Inhibition of Local and Metastatic Hepatoma Growth and Prolongation of Survival after Induction of Hypothyroidism


ABSTRACT

The local growth rate of Morris Hepatoma 44 (generation time, 6 months) was inhibited by 66 to 87%, and host survival was prolonged by 36 to 78% after the induction of hypothyroidism within 2 weeks of tumor implantation by propylthiouracil (0.1% in Purina chow), $^{31}$I (1 mCi/100 g body weight i.p.), or surgical thyroidectomy. In additional experiments, we studied the effects of inducing hypothyroidism ($^{31}$I) at different stages in the natural history of Morris Hepatoma 44 on local and metastatic growth as well as on host survival. Induction of hypothyroidism within 2 weeks of tumor implantation (Group I) reduced local tumor growth as well as the number and size of pulmonary metastases, and prolonged survival by 70 to 80%. Induction of hypothyroidism at 6 weeks postimplantation when tumors were palpable (Group II) inhibited local growth by 39%, reduced the number and size of pulmonary metastases by approximately 80%, and prolonged host survival by 35%. Initiation of $^{31}$I treatment at 11 weeks when microscopic pulmonary emboli were present in most animals (Group III) reduced local growth by 19% and the number and size of pulmonary metastases by 72 and 50%, respectively. In this case, survival was prolonged by 17%. We conclude from these results that the local and metastatic growth of Morris Hepatoma 44 as well as host survival are thyroid hormone-dependent processes. The mechanisms responsible for these observations remain to be explained.

INTRODUCTION

At present, at least 7 cancer types are known to be responsive to hormonal manipulation. These include tumors of breast, prostate, thyroid, endometrium, kidney, and seminal vesicle, and also include lymphoma and leukemia (5). In most cases, shrinkage of the cancer is associated with improved clinical status of the host.

In spite of the fact that the growth and metabolic function of adult and regenerating liver in vivo and in vitro are strongly influenced by a variety of hormones (6, 9, 11, 24), there have been few studies of the possible hormonal responsiveness of hepatomas. In 3 studies reported during the 1950s, it was noted that induction of diabetes inhibited the growth of established experimental hepatomas (2, 3, 23). During the same period, Miller and Bauman (13) reported that induction of moderate hypothyroidism with 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. While other studies have established that hypothyroidism will inhibit hepatocarcinogenesis (1, 19, 20), until recently, there were no further studies on the effect of thyroid function on the growth of established experimental or human hepatomas.

We have recently reported that the induction of hypothyroidism significantly inhibited the local growth of Morris Hepatoma 44, a slow-growing hepatoma implanted into the hind limbs of Buffalo rats (14-16). After 11 weeks of treatment, hepatoma weight was reduced by 66, 86, and 75% (after correction for total body weight) relative to controls, in PTU3-fed, $^{31}$I-injected, and thyroidectomized rats, respectively. In each case, exogenous thyroxine (8 µg/kg body weight i.p.) reversed these inhibitory effects. In this manuscript, we report on the results of simultaneous experiments using littermate rats in which we studied the effects of the above-mentioned treatment modalities on the survival as well as on the local growth of Morris Hepatoma 44. In addition, we have studied the effect of the timing of $^{31}$I-induced hypothyroidism on the above-mentioned parameters as well as on the number and size of pulmonary metastases.

MATERIALS AND METHODS

PTU and carrier-free potassium $^{31}$Iodide were generous gifts of the Charles Frosst Co., Ltd. (Pointe Claire, Quebec, Canada). Stock solutions of L-thyroxine (Sigma Chemical Co., St. Louis, Mo.) 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 before the time of injections.

Female Buffalo rats (Simonsen Laboratories, Gilroy, Calif.) were shipped to Howard University (Washington, D. C.) for implantation of Morris Hepatoma 44 into the thigh musculature of both hind limbs. Within a few days of inoculation, the animals were transferred to Montreal. Animals were housed in pairs in wire-bottomed cages, fed powdered Purina rat chow, and given tap water ad libitum. Animals were weighed once every week, and tumor size was estimated by using blunted calipers to measure the longest diameter of the tumor. Studies were initiated within 10 to 14 days of tumor implantation. PTU (0.1% mixed into Purina rat chow) was found to be superior to either 0.03 or 0.4% mixtures in prolonging the survival of Buffalo rats bearing Hepatoma 7800 (17). $^{31}$I was administered as a single i.p. dose (1 mCi/100 g body weight). Animals receiving $^{31}$I were housed and cared for in a separate room for 2 weeks

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3 The abbreviation used is: PTU, propylthiouracil.
after injection until levels of radioactivity became insignificant. Surgical thyroidectomy was performed by one of the authors. Exogenous thyroxine was administered in a dose of 8 μg/kg body weight i.p. on 6 of 7 days. No surgical procedures or blood sampling were carried out on these animals.

In Experiment 2 (Table 2), post mortem examination was carried out in all cases. The lungs were inflated before removal by the intratracheal infusion of 4% formalin. In agreement with the observations of Morris and Slaughter (18), metastatic lesions were found only in the lungs. Quantitation of the number and size of pulmonary metastases was carried out as follows. The right lung was sectioned transversely at the same levels to yield 3 slices, each approximately 5 mm thick. The first sample was taken from the top of the superior lobe and embedded apex-down in order to preserve the proper polarity during the sectioning. The second and third samples, constituting cross-sections of the intermediate lobe and basal margin of the inferior lobe, respectively, were treated in a similar manner. The tissues were embedded in paraffin blocks, cooled on ice, and 5-μm sections were cut with a rotary microtome. A routine hematoxylin and eosin stain was performed prior to morphometrical analysis. Photomicrographs of the slides were made, and the data were then compiled using a Carl Zeiss MOP III Digitizer.

Cumulative percentage distribution of mortality was compared using a Kolmogorov-Smirnoff 2-sample test (Chart 2). The Kolmogorov-Smirnoff test, which is a nonparametric approach involving only minimal assumptions which were met, was used to test that the distribution of mortality was identical. Mean values were compared via Student’s t test (Tables 1 to 3).

RESULTS

The survival of hepatoma-bearing Buffalo rats was significantly prolonged by the induction of hypothyroidism (Table 1). The mean survival of PTU-treated, thyroidectomized, and 131I-injected rats significantly exceeded that of controls by 49, 36, and 50%, respectively. The survival of thyroidectomized rats was reduced by early surgery-related mortality. The administration of low-dose exogenous thyroxine significantly reduced but did not neutralize the protective effect of hypothyroidism. The reduction in tumor size observed in hypothyroid rats was neutralized by the administration of exogenous thyroxine (Chart 1). Animal weights were reduced in the hypothyroid rats as noted previously (16, 17). During the last 3 weeks of life, the weight of each animal fell by 28 to 42%. Prior to this, body weight had been maintained relatively stable for at least 4 weeks (depicted as "plateau weight" in Tables 1 and 2).

In the subsequent experiment, hypothyroidism was induced by administering 131I at 3 different stages in the natural history of the growth of Morris Hepatoma 44: (a) Group I, 2 weeks after hepatoma implantation, the time of administration used in the previous experiments; (b) Group II, 6 weeks postimplantation, at which time hind limb tumors were easily palpable; and (c) Group III, 11 weeks postimplantation when microscopic pulmonary metastases were present in most untreated animals (~70%). It should be noted that the earliest mortality of untreated euthyroid animals occurred at this time (Chart 2).

The early induction of hypothyroidism (Group I) prolonged survival by 78% (Table 2; Chart 2) while the mean survival of animals rendered hypothyroid at 6 weeks (Group II) and at 11 weeks (Group III) was also significantly increased by 35 and 17%, respectively. In each of the above groups, local tumor growth was inhibited within 2 weeks of 131I administration (Chart 3). The data depicted in Chart 3 reflect the fact that no measurements of tumor size were made during the 2- to 3-week period of isolation following the injection of 131I. At 16 weeks, when the effects of hypothyroidism were manifest in all

### Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plateau</th>
<th>Terminal</th>
<th>Days</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (9)</td>
<td>244.0 ± 4.6</td>
<td>156.2 ± 6.7</td>
<td>125 ± 11.1</td>
<td>+49</td>
</tr>
<tr>
<td>PTU 0.1% (10)</td>
<td>170.9 ± 2.8</td>
<td>124.2 ± 5.3</td>
<td>186 ± 15.2</td>
<td>+36</td>
</tr>
<tr>
<td>Thyroidectomy (9)</td>
<td>214.8 ± 5.3</td>
<td>143.8 ± 8.5</td>
<td>170 ± 19.7</td>
<td>+19</td>
</tr>
<tr>
<td>Thyroidectomy + thyroxine (11)</td>
<td>250.5 ± 9.1</td>
<td>166.9 ± 9.0</td>
<td>149 ± 6.4</td>
<td>+19</td>
</tr>
<tr>
<td>Controls (11)</td>
<td>256.4 ± 4.6</td>
<td>160.0 ± 4.6</td>
<td>145 ± 16.1</td>
<td>+50</td>
</tr>
<tr>
<td>131I (12)</td>
<td>198.7 ± 5.8</td>
<td>115.0 ± 2.1</td>
<td>219 ± 10.8</td>
<td>+15</td>
</tr>
<tr>
<td>131I + thyroxine (12)</td>
<td>258.1 ± 9.2</td>
<td>159.2 ± 8.2</td>
<td>166 ± 10.0</td>
<td>+15</td>
</tr>
</tbody>
</table>

*a* Stable animal weight maintained for at least 4 weeks prior to terminal weight loss which occurred in the last 3 weeks of life.

*b* Animal weight within the last week of life.

*c* Numbers in parentheses, number of animals in whom the hind limb implants "took." Originally there were 15 animals/group.

*d* Mean ± S.E.

*e* Statistically significant difference (p ≤ 0.05) versus controls using Student’s t test as well as the Kolmogorov-Smirnoff test.
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Chart 1. Effect of hypothyroidism on the primary growth of Morris Hepatoma 44 implanted into the hind limbs of female Buffalo rats. Hepatoma diameter was measured using blunt calipers. Each point represents the mean value of measurements in a given group.

Chart 2. Effect of the timing of $^{131}$I-induced hypothyroidism on the cumulative mortality of Buffalo rats bearing Morris Hepatoma 44. Symbols correspond to those used in Chart 2. Hepatoma diameter was measured using blunt calipers. Each point represents the mean value of measurements in a given group.

Chart 3. Effect of the timing of $^{131}$I-induced hypothyroidism on the primary growth of Morris Hepatoma 44. Symbols correspond to those used in Chart 2. Hepatoma diameter was measured using blunt calipers. Each point represents the mean value of measurements in a given group.

Groups, tumor size was reduced by 74% in Group I, by 39% in Group II, and by 19% in Group III. The apparent reduction in mean tumor diameter noted in Group III animals after 17 weeks (Chart 3) does not reflect regression of tumor size but is due to the dying off of animals bearing the larger tumors. The effect of the timing of $^{131}$I-induced hypothyroidism on pulmonary metastatic lesions is summarized in Table 3 and Fig. 1. The incidence of pulmonary metastases was greatest in untreated controls (80%) and Group III (88%) compared to an incidence of 30% and 40% in Groups I and II, respectively. The absolute number of pulmonary metastases was significantly reduced by at least 70% in all treatment groups. The surface area of metastatic lesions was reduced by more than 80% in Groups I and II, while a 51% reduction was noted in Group III. Expressed differently, the percentage of lung surface area occupied by tumor metastases was 25% in untreated controls compared to 4% in Groups I and II and 13% in Group III.

DISCUSSION

The results of these studies document that the induction of hypothyroidism by 3 different methods in female rats bearing growing Morris Hepatoma 44 (generation time, 6 months) inhibited local tumor growth by 66 to 87% and prolonged host survival by 36 to 78%. We have documented similar effects of hypothyroidism on primary tumor growth using other slow-growing Morris Hepatomas 9633 and 7787 (15) and have noted that these results were independent of the sex of the
host animal (15, 16). In the case of the more rapidly growing Morris Hepatoma 7800 (generation time, 1.3 months), induction of hypothyroidism 2 weeks after tumor implantation did not affect local growth but did prolong survival by 25% (17). Short et al. (26) have recently documented an inhibition of the local growth of Morris Hepatomas 7777 and 5123 implanted into male Buffalo rats 5 days after surgical thyroidectomy. In an attempt to make our experiments more relevant to the clinical situation, we studied the effect of inducing hypothyroidism at different stages in the natural history of Morris Hepatoma 44. Induction of hypothyroidism at a time when primary hind limb tumors were easily palpable (Group II) inhibited the growth rate of the primary tumor and prolonged survival by approximately 35% (Table 2; Charts 2 and 3). In addition, only 40% of rats in this group were found to have pulmonary metastases in contrast to an incidence of 88% in euthyroid controls (Table 3). The number and area of pulmonary metastases in Group II was reduced by approximately 80% (Table 3; Fig. 1). In rats receiving $^{131}$I after microscopic pulmonary metastases were present (Group III), local hepatoma growth was inhibited by approximately 17%, and survival time was prolonged to a similar degree. While the 80% incidence of pulmonary metastases in this group was not significantly different from untreated controls, the absolute number of metastases as well as the area occupied by these lesions were reduced by 71 and 51%, respectively, compared to controls (Table 3; Fig. 1).

In recent studies, Kumar et al. (8) demonstrated that 2 syngeneic mouse tumors (Sarcoma 1 and Lewis fibrosarcoma) are thyroid dependent in a manner analogous to the Morris hepatoma. The authors postulated that the stimulatory effect of thyroxine on the local and metastatic growth of these tumors might be due to its interaction with lymphocyte receptors, leading to an altered immune response together with its direct metabolic effect on tumor cells.

We are attempting to define the mechanisms responsible for the above-mentioned observations. We presume that these effects are mediated by nuclear and/or cytoplasmic receptors for triiodothyronine which we have demonstrated in Morris hepatoma tissue (21). It would seem that thyroid hormones, either by direct or indirect action via other hormones, constitute important growth-promoting factors for this rapidly growing tumor. Evidence for a direct effect of thyroid hormones on hepatoma cell growth has come from preliminary experiments with cell cultures prepared from Morris Hepatomas 44 and 7787. Addition of triiodothyronine (20 x 10$^{-9}$ M) to medium containing fetal calf serum depleted of thyroid hormone increased the cell population significantly. The effect of hormonal stimulation on the growth of hepatocytes has been well documented. The work of Leffert and Koch (6, 9, 11, 12) and Short et al. (25, 26) indicate that iodothyronines stimulate the growth of Morris hepatomas selectively to lung has not been elucidated. One could postulate that short-term stimulation of tumor growth might be related to differences in the rate of utilization and turnover of thyroid hormones in the nongrowing and proliferative state (10). It is possible that thyroid hormones could also influence hepatoma growth indirectly by affecting the rate of synthesis and release of hormones and other substances which influence the growth of hepatoma tissue. For example, growth hormone, the synthesis of which is influenced by thyroid status (4), will stimulate the local growth of Morris Hepatoma 44. The mechanism by which Morris hepatomas metastasize selectively to lung has not been elucidated. One could postulate

Table 3

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of animals</th>
<th>% of incidence of metastases</th>
<th>Av. no. of lesions</th>
<th>Area of lesions (sq µm x 10$^3$)</th>
<th>% of lung area as tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>88</td>
<td>42.6 ± 7.8</td>
<td>163.1 ± 26.6</td>
<td>25</td>
</tr>
<tr>
<td>Group I (2 wk)</td>
<td>13</td>
<td>30</td>
<td>12.6 ± 2.4</td>
<td>31.9 ± 11.7</td>
<td>4</td>
</tr>
<tr>
<td>Group II (6 wk)</td>
<td>12</td>
<td>40</td>
<td>7.2 ± 1.4</td>
<td>79.7 ± 9.6</td>
<td>13</td>
</tr>
</tbody>
</table>

* Based on the microscopic and morphometric analysis of 3 standardized sections from the right lung of each rat.
* Represents only animals in whom the hind limb implants "took." Originally, there were 15 animals/group.
* Statistically significant difference (p ≤ 0.05) versus control using Student’s t test.
that the reduced number of pulmonary metastases in hypothyroid animals is related to the retarded growth of the primary hepatoma.

The mechanisms responsible for the increased survival of the hypothyroid rats has not been elucidated. As of this time, there have been no significant differences in determinations of routine biochemical parameters, blood gases, and pH, as well as preliminary measurements of cell-mediated immunological status between different groups. Elucidation of the pathophysiological mechanisms responsible for the dramatic weight loss before death may provide useful clues to explain the differences in survival among the different experimental groups.

The experimental studies reported in this and previous communications (14–17) establish the Morris hepatoma as a hormone-responsive tumor. Induction of hypothyroidism may be worthy of consideration as an adjunct to the therapy of hepatomas in humans.

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REFERENCES

Fig. 1. Histological sections of lung (inflated post mortem specimens) from A, untreated controls; B, Group I; C, Group II; D, Group III. See legend of Chart 2 for details. H & E.
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