Increased Survival of Rats Bearing Morris Hepatoma 7800 after Induction of Hypothyroidism

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ABSTRACT

The survival of Buffalo rats bearing Morris Hepatoma 7800 was increased significantly (23 to 31%) after induction of hypothyroidism by propylthiouracil (PTU) (0.1% in Purina rat chow) or 131I. The concentration of PTU used was in the optimal range as 0.03% PTU was less effective than 0.1%, while 0.4% PTU appeared to be toxic. Exogenous thyroxine (8 μg/kg body weight) reversed the effects of PTU and actually shortened survival. Because food consumption and body weights of hypothyroid rats were decreased, the survival of pair-fed controls was studied and found to be the same as in untreated controls. We conclude that the hypothyroid state increases the survival of rats bearing Morris Hepatoma 7800. We have not yet been able to define any anatomical or biochemical parameters which may be responsible for this effect.

INTRODUCTION

The effect of thyroid status on the induction and growth rate of experimental hepatomas, as well as on the survival of animals bearing these tumors, has received very little attention. Paschkis et al. in 1948 (26) reported that "thiouracil protected the liver of rats against the carcinogenic action of acetaminofluorene." In 1952, Bielschowsky and Hall (2) reported that thyroidectomy inhibited liver tumor formation in rats fed either acetaminofluorene or 2-aminofluorene, while the incidence of extra hepatic tumors was unaffected. These results were later confirmed by Goodall in 1966 (10). In contrast, in the studies of Miller and Baumann (19), administration of either thiouracil or 6-n-propylthiouracil did not alter the development of azo dye-induced liver tumors nor the survival of rats who had developed hepatomas. In contrast to the above-mentioned studies in which antithyroid agents were administered continuously, Miller and Baumann fed their rats 0.1% PTU3 for 8 days, followed by 0.02% PTU for another 18 days. There is reason to believe that the animals in this study were not rendered severely hypothyroid. Reported effects of hypothyroidism on the induction of mammary tumors range from enhancement (5, 12) to a reduction of carcinogenesis (6, 15, 24). It is of interest that, when studied, the protective effect of PTU was neutralized by the administration of T₄ (24). Shellabarger (30) interpreted his data to mean that hypothyroidism does not interfere with the initiation phase of carcinogenesis in female rats but inhibits the growth of mammary adenocarcinomas subsequent to their initiation. Shoemaker et al. (31) observed an inhibition of the growth of mammary adenocarcinomas implanted into hypothyroid adult mice as well as an increased survival of these tumor-bearing animals.

We have studied the effect of hypothyroidism on the survival of rats bearing Morris Hepatoma 7800. The survival of animals bearing this rapidly growing tumor is approximately 5 to 8 weeks, which was well suited for short-term survival studies. The present study attempted to define the effect of PTU on the growth rate of Morris Hepatoma 7800 as well as on the survival of these hepatoma-bearing rats. We have determined the effect of various doses of PTU as well as the effect of exogenous T₄, given along with PTU, on the survival of these animals. The effect of 131I-induced hypothyroidism was compared to that of PTU to determine whether the effects observed were dependent upon the hypothyroid state per se or specific extrathyroid actions of PTU. The extrathyroid effects of PTU include the inhibition of many peripheral actions of T₄ and of the conversion of T₄ to T₃, as well as of the overall rate of T₄ degradation (9, 27). In contrast, while PTU has been shown to slow the fractional deiodination and degradation rate of T₃ (25), it has not been found to inhibit the biological actions of T₃ (23).

MATERIALS AND METHODS

PTU and carrier-free potassium [131I]iodide were generous gifts of the Charles Frost Co., Ltd., Pointe Claire, Quebec, Canada. L-Thyroxine was purchased from Sigma Chemical Co., St. Louis, Mo. Stock solutions containing 1.6 mg/ml were made up and frozen in aliquots of 50 μl, which were thawed and diluted to 10 ml with 0.9% NaCl solution prior to the time of injections.

Buffalo rats (160 to 250 g; supplied by Simonsen Laboratories, Gilroy, Calif.) were shipped to Howard University, Washington, D. C., for implantation of Morris Hepatoma 7800 into the muscle of both hindlimbs. Within a few days of inoculation, the animals were transferred to Montreal for the experimental studies. All animals were housed singly in individual wire-bottomed cages, fed powdered Purina rat chow, and were given tap water ad libitum. Animals were weighed once every week, and tumor size was estimated by measuring the longest diameter, using bluntened calipers.

Two survival experiments were carried out using littermate female Buffalo rats. The first experiment involved 35 animals divided into 3 groups (see Charts 1 and 2): (a) tumor-bearing controls (12 rats); (b) PTU, 0.1% by weight mixed into Purina rat chow (13 rats); and (c) pair-fed tumor-bearing controls (10 rats). Mean tumor diameter at the onset of this study was approximately 13 mm. In pair feeding, animals of the same weight and age were matched. One animal is fed the PTU diet

1 Supported by grants from the Canadian Medical Research Council (MA 5474), Charles Frost Co., Ltd., and USPHS (Grant Ca 10729).
2 Recipient of a Chercheur-Boursier award of the Quebec Medical Research Council. To whom requests for reprints should be addressed, at Division of Gastroenterology, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1.
3 The abbreviations used are: PTU, propylthiouracil; T₄, thyroxine; T₃, triiodothyronine.

Received August 14, 1978; accepted March 22, 1979.
ad libitum, and the other (pair fed) is given the control Purina diet. The amount of control diet is restricted to that consumed by the matched animal on the PTU diet.

In the second experiment, begun within 14 days of tumor implantation when tumor was not yet palpable, 90 animals were divided into 6 equal groups: (a) tumor-bearing controls; (b) 0.03% PTU; (c) 0.1% PTU; (d) 0.4% PTU; (e) 131I (1 mCi/100 g body weight i.p.); and (f) 0.1% PTU + T₄ (8 µg/kg body weight i.p. on 6 of 7 days). The dose of T₄ used is equivalent to the daily excretion of T₄ as determined by Taurog and Chaikoff (35) and corresponds to the dose used by other authors (16, 17). The serum levels of T₃ and T₄ in hypothyroid animals receiving this dose of T₄ were similar to those of euthyroid controls. For ease of presentation, the results of this latter experiment are depicted in 2 charts (Charts 3 and 4). In all experiments, the initial mean weight ± S.E. of each experimental subgroup did not differ significantly. As the above studies were concerned only with animal survival, no samples of any kind were collected before death. Cages were inspected daily for dead animals, the carcasses of which were autopsied immediately or were frozen at −20°C for later examination. At necropsy, the abdomen and thorax were opened, and visual observations were made on various organs. In some cases, tissue was removed for histological examination.

In a separate experiment, 20 female Buffalo rats fed either powdered Purina rat chow or rat chow containing 0.1% PTU were sacrificed after 4.5 weeks of treatment. In addition to the data collected in the previous experiments, we obtained absolute values for the weights of liver, tumor, and thyroid tissue (see Chart 5). Sera as well as tumor and hepatic tissue were fast frozen at −70°C for future analysis. From the above and other experiments involving other Morris hepatomas (to be described in future communications), we have been able to confirm that caliper measurements of tumor diameter correlate very well with absolute measurements of tumor weight (r = 0.91; n = 53; p < 0.001).

Cumulative percentage distribution of mortality was compared using a Kolmogorov-Smirnoff 2-sample test. The Kolmogorov-Smirnoff test, which is a nonparametric approach not involving any assumptions, was felt to be more valid than Student's t test for the analysis of mortality data. The significance of other results was compared using Student's t test with a p value of <0.05.

RESULTS

Tumor-bearing animals fed 0.1% PTU showed a significant increase in survival over either pair-fed controls or tumor-bearing controls fed Purina diet (Charts 1 and 2). The cumulative mortality of the control group was significantly higher than that of the PTU-treated group (p < 0.05). This effect was also observed in the second experiment, where the cumulative mortality of the tumor-bearing controls was significantly higher than that of the PTU-treated group (p < 0.05).

Chart 4. The effect of different methods of inducing hypothyroidism on cumulative mortality. Cumulative mortality curves of Purina (Pur.)-fed controls and rats treated with 131I, 0.1% PTU, and 0.1% PTU + T₄ are derived from the data of Experiment 2. Statistical significance was determined by the Kolmogorov-Smirnoff 2-sample test (Table 1). Inset, mean tumor size for each group.
bearing controls fed ad libitum (Chart 1). The mean survival time for the PTU-treated animals was 61.8 ± 2.4 S.E. days compared to 46.9 ± 1.7 days and 44.2 ± 1.6 days for the tumor-bearing and pair-fed controls, respectively (Table 1). The mean body weights of these animals are depicted in Chart 2. The range of daily food intake was 11 to 14 g for tumor-bearing controls and 3 to 7 g for PTU-treated rats. These amounts were reduced when the animals became terminal. The effect of 0.1% PTU in prolonging survival was confirmed in the second experiment, which also showed that the dose of 0.1% PTU was an appropriate choice (Table 1; Chart 3). The mean survival of animals treated with 0.03% PTU for 62.9 days was similar to that of rats fed 0.1% PTU for 65.0 days (Table 1). Both values for mean survival were significantly greater than that of controls, 53.0 days. However, when the cumulative percentage distribution of mortality curves for these groups were compared, using the Kolmogorov-Smirnoff method, the results obtained when rats bearing Morris Hepatoma 7800 were sacrificed after 4.5 weeks of treatment with 0.1% PTU (left). Ratios correcting for changes in body and liver weights are shown (right). TBW, total body weight.

**Table 1**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Survival (days)</th>
<th>Mean survival</th>
<th>Cumulative mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Control (12)</td>
<td>46.9 ± 1.7</td>
<td>NS*</td>
</tr>
<tr>
<td>0.1% PTU (13)</td>
<td>61.8 ± 2.4</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pair fed (10)</td>
<td>44.3 ± 1.6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Control (15)</td>
<td>53.0 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>0.03% PTU (15)</td>
<td>62.9 ± 2.9</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>0.1% PTU (15)</td>
<td>65.0 ± 1.9</td>
<td>&lt;0.005</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>0.4% PTU (15)</td>
<td>54.4 ± 1.7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>0.1% PTU + T₄ (15)</td>
<td>46.0 ± 2.6</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>¹³¹I (15)</td>
<td>65.0 ± 1.4</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* p determined by comparing mean survival to that of controls using Student's t-test.
* p determined by comparing cumulative mortality curves shown in Charts 3 and 4 to that of controls using the Kolmogorov-Smirnoff 2-sample test.
* Numbers in parentheses, number of animals in each experimental group.
* Mean ± S.E.
* NS, not significant.

in thyroid weight shows that 0.1% PTU had rendered the animals in this group hypothyroid (6). Mean values ± S.E. for T₃ in 5 control and 5 PTU-treated animals were 116.8 ± 7.2 and 2.8 ± 1.1 ng/100 ml, respectively, while the corresponding values for T₄ were 4.5 ± 0.3 and 0.1 ± 0.1 µg/100 ml, respectively. Similar values have been measured after feeding PTU (0.1%) to Buffalo rats bearing Morris Hepatoma 44. In these experiments, mean T₃ values in 40 control and 33 PTU-treated rats were 119.5 ± 7.3 and 0.6 ± 0.6 ng/100 ml, respectively, while the corresponding T₄ values were 4.5 ± 0.2 and 0.2 ± 0.1 µg/100 ml. Serum T₃ and T₄ values after ¹³¹I thyroid ablation (n = 15) were 0.1 ± 0.01 ng/100 ml and 0.2 ± 0.0 µg/100 ml, respectively. Following the daily injection of T₄ (8 µg/kg body weight) to ¹³¹I-treated rats (n = 5), the corresponding values were 2.3 ± 2.3 ng/100 ml and 2.2 ± 0.3 µg/100 ml, respectively. Minimal reduction in liver weight after PTU treatment was not significant when corrected for reduced body weight. While absolute mean tumor weight was significantly less in the PTU-treated animals, this difference was not statistically significant after correcting for body or liver weight change. Serum samples submitted for routine clinical

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* Assuming an average intake of 5 g of Purina per day containing 0.1% PTU, the average dose of PTU was 5 mg/rat/day, which is well within the effective dose range to induce hypothyroidism (32, 36).
biochemical screen (sequential multiple analyzer computer) revealed that in animals treated with PTU (0.1%) there was a significant increase in serum cholesterol and urea while uric acid levels were reduced. In the 5 control tumor-bearing rats, the mean serum concentrations of cholesterol, urea, and uric acid were 130.2 ± 3.8, 22.8 ± 0.7, and 2.7 ± 0.1 mg/ml, respectively, while the corresponding concentrations in the 5 PTU-treated hepatoma-bearing rats were 267.8 ± 15.5, 44.5 ± 3.4, and 1.2 ± 0.2, respectively. Analysis of blood gases in heparinized blood did not reveal any differences between treated and untreated animals.

DISCUSSION

To the best of our knowledge, this study is the first to document a prolongation of survival of hepatoma-bearing rats following the induction of hypothyroidism by means of PTU or 

\[ ^{131} \text{I} \]. In addition, our results indicated that PTU in a concentration of 0.1% by weight in Purina rat chow is in the optimal dose range to achieve an increase in the survival of these animals (Chart 3). It has been documented that PTU administered in a concentration of 0.03% or greater in the diet is an effective goitrogenic dose (16, 32, 36). In view of the fact that PTU may have toxic effects separate from its antithyroid actions such as agranulocytosis and increased susceptibility to infection (33), it was not surprising that the increased survival observed after feeding 0.1% PTU was not observed in the group fed 0.4% PTU. It is unlikely that the action of PTU is a direct extrathyroid effect, as identical results were achieved with 

\[ ^{131} \text{I} \] thyroid ablation (Chart 4). This conclusion is further strengthened by the observation that exogenous T4 reversed the protective effect of PTU on survival (Chart 4). At present, we are not able to explain why the survival of animals receiving PTU and T4 was significantly shorter (according to the Kolmogorov-Smirnoff analysis) than that of tumor-bearing controls. Serum T3 and T4 values measured in hypothyroid Buffalo rats bearing Morris Hepatoma 44 and receiving the same dose of exogenous T3 were not greater than those of tumor-bearing controls. We can only speculate whether PTU-treated hepatoma-bearing rats are either very sensitive to exogenous T3 or have a reduced hepatic clearance of this hormone, in contrast to tumor-free PTU-treated animals who require larger doses of T4 to reverse the antithyroid effects of PTU (36).

Since PTU-treated rats consumed less food and had a slower weight gain than untreated animals, we studied pair-fed tumor-bearing controls, the weight gain of which was similar to that of PTU-treated rats. The survival of these animals did not exceed that of tumor-bearing controls (Chart 1). We have noted in this and other experiments that PTU-treated rats usually weigh less than pair-fed controls. We presume that this additional weight loss is related to the metabolic consequences of hypothyroidism (6) resulting from a general decrease in the metabolic rate, including a decrease in protein and RNA synthesis (1, 34). These experiments also indicated that the reduced food intake and weight loss seen in PTU-treated animals did not contribute to the PTU-mediated effects on animal survival. In a recent study comparing the survivors of rats bearing Morris Hepatoma 7777 to total parenteral hyperalimentation and to ad libitum feeding (4), it was noted that in spite of marked weight loss in the latter group there was no significant difference in the length of survival.

Why did we not observe an inhibition in tumor growth in addition to increased survival after PTU? It is possible the growth rate of Morris Hepatoma 7800 (generation time, 1.3 months) is too rapid to be inhibited by hypothyroidism induced after tumor implantation. In contrast, we have noted that the growth rate of several slower-growing Morris Hepatomas is significantly inhibited by PTU- or 

\[ ^{131} \text{I} \]-induced hypothyroidism (20). Studies in progress in a slower-growing hepatoma indicate that the survival of these tumor-bearing rats is also prolonged after induction of hypothyroidism.

While it has been documented that thyroid deficiency interferes with hepatocarcinogenesis (10, 26), we have not been able to find any studies on the effect of thyroid status on the growth rate of established liver tumors in vitro or in vivo.

In tissue culture experiments, thyroid hormone has been observed to stimulate MCF-7 cells (3), which were derived from a pleural effusion in a postmenopausal woman with metastatic breast cancer. Treatment of these cells with T3 resulted in increased growth as well as increased estrogen receptor levels. The nuclei of these cells also contain receptor sites for thyroid hormones.

The correlation of thyroid status with the incidence and progression of human neoplasms is not clear. A number of epidemiological studies have suggested that breast cancer is associated with abnormalities of thyroid function (37). Although the most frequent association has been with hypothyroidism, a careful study by Schottenfeld (28) on his own patients (including measurement of protein-bound iodine levels) and of the literature up to that time (1968) failed to confirm a relationship between hypothyroidism and breast cancer. In a recent editorial, the American Thyroid Association stated that it is not yet possible to define the exact relationship between thyroid status and breast carcinoma (11). In spite of this statement, it is of interest that the majority of studies that have investigated serum thyroid hormone concentrations have found a tendency toward elevation of T4 levels in cancer patients (37). That breast cancer patients may indeed be slightly hyperthyroid as a group is suggested by the recent observation (37) that these patients have slightly increased cortisol production, a known physiological consequence of hyperthyroidism (13). Studies on the possible relationship between thyroid function and prostatic cancer have not been conclusive (37).

It should be noted that the postulated effect of thyroid status on a given tumor or target organ may be due either to the direct effect of thyroid hormones or the indirect effects resulting from altered production of thyroid-stimulating hormone (21), growth hormone (14), and prolactin (22), as well as estrogenic (7) and androgenic (8) steroids. It is of interest that recent studies have demonstrated that both estrogen and T4 are important for the development of carcinogen-induced skin tumors (18).

The mechanism by which hypothyroidism increased the survival of hepatoma-bearing rats remains unexplained at present. To date, we have not been able to define any anatomical or metabolic alterations which might be responsible for the protective effect of hypothyroidism. The possibility that these results can be explained on an immunological basis has not been ruled out. These studies and others in progress raise the possibility that experimental hepatomas may be hormone dependent in a manner analogous to mammmary carcinomas (29).

ACKNOWLEDGMENTS

We acknowledge the excellent assistance of Francon Lenoff and Raphael Pollack, and we thank Dr. C. Bastomsky for measuring serum T3 and T4 levels,
REFERENCES

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