de la dosis de radiación administrada; todo ello sin alteraciones ultraestructurales patológicas en sus glándulas tiroides.

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