Thyroid Hormone in 7,12-Dimethylbenz(a)anthracene-induced Leukemia in Rats

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ABSTRACT

Thyroidectomy reduces the incidence of hormone-dependent stem cell leukemia elicited by 4 biweekly doses of 7,12-dimethylbenz(a)anthracene to Long-Evans rats (22% compared with 46% in control animals). There is a high incidence of early death due to aplastic anemia in the thyroidectomized rats (70%) compared with controls (34%). In established leukemia, neither thyroidectomy nor thyroxine excess produces any lasting benefit. In all experiments, the thyroid status of each rat is continuously measured by the free thyroxine index.

INTRODUCTION

A stem cell leukemia can be induced rapidly by a series of p.o. feedings of DMBA to Long-Evans rats (8). A remarkable feature of this leukemia is its rapid regression following hypophysectomy in substantial numbers of rats with primary leukemia or bearing transplants of leukemia cells (7, 11, 19).

Which single hormone or combination of hormones under pituitary control is responsible for this effect is not known. Prolactin has been found to be unimportant in the induction and development and growth of tumors have been sporadic, and in none of these studies, however, was the thyroid status of each rat monitored during the period when altered hormone levels were assumed to be present. This paper, therefore, represents the first controlled study of the influence of thyroid activity on the induction and maintenance of a hydrocarbon-induced leukemia where hormone levels have been carefully monitored throughout the period of experimental observation.

MATERIALS AND METHODS

Animals. Long-Evans rats were studied exclusively. They were taken from a random-bred closed colony of rats which has been maintained for 14 years. The rats were housed in stainless steel or plastic cages in air-conditioned rooms at 25 ± 1 °C (S.D.), artificially lit for 12 hr/day. They were fed commercial ration [Rockland Mouse/Rat diet (Teklad, Monmouth, Ill.) or Diet 41 (William Shearer Ltd., Glasgow, Scotland)]. Tap water was given freely. Ether was used as anesthetic when required. Vaginal smears were examined daily.

Induction of Leukemia. In experiments to assess the effect of thyroidectomy on the induction of leukemia, 15% (w/v) emulsion of DMBA (kindly supplied by Dr. Paul Schurr, The Upjohn Company, Kalamazoo, Mich.) was injected into the tail vein of 44- to 54-day-old rats (designated Day 0). This was repeated on a further 3 occasions at 14-day intervals; the dose was fixed at 30 mg/kg body weight. In experiments on established leukemia, the leukemia was induced by p.o. administration of a solution of refined 0.5% (w/v) DMBA in sesame oil, as described previously (11), to animals ages 28 to 35 days in a dose of 175 mg/kg body weight, and on 3 further occasions at 14-day intervals in a dose of 10 mg to each rat.

Diagnosis of Leukemia. The most convenient method of early diagnosis of this leukemia is its appearance on open liver biopsy as described earlier (8). The first diagnostic liver biopsy was performed 14 days after the final exposure to carcinogen (i.e., Day 56), and on a further 7 occasions at 14-day intervals thereafter.

Hematological Studies. Blood for hematological procedures was obtained by cardiac puncture into a heparinized syringe and examined by conventional hematological techniques. Erythropoiesis was estimated by 59Fe uptake into bone marrow 2 hr after injection of a dose of 1 μCi/g body weight (17).

Thyroidectomy. Thyroparathyroidectomy was performed by surgical avulsion. In induction experiments, this preceded the first dose of carcinogen by 6 to 7 days in all cases. All thyroparathyroidectomized rats were given 1% calcium lactate in the drinking water to avoid tetany. Completeness of thyroidectomy was assessed by assay of the free thyroxine index. This index is derived from multiplying the product of the free thyroxine level (μg/100 ml), based on a competitive binding technique (16), and the ratio of the uptake of thyroxine onto resin beads compared with standard sera. Serum from all animals was assayed at approximately 6 weekly intervals. Assay of 85 normal rats established a normal range of 5.46 ± 1.46 (S.D.). Thyroidectomy was considered incomplete if the free thyroxine index was greater than 1.0. L-Thyroxine (Sigma Chemical Co., St. Louis, Mo.) was administered in a dose of 10 μg/day s.c.

RESULTS

Anatomical and Hematological Effects of Thyroidectomy. Female rats were used for this study. Thyroidectomy was performed at age 21 days. The animals became sluggish,
developed coarse fur, and showed retardation of body growth (Chart 1). Opening of the vaginal plate was not delayed, and periodicity of the estrus cycle was normal. A mild nonprogressive anemia developed around the 35th postthyroidectomy day. No persistent significant differences in peripheral WBC or platelet count were noted. At the 77th postthyroidectomy day, a small but significant reduction \( (p < 0.05) \) in spleen size was observed: \( 0.18 \pm 0.02 \) versus \( 0.21 \pm 0.01 \) g/100 g body weight (10 rats in each group).

Erythropoiesis as measured by \( ^{59} \text{Fe} \) incorporation into bone marrow 77 days postthyroidectomy was significantly reduced \( (p < 0.05) \) in the thyroidectomized group (1148 ± 324 versus 1773 ± 402 cpm, wet weight, bone marrow; 8 rats in each group).

The Effect of Thyroidectomy on Induction of Leukemia. In the series of experiments to assess the effect of hypothyroidism on the induction of leukemia, 217 adequately thyroidectomized rats and 138 intact littermates were given 4 doses of carcinogen at biweekly intervals. Two major effects were noted.

First, there was a significantly higher incidence of early death in the thyroidectomized group, compared with controls. Only 45 of the original 219 thyroidectomized animals became "effective," i.e., survived until the day of the first diagnostic liver biopsy (Day 56), compared with 90 of 138 controls \( (p < 0.001) \).

The majority of deaths occurred following the second or third injection of carcinogen and appeared to be due, in the majority of cases, to aplastic anemia. This was characterized by loss of body weight, loss of normal estrus periodicity in females, reduced body temperature, peripheral blood pancytopenia, and marrow hypoplasia or aplasia.

Second, of the 45 "effective" thyroidectomized animals, 10 (22%) developed leukemia by Day 140 compared with 41 (46%) of the 90 "effective" controls \( (p < 0.01) \) (Table 1). Since several animals from each group succumbed to the stress of repeated diagnostic liver biopsy, the incidence of leukemia was assessed on each biopsy day (Table 1). There was a statistically significant difference \( (p < 0.05) \) on Day 56, but on each other day the differences failed to achieve significance. However, the total number of rats developing leukemia by Days 56, 70, 84, 98, and 112 was significantly greater in controls compared with thyroidectomized rats \( (p < 0.06; Day 70, p < 0.05; Day 84, p < 0.01; Day 98, p < 0.05, Day 112, p < 0.01) \).

There were no significant differences in the time taken to develop leukemia between the groups (thyroidectomy, 91 ± 29 days; controls, 78 ± 26 days). The statistical test used in these experiments was \( \chi^2 \) with Yates correction for small numbers.

Effect of Thyroidectomy on Stem Cell Leukemia. None of the animals with established primary leukemia which were subjected to thyroidectomy showed any reduction in growth of leukemia. In every case, peripheral blood values deteriorated, and liver biopsy on the 14th postoperative day exhibited more extensive infiltration than preoperatively (Table 2). Survival in the thyroidectomized animals was shorter \( (21 \pm 9.9 \text{ days}) \) than in controls \( (29.4 \pm 14.0 \text{ days}) \).

Effect of Thyroxine Excess on Stem Cell Leukemia. Of the 20 animals with primary leukemia that received large doses of thyroxine, 18 showed clear deterioration (Table 3). Peripheral blood values had deteriorated, and liver histology exhibited marked deterioration. Two animals, however, showed improvement in peripheral blood indices. In these cases, liver biopsy showed apparent complete regression in one, and partial

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**Chart 1.** The effect of thyroidectomy on peripheral blood and body weight. Thyroidectomy performed at 21 days of age (Day 0). Each point is the mean of 8 females.
regression in the other. Therapy was discontinued in these 2 animals and subsequent liver biopsy, 14 days later, showed clear extension of the tumor. Survival in the treated group was not prolonged compared with controls. The statistical test applied to the experiments in established leukemia was Student’s t test.

**DISCUSSION**

A remarkable feature of this (Huggins) leukemia is its regression following hypophysectomy. Which hormone under pituitary control is responsible for this effect is not yet known. From the results described, the thyroid axis does not contribute lasting benefit.

It is also unlikely that thyrotrophin levels are important since they bear a reciprocal relationship to thyroxine levels in the blood. Temporary improvement, however, of a small number of rats receiving thyroxine justifies further examination of the effect of thyroxine excess. The repeated doses of carcinogen given to hypothyroid rats had 2 effects in this model. First, there was a significant reduction in the incidence of leukemia compared with control littermates. An additional unexpected finding was the high incidence of early death (172 of 217) compared with controls (48 of 138); p < 0.001. Most animals succumbed because of aplastic anemia.

The reasons for this undue susceptibility to carcinogen in hypothyroid animals is not yet clear. The dose of carcinogen given to each animal determined on a body weight basis, which makes allowance for the fact that in hypothyroid animals growth is retarded. Surgical interference per se has not had this effect in other experiments, e.g., splenectomy or in a group of 42 of the control animals which had sham thyroidectomies.

In the testis, DMBA attacks selectively cells which synthesize DNA (5), probably by intercalation into DNA. Incorporation of $^{59}$Fe into the bone marrow of hypothyroid animals was reduced compared with controls. This may indicate that there is less opportunity for the carcinogen to be effective. A single dose of DMBA causes moderate pancytopenia in the peripheral blood by the tenth day after injection; this is accompanied by temporary marrow hypoplasia (2), but the hypoplasia has almost completely disappeared 4 days later. In hypothyroid animals, the pancytopenia is more prolonged, and full recovery is not usual until the 17th postinjection day. This probably reflects the inhibition of erythropoiesis demonstrated in hypothyroid animals and the susceptibility of this group to develop fatal aplastic anemia.

Inhibition of breast carcinogenesis by DMBA in thyroidectomized Sprague-Dawley rats (12) was attributed to reduced caloric intake. This aspect was not controlled in these experiments.
The metabolism of DMBA may be retarded in thyroidectomized animals, permitting its toxic effects on the bone marrow to be enhanced. Hypothyroidism is known to inhibit hepatic pyridine nucleotide-linked dehydrogenases (10). Administration of DMBA causes an increase in hepatic menadione reductase (9). Our initial experiments, however, indicate that the increase in the hepatic level of this enzyme following administration of carcinogen is no different in hypothyroid animals. Investigation of this aspect continues.

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