Biological Behavior of MM1 Hamster Melanoma

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ABSTRACT

We have reported evidence recently for a high-affinity receptor for glucocorticoid Malignant Melanoma No. 1 hamster melanoma and suggested that tumor growth was facilitated by adrenal steroids. This report characterizes the behavior of Malignant Melanoma No. 1 following manipulation of the pituitary-adrenal axis in vivo. Bilateral adrenalectomy significantly retarded tumor growth. Hypophysectomy also significantly reduced tumor growth. Silastic implants of hydrocortisone in intact hamsters produced a dose (7 to 28 μg/day)-related increase in tumor growth. Implants releasing a low dose (3 μg/ day) of dexamethasone also increased tumor growth. Chronic exposure of adrenalectomized and intact hamsters to a high dose (125 μg/day) of desoxycorticosterone acetate also produced a significant increase over adrenalectomized and sham-adrenalectomized controls. In contrast, chronic administration of adrenocorticotropic hormone and α-melanocyte-stimulating hormone to intact hamsters did not significantly alter melanoma growth. These observations support the suggestion that adrenocorticosteroids influence the growth of Malignant Melanoma No. 1 hamster melanoma and provide a model for studying the regulation of growth of a glucocorticoid-positive neoplasm originating outside the reticuloendothelial system.

INTRODUCTION

Clinical and epidemiological studies suggest that sex (9, 18, 23), the advent of puberty (1), and pregnancy (24) influence the natural history of human melanoma. Recent reports also suggest that human melanoma contains cytoplasmic receptors for gonadal and adrenal steroid hormones (2, 4, 6, 7, 10, 21). In addition, the presence of receptor for estrogen is directly correlated with those human cell lines responding to estradiol in vitro (23, 24, 25). The present study investigates the effect of chronic exposure of pituitary tumors in vivo. Bilateral adrenalectomy significantly inhibited tumor growth. Hypophysectomy also significantly reduced tumor growth. Silastic implants of hydrocortisone in intact hamsters produced a dose (7 to 28 μg/day)-related increase in tumor growth. Implants releasing a low dose (3 μg/day) of dexamethasone also increased tumor growth. Chronic exposure of adrenalectomized and intact hamsters to a high dose (125 μg/day) of desoxycorticosterone acetate also produced a significant increase over adrenalectomized and sham-adrenalectomized controls. In contrast, chronic administration of adrenocorticotropic hormone and α-melanocyte-stimulating hormone to intact hamsters did not significantly alter melanoma growth. These observations support the suggestion that adrenocorticosteroids influence the growth of Malignant Melanoma No. 1 hamster melanoma and provide a model for studying the regulation of growth of a glucocorticoid-positive neoplasm originating outside the reticuloendothelial system.

The abbreviations used are: DEX, dexamethasone; MM1, Malignant Melanoma No. 1; DOCA, desoxycorticosterone acetate; HC, hydrocortisone; MSH, α-melanocyte-stimulating hormone; ACTH, adrenocorticotropic hormone.

Received April 23, 1981; accepted January 5, 1982.

1 This study was supported by the Cancer Research Fund, Carol Thomas Brigham Fund, and NIH Grant BRSG 7970. This is Paper 1, Effect of Adrenal Manipulation of Tumor Growth, of a series.
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Materials and Methods

Tumor. The MM1 (11) was provided by Dr. J. Fortner (Memorial Sloan Kettering, New York, N. Y.) and maintained by serial transplantation in male Syrian hamsters. MM1 is a solid tumor composed of a heterogeneous melanoma cell population. The heterogeneous nature of this tumor can result in some interexperiment variability in tumor growth. This variable was controlled within experiments by giving all animals injections of a cell suspension derived from the same tumor.

Animals. Male Golden Syrian hamsters, 4 to 6 weeks of age, (Engle Laboratories, Farmersberg, Ind.) were housed 2/cage in disposable plastic cages maintained at 23°, 40% humidity, under a 14-hr light:10-hr dark lighting regimen. Bilateral adrenalectomy was performed using clean but not sterile techniques. Surgically treated and sham-operated control animals were allowed to recover for 1 week prior to inoculation of 5 x 10⁶ viable (trypan blue exclusion) melanoma cells to the right flank. Those adrenalectomized animals not receiving DOCA were maintained on 1% NaCl solution and glucose with a dietary supplement of green leafy vegetables. Adrenalectomized animals were not maintained more than 21 days as morbidity and mortality were extremely high after this date. Male hamsters 5 to 6 weeks of age were hypophysectomized (Charles River Breeding Laboratories, Wilmington, Mass.) and allowed to recover for 1 week prior to tumor cell inoculation. The sela turcica of all animals was examined at autopsy to insure completeness of surgery. Those animals bearing pituitary remnants were excluded from the study.

Drugs. HC, DEX, DOCA, and MSH were obtained from Sigma Chemical Co. (St. Louis, Mo.). ACTH in a repository gel form was purchased from Armour Pharmaceutical (Phoenix, Ariz.). All steroids were administered by silastic capsules (1.5 to 6.0 x 0.15 cm, inner diameter) implanted s.c. Daily release rates of steroid hormones were determined by weighing the capsules to constant weight prior to implantation and following removal and dividing the difference by the number of days of exposure. To obtain a sustained release of pituitary hormones, ACTH in gel and MSH dissolved in 1% NaCl solution with 50% polyvinyl pyrolidone (K & K Laboratories, Planview, N. Y.) were administered daily by s.c. injection. All hormone administration was initiated 24 hr after tumor cell inoculation.

Measurements. Tumor volume was calculated as described by Mori et al. (20). Three dimensions (length, width, and height) of each tumor were isolated, washed free of blood and debris with ice-cold 1% NaCl solution, and frozen in liquid nitrogen for assay of glucocorticoid receptor.

Statistics. Statistical comparisons between treatment and control groups were made using Student’s t-test when single group comparisons were made (27). Single-classification analysis of variance was followed by an a posteriori multiple-comparison (Student-Newman-Keuls) test (27) when more than 2 groups were compared.
RESULTS

Adrenalectomy. Bilateral adrenalectomy significantly decreased tumor growth in melanoma-bearing hamsters. Mean tumor volumes were significantly decreased in the adrenalectomized group (Chart 1) as early as 15 days following tumor transplantation, and the final tumor weights were significantly lower compared to sham-adrenalectomized controls (Table 1).

Hypophysectomy. The effect of hypophysectomy on melanoma-bearing hamsters is summarized in Table 2. Hypophysectomy resulted in significant reduction in adrenal and tumor weight when compared to sham-operated controls 21 days following melanoma transplantation.

DOCA. Chart 2 demonstrates the effect of DOCA (125 μg/day) on melanoma growth in adrenalectomized and sham-adrenalectomized hamsters. Tumor volumes were significantly greater in DOCA-treated animals compared to sham-operated controls, regardless of the presence or absence of the adrenal glands. Final tumor weights were also significantly larger in DOCA-treated hamsters compared to untreated controls (Table 3).

HC. The effect of HC on melanoma growth is shown in Chart 3. Three doses of HC (7.14 and 28 μg/day) were released continuously from silastic capsules. Mean tumor volume increased with increasing doses of HC. Table 4 demonstrates that HC increased tumor weight in a similar, dose-related manner. The group exposed to the highest dose of HC (28 μg/
day) had significantly larger tumors compared to controls bearing empty capsules.

DEX. The administration of DEX by a silastic capsule significantly increased mean tumor volume (Chart 4) and final tumor weight (Table 5).

**Table 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (ug/day)</th>
<th>n</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>8</td>
<td>2.68 ± 0.31c</td>
</tr>
<tr>
<td>HC</td>
<td>7.0</td>
<td>7</td>
<td>3.92 ± 0.86</td>
</tr>
<tr>
<td>HC</td>
<td>14.0</td>
<td>7</td>
<td>6.50 ± 1.99</td>
</tr>
<tr>
<td>HC</td>
<td>28.0</td>
<td>8</td>
<td>9.32 ± 1.36d</td>
</tr>
</tbody>
</table>

*a* Five-week-old male hamsters were killed 37 days posttransplantation.

**Table 5**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Tumor wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>26.78 ± 2.44m</td>
</tr>
<tr>
<td>DEX</td>
<td>9</td>
<td>33.81 ± 2.74c</td>
</tr>
</tbody>
</table>

*a* Five-week-old male hamsters were killed 42 days posttransplantation. DEX release rate = 3 /ug/day.

**Chart 4.** Effect of DEX acetate on melanoma growth in 6-week-old male hamsters. Control, n = 10; DEX treated, n = 9. Mean tumor volumes significantly different on Day 42 by the Student t test, p < 0.05. DEX acetate administered by silastic capsule implant with release rate approximately 3 /ug/day. Bars, S.E.

**ACTH and MSH.** Table 6 summarizes 3 experiments which investigated the effect of continuous exposure of male hamsters to ACTH and MSH on melanoma growth. ACTH (4 units/day) administered over 21 or 35 days significantly increased adrenal size without significantly influencing tumor weight. MSH, either 3 µg/day for 21 days or 5 µg/day for 35 days had no statistically significant effect on either adrenal size or tumor weight.

**DISCUSSION**

Initial reports that 2 hamster melanoma lines, which contain cytoplasmic macromolecules apparently specific for glucocorticoids (13, 17, 30, 31), were responsive to adrenal steroids in vitro (13, 18) and in vivo (28-31, 33) prompted this investigation of the influences of adrenal manipulation on the growth of hamster MM1 in vivo. Bilateral adrenalectomy significantly retarded the development of melanoma growth in male hamsters. This observation implied that either the adrenals secreted a factor which facilitates tumor growth or that adrenalectomy elevated the level of a regulatory pituitary hormone which in turn acted to inhibit tumor growth.

Since ACTH regulates adrenocorticotoid secretion via a negative feedback mechanism, adrenalectomy results in elevated circulating levels of ACTH. ACTH has been shown to increase pigment production (5, 8, 16, 21) and inhibit murine melanoma growth in culture (8). It seemed reasonable, therefore, that adrenalectomy might retard melanoma growth by increasing circulating ACTH levels. Indeed, ACTH administration to animals with intact adrenals, in a dose sufficient to produce adrenal hypothyropathy, produced a slight reduction (14 to 27%) in tumor growth, which was not statistically significant. MSH, which comprises the first 13 amino acids of ACTH, is a potent melanotropic agent that inhibits the proliferation of murine melanoma cells plated at high density in vitro (8, 12, 19) and stimulates cortisol release under certain conditions (14). MSH, like ACTH, did not significantly alter MM1 tumor growth in intact hamsters. A slight reduction of tumor growth following exposure to high doses of MSH has been reported previously for murine melanoma (15).

The failure of MSH and ACTH to significantly influence melanoma growth suggests that adrenalectomy may decrease tumor growth by removing an adrenal factor which supports melanoma growth. This suggestion is supported by the significant reduction in tumor weight produced by hypophysectomy.

**Table 6**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Dose/day</th>
<th>Duration (days)</th>
<th>n</th>
<th>Tumor wt (g)</th>
<th>Adrenal wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>21</td>
<td>10</td>
<td>6.72 ± 1.66c</td>
<td>18.5 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>ACTH</td>
<td>4 IU</td>
<td>21</td>
<td>10</td>
<td>5.77 ± 0.92</td>
<td>27.8 ± 1.1d</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>35</td>
<td>7</td>
<td>4.12 ± 1.07</td>
<td>28.6 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>MSH</td>
<td>21</td>
<td>6</td>
<td>2.68 ± 1.05</td>
<td>30.5 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ACTH</td>
<td>4 IU</td>
<td>35</td>
<td>11</td>
<td>15.15 ± 2.31</td>
<td>37.7 ± 2.6d</td>
</tr>
<tr>
<td>6</td>
<td>MSH</td>
<td>5 µg</td>
<td>35</td>
<td>10</td>
<td>21.89 ± 2.14</td>
<td>27.1 ± 1.0</td>
</tr>
</tbody>
</table>

*a* Five- to 6-week old male hamsters at time of tumor transplantation.

b Hormones administered by daily s.c. injection for duration of the experiment.

c Mean ± S.E.

d Mean adrenal weight of ACTH group significantly greater than control by analysis of variance followed by the Student-Newman-Keuls test, p < 0.005.
Exposure of intact male hamsters to HC increased tumor growth in a dose-related manner. This observation is compatible with the inhibition of tumor growth following adrenalectomy. Because HC exhibits gluco- and mineralocorticoid activity (14), the effects of DEX, a synthetic glucocorticoid devoid of any significant mineralocorticoid activity, are particularly important. A low dose of DEX (3 μg/day) significantly increased tumor growth. Mineralocorticoids also apparently affect melanoma growth. DOCA, a mineralocorticoid devoid of glucocorticoid activity at normal doses (0.05 mg/kg/day in humans) (14), significantly increased MM1 growth following exposure to high doses (125 μg/day = 1.25 mg/kg/day). Further, when administered to adrenalectomized animals, DOCA actually increased tumor growth compared to untreated sham-operated controls. Interestingly, DOCA exacerbates corticosterone-induced hyperpigmentation in patients with adrenal insufficiency (25). Since low doses of HC or DEX and high doses of DOCA significantly enhance tumor growth, MM1 is either responsive to a variety of C-21 adrenocorticosteroids, or DOCA in high doses may be acting via the same mechanism as glucocorticoids. Work in our laboratory suggests that MM1 contains a cytoplasmic macromolecule with a high affinity for glucocorticoids. Work in our laboratory suggests that MM1 contains a cytoplasmic macromolecule with a high affinity for glucocorticoids. A similar molecule has been reported in RPMI 3460 hamster melanoma cytosol (13, 17). The present results also confirm earlier studies which suggest that adrenalectomy inhibits B16 melanoma growth in male mice (32) and initial evidence that a cloned line (B1) of MM1 responds to high doses (10^{-6} M) of corticosterone with a slight increase in proliferation in vitro (33).

The increase in MM1 tumor growth in vitro and in vivo following exposure to glucocorticoids is at variance with the inhibitory effects of glucocorticoids in the growth of RPMI 3460 and B16 melanoma cells in vitro (3, 13, 17). The basis for this discrepancy may reside in host species and strain differences, differences in how each cell line responds to glucocorticoids, or the suprapharmacological doses of DEX used to suppress B16 melanoma growth in male mice (3).

Taken together, our results suggest that the MM1 cell line, like B16 murine melanoma and RPMI 3460 hamster melanoma, is an adrenocorticotoid-responsive neoplasm. As such, the MM1 melanoma may provide a model for investigating the role of adrenal steroids in the biological behavior of melanoma.

REFERENCES

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