Propylthiouracil-Induced Hypothyroidism Reduces Xenograft Tumor Growth in Athymic Nude Mice

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BACKGROUND. Thyroid hormones are endocrine modulators of several vital processes that are crucial to tumor growth and differentiation. Several anecdotal reports in the literature suggest that some histologic types of carcinoma may remain in a dormant state for prolonged periods of time in patients with hypothyroidism, with eventual progression of the disease once the decreased thyroid function is identified and corrected.

METHODS. Oral propylthiouracil (PTU) was used to induce hypothyroidism in athymic nude mice that were subsequently inoculated with lung adenocarcinoma and prostate adenocarcinoma cells. Mice were also treated with a combination of PTU and thyroxine, which resulted in hyperthyroid levels of T_4.

RESULTS. Subcutaneous lung and prostate xenografts grew significantly more slowly in hypothyroid mice treated with PTU than in euthyroid or hyperthyroid mice, regardless of treatment with PTU. Tumors grew well in groups of mice that were changed from a hypothyroid state to a euthyroid state by withdrawal of oral PTU. Administration of PTU 3 weeks after tumor inoculation also caused the tumor growth to slow significantly compared with tumors in mice that did not receive PTU. Mice that received PTU and thyroxine had tumors that grew as well as the tumors in euthyroid control animals.

CONCLUSIONS. Our study indicates that human lung and prostate tumors do not grow well in hypothyroid nude mice, and that rendering these animals euthyroid has a significant impact on the growth rate of these tumors. Furthermore, in vitro and in vivo data indicated that this was not a result of an interaction of the tumor cells with PTU, but rather a result of the hypothyroid state. ***Cancer* 1999;86: 1596–601.** © *1999 American Cancer Society.*

KEYWORDS: hypothyroidism, propylthiouracil, T_4, prostate carcinoma, nonsmall cell lung carcinoma.

Several case reports have indicated that patients with cancer who have clinical or subclinical hypothyroidism may have a prolonged course compared with euthyroid patients, suggesting that the presence of hypothyroidism may be associated with prolonged survival, with the exception of patients with thyroid tumors.[1–4] Furthermore, animal experiments also have suggested that low levels of circulating thyroid hormones may result in increased response to chemotherapy.[5] In a transplantable mouse mammary carcinoma model, a significant increase of complete responses was observed in mice treated with 5-fluorouracil that had been rendered hypothyroid.[5]

The mechanisms through which decreased levels of thyroid hormones may influence tumor growth have not been elucidated. It is conceivable that the thyroid hormones may modulate metabolic pathways essential to tumor growth.

To date, the role of thyroid hormones in tumor growth has been

investigated only in mammary carcinoma,[6] fibrosarcoma,[7] chondrosarcoma,[8] colon carcinoma,[9] and hepatoma.[10] Due to the preponderance of lung and prostate carcinomas in the United States population, we evaluated the effects of hypothyroidism induced by 6-n-propyl-2-thiouracil (propylthiouracil, or PTU) on the growth of PC-3 prostate carcinoma cells and 201T lung adenocarcinoma cells in athymic nude mice.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

The human tumor cell line PC-3 was established from human prostate adenocarcinoma.[11] The poorly differentiated human lung adenocarcinoma 201T was developed using the method described by Siegfried and Owens.[12] These cell lines have been described previously.[11,13,14] The 201T cells were cultivated in culture medium conditioned by the bronchioalveolar carcinoma cell line A549. Culture medium was produced by blending conditioned basal Eagle's medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 1% fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT) with fresh Ham's F-12 medium (Life Technologies) supplemented with 1% FBS. The basal Eagle's medium was conditioned for 48 hours on a confluent monolayer of A549 cells in an 850 cm^2 roller bottle (100 mL medium/bottle). Medium was sterilized by filtration through a 0.22 μm filter. Cells were maintained in humidified incubators at 37 °C in an atmosphere of 7.5% CO_2. PC-3 cells were maintained in RPMI 1640 medium (Life Technologies) supplemented with 10% FBS. The mean levels of T_3 and T_4 in the FBS were 131.3 $\pm$ 42.9 ng/dL and 12.4 $\pm$ 1.6 μg/dL, respectively. Therefore, the levels of T_3 and T_4 in the 201T medium were approximately 1.31 ng/dL and 0.12 μg/dL, respectively. The levels of T_3 and T_4 in the PC-3 medium were approximately 13.1 ng/dL and 1.2 μg/dL, respectively. The 201T and PC-3 cells approaching 75% confluency were harvested using the DeLarco formulation of trypsin–ethylenediaminetetraacetic acid (Life Technologies) and were subcultured for serial passage. The cells were isolated by centrifugation at 500 xg for 15 minutes, washed once in medium supplemented with 1% FBS, and washed an additional 2 times with phosphate-buffered saline (PBS) (Life Technologies). Cells were suspended in Hanks' Balanced Salt Solution (HBSS) (Life Technologies).

Experimental Animals

Specific pathogen free male Ncr-nude mice ages 4–6 weeks obtained from Taconic (Germantown, NY) were housed in sterilized, filter-topped cages kept in laminar flow isolators (Forma Scientific, Marietta, OH) and fed autoclaved food and water ad libitum. Four animals were housed in each cage. The water given to the treated animals contained 0.05% (weight per volume) PTU (Sigma Chemical Co., St. Louis, MO) or 0.003% thyroxine (Sigma Chemical Co.), or both. Mice were acclimated to our vivarium for 1 week prior to their use in study protocols. All procedures involving the animals were performed under sterile conditions in a laminar flow hood (Forma Scientific). All studies were approved by the Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee.

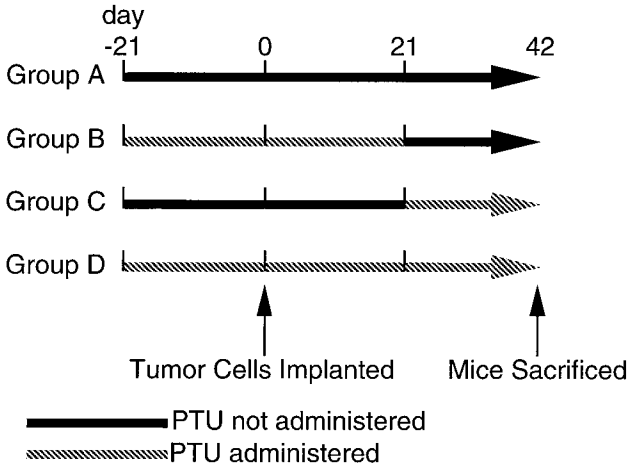

FIGURE 1. The propylthiouracil (PTU) treatment schedule used in this study for nude mice is shown. Mice were divided into Groups A–D (n = 8 for each group). Mice in Groups B and D were pretreated for 21 days with PTU prior to tumor cell implantation. Animals in Groups A and C were not pretreated with PTU prior to tumor cell implantation. All mice were implanted with tumor on Day 0. The animals in Group B were removed from PTU on Day 21; the animals in Group C were administered PTU starting on Day 21. All mice were sacrificed on Day 42.

Tumor Implantation and Growth

PC-3 prostate carcinoma cells and 201T lung adenocarcinoma cells (2×10^6 cells) were inoculated subcutaneously into the flanks of nude mice in 0.1 mL HBSS. For the first experiment, mice were segregated into 4 groups, each of which consisted of 8 animals (Fig. 1). Group A consisted of animals that were inoculated with tumor but were not given PTU in their water. Group B consisted of animals that had been given PTU for 3 weeks prior to inoculation, were subsequently inoculated with 2×10^6 cells, and continued to receive water containing PTU for the first 21 days after the inoculation. Starting on Day 22, Group B animals received water without PTU. Group C consisted of 8 animals that were inoculated with tumor and received PTU starting on Day 22 after inoculation.

Finally, Group D consisted of 8 animals that began receiving PTU 21 days prior to inoculation and continued receiving it throughout the experiment. The animals were inspected 3 times per week for evidence of tumor development, and tumor dimensions were measured using a dial caliper. The first measurements were taken on Day 13 after tumor inoculation and repeated twice weekly thereafter. All surviving animals were sacrificed on Day 42, and tumors were removed and weighed. The experiment was repeated twice for the PC-3 cell line. In the second experiment, 38 mice were segregated into 4 groups, so that 10 mice received oral PTU in their water, 8 mice received oral thyroxine, 10 mice received both PTU and thyroxine, and 10 mice received placebo. All mice were treated for 21 days prior to tumor implantation and were sacrificed 42 days postimplantation.

T_4 Assay

To measure thyroid function, 8 separate mice were given PTU for 21 days. PTU was subsequently removed from their water. Serial measurements of the thyroid hormone T_4 were obtained during the 21-day period during which the animals received PTU, and for 3 weeks after discontinuation of the drug. Blood samples were obtained with a small glass capillary tube positioned behind the eye in the ophthalmic venous plexus. Serum T_4 levels were determined using a T_4 radioimmunoassay kit obtained from ICN Pharmaceuticals (Costa Mesa, CA). An additional 38 mice were treated with PTU (n = 10), thyroxine (n = 8), PTU and thyroxine (n = 10), or placebo (n = 10), and blood samples were taken on Days 0, 10, 17, 29, 43, 55, and 69. Again, serum T_4 levels were determined using radioimmunoassay.

In Vitro Concentration Response Assay

PC-3 cells were exposed to 0, 0.1, 1, 10, and 100 μM PTU in RPMI medium supplemented with 10% FBS and [^{3}H]thymidine (Amersham Life Science, Arlington Heights, IL). The cells were allowed to propagate for 5 days in the presence of PTU or placebo, followed by the measurement of acid-precipitable [^{3}H]DNA by liquid scintillation counting.

RESULTS

To determine whether the administration of PTU and/or thyroxine suppressed thyroid function in nude mice, T_4 levels were measured in two separate experiments. Figure 2A outlines the results of the serial T_4 measurements obtained from 8 mice treated with PTU alone. The T_4 level dropped from a mean value of 26.04 ng/mL onDay 0 to a nadir of 8.72 ng/mL on Day 21. Once PTU was discontinued, the level returned to

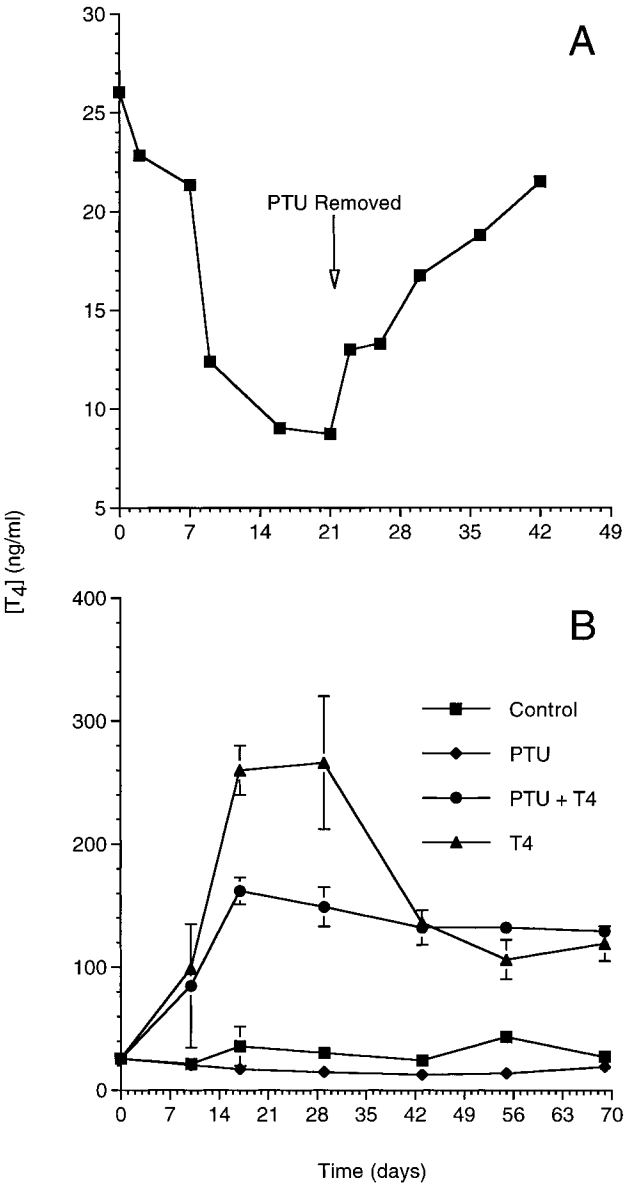

FIGURE 2. Serum T_4 levels are reduced in mice treated with oral propylthiouracil (PTU). (A) Mice (n = 8) were treated for 3 weeks with PTU. After treatment, mice stopped receiving PTU and serum T_4 levels were measured for an additional 3 weeks. (B) Mice (n = 38) were treated with placebo, PTU, PTU and thyroxine, or thyroxine alone for 69 days.

a mean of 21.47 ng/mL within 21 days. The concomitant treatment of mice with PTU and thyroxine resulted in levels of serum T_4 comparable to those in hyperthyroid mice treated with thyroxine alone (Fig. 2B). The mean level of serum T_4 in control (placebo-treated) mice was 27.0 ng/mL over the entire measurement period (69 days). The mean level of serum T_4 in PTU-treated mice was 14.9 ng/mL; the nadir for this group was 10.9 ng/mL, which was slightly higher than was observed in the previous experiment. The reduc-

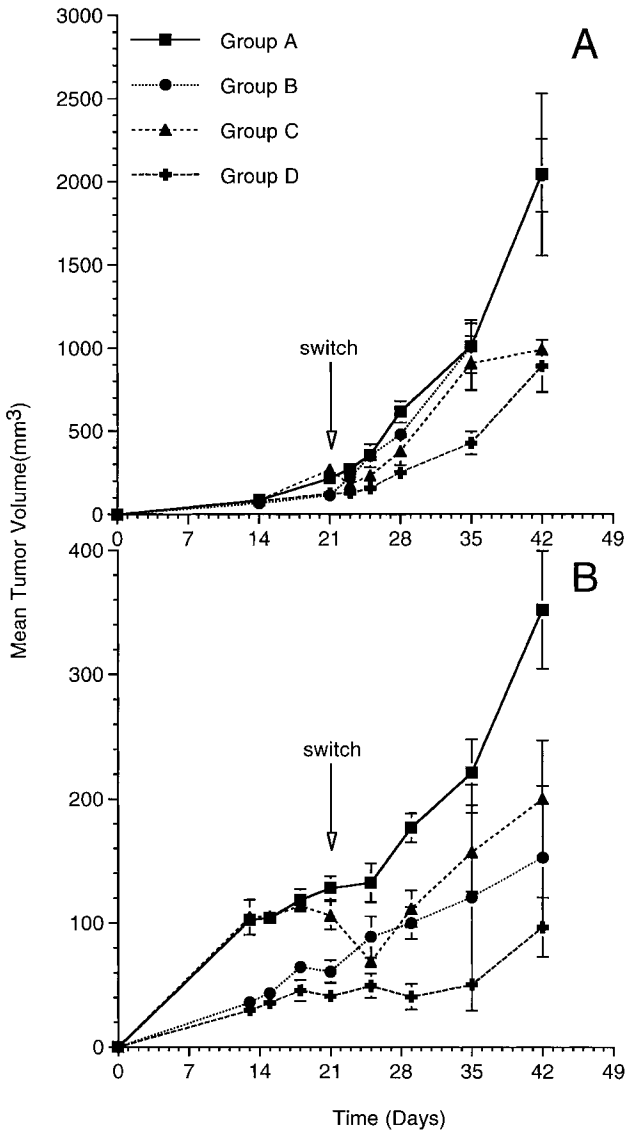

FIGURE 3. The mean tumor volume was measured in mice containing subcutaneous PC-3 prostatic tumor xenografts (A) or 201T lung adenocarcinoma xenografts (B). The mice in Group A never received propylthiouracil (PTU) (controls). The mice in Groups B and C were either removed from oral PTU (Group B) or started on PTU (Group C) 21 days postimplantation (switch). The mice in Group D received PTU throughout the entire experimental period. The error bars are ± the standard error of the mean.

tion of serum T_4 levels in PTU-treated animals was significantly lower than the level found in control mice (P 0.0005, Fisher exact test). The mean level of serum T_4 in mice treated with PTU and thyroxine was 124.4 ng/mL, which was well above the euthyroid levels. This was comparable to the mean levels detected in thyroxine-treated mice, which was 150.0 ng/mL.

To determine whether hypothyroidism would suppress tumor growth, mice were treated as shown in Figure 1. Figure 3A shows the mean tumor volume of PC-3 human prostatic adenocarcinoma cell xenografts. Animals in Groups B and D received PTU for 21 days prior to tumor cell inoculation. Although tumors eventually grew in all animals, the mice that received PTU throughout the experiment and the mice that began receiving PTU 21 days after tumor inoculation (Groups C and D) exhibited diminished tumor growth compared with the animals that did not receive PTU, and compared with the animals that were removed from PTU 21 days after tumor inoculation (Groups A and B). Because three animals in Group C did not survive until Day 42, the mean tumor volume for Group C is based on the 5 animals that were sacrificed on that day. All other animals survived until Day 42, at which point they were sacrificed. Figure 3B shows the results with the human lung adenocarcinoma cell line 201T. The animals in Groups B and D received PTU for 21 days prior to tumor cell inoculation. Here again, mice that received PTU throughout the experiment and mice that began receiving PTU on Day 21 (Groups C and D, respectively) exhibited diminished tumor growth compared with the animals that did not receive PTU or received PTU only for 21 days after inoculation. The Group A data were drawn from observing six mice. All other groups contained eight mice.

To demonstrate that the reduced tumor growth was a result of hypothyroidism and not a direct result of an antitumor effect of PTU, in vitro and in vivo experiments were conducted. As seen in Figure 4, concentrations of PTU from 0 to 100 μM had no effect on the ability of PC-3 cells to incorporate [^{3}H]thymidine. To demonstrate further that reduced tumor xenograft growth was as a result of hypothyroidism, mice were treated with either PTU and/or thyroxine for 21 days prior to xenograft implantation. Treatment continued throughout the experiment. As seen in Figure 5, the mice that received PTU and thyroxine, thyroxine, or placebo had comparable tumor growth; however, the hypothyroid mice treated with PTU alone had reduced tumor growth. These data indicate that PTU does not act directly on the tumor cells.

DISCUSSION

The role of the thyroid hormones T_3 and T_4 in carcinogenesis has not been elucidated. In vivo data from animal experiments suggest that the development of cancer may be a thyroid hormone–dependent phenomenon. Studies similar to ours have involved mice with human mammary carcinoma,[6] fibrosarcoma,[7] chondrosarcoma,[8] colon carcinoma,[9] and hepatoma.[10] These studies have also demonstrated that hypothyroidism slows the neoplastic pro-

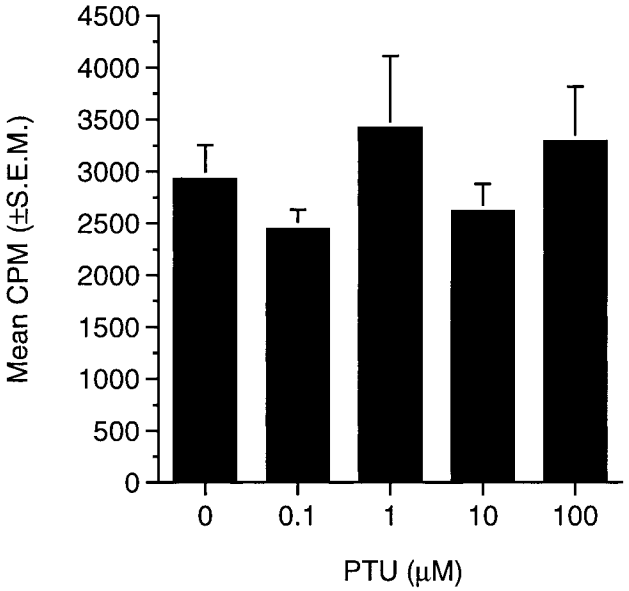

FIGURE 4. Propylthiouracil (PTU) does not have an affect on in vitro cell growth. PC-3 prostatic cells were exposed to increasing concentrations of PTU in growth medium supplemented with [^{3}H]thymidine. The cells were allowed to propagate for 5 days in the presence of PTU or placebo, followed by the measurement of acid-precipitable [^{3}H]DNA by liquid scintillation counting. The error bars are ± the standard error of the mean.

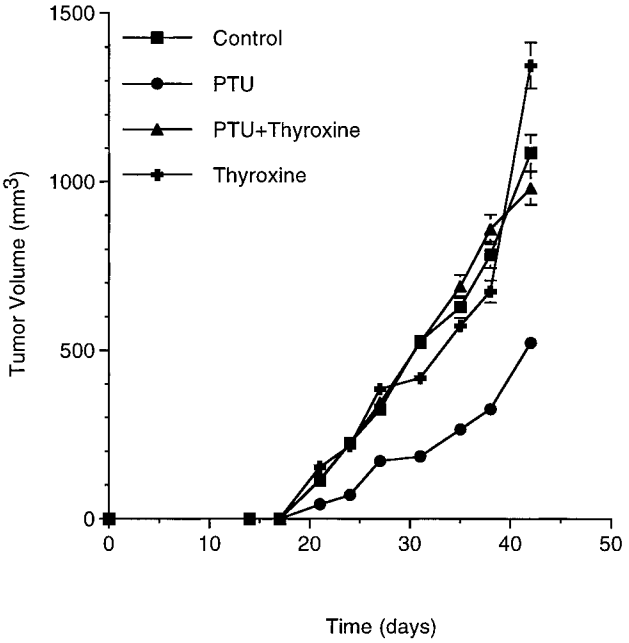

FIGURE 5. Hyperthyroid mice receiving propylthiouracil (PTU) and thyroxine have tumor growth comparable to that of control mice. Mice were treated with placebo (n = 10), PTU (n = 10), PTU and thyroxine (n = 10), or thyroxine alone (n = 8). Control mice received only ethanol in the drinking water. Mice that were hypothyroid by administration of PTU had slower tumor growth than hyperthyroid mice treated with PTU and thyroxine or euthyroid mice treated with placebo.

cess, whereas administration of thyroid hormone preparations restores tumor growth rates. The results of our studies show for the first time that the development of prostate and lung carcinoma is also thyroid hormone–dependent. It is difficult to determine, however, whether there was an in vitro selection for cell lines dependent on T_3 and T_4 supplied by the FBS in the culture media.

The mechanisms of this apparent inhibition of tumor growth have not been elucidated. Possible explanations include modulation of several paracrine growth factors, including epidermal growth factor,[15] insulin-like growth factor-I,[16] and nerve growth factor[17] by thyroid hormones. Thyroid hormones are also known to modulate microtubule assembly protein[18] and mitochondrial biogenesis[19] and to regulate cathepsin D, which facilitates tumor invasion and metastasis.[20] Finally, there is indirect evidence that neoangiogenesis may be decreased in a hypothyroid state;[21] however, microscopic examination of xenografts from our study failed to demonstrate a difference in neovascularization (data not shown).

Both prostate carcinoma and lung carcinoma are significant public health problems. Lung carcinoma is the leading cause of death from neoplasia for both men and women, and the estimated lung carcinoma mortality rate for 1997 was 160,400. The median survival for patients with metastatic lung carcinoma is less than 1 year and does not substantially increase with chemotherapy.

Prostate carcinoma is the most common cancer in males, with 113 deaths occurring daily in the United States alone.[22] The median survival for patients with hormone-refractory prostate carcinoma is only 1 year, and no available therapeutic modality has ever been shown to increase survival. Induction of a hypothyroid state with PTU may be a therapeutic alternative for both diseases that may warrant further investigation, although this has not yet been observed clinically in human patients. PTU may induce myxedema coma, which can be life-threatening; however, a low but clinically acceptable hypothyroid state that averts development of myxedema may be achieved with the combination of PTU and a low dose of thyroxine. Whether such an approach will benefit patients with advanced stage malignancies remains to be determined.

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