Glucocorticoid Receptor-mediated Effects on Rat Fibrosarcoma Growth

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ABSTRACT

Glucocorticoid receptors are present in most normal and malignant mammalian cells. To examine the hypothesis that the growth of methylcholanthrene-induced malignant sarcoma is glucocorticoid dependent, we evaluated the behavior of malignant fibrosarcoma (MCA) in adrenalectomized rats treated with either normal saline or deoxycorticosterone acetate and in intact rats treated with placebo or with the glucocorticoid receptor antagonist RU 486. Survival, tumor weight, and loss of body weight (an index of cachexia) were measured. In MCA-bearing rats, neither survival nor loss of body weight was affected by bilateral adrenalectomy or by treatment with RU 486. Tumor weight and time-integrated tumor volume, however, were significantly less in bilaterally adrenalectomized rats without deoxycorticosterone acetate replacement than in animals treated with deoxycorticosterone acetate. Similarly, tumor weight and time-integrated tumor volume were less in intact animals treated with RU 486 than in intact animals treated with placebo. The glucocorticoid receptors in the tumor cells had similar binding capacity \( R_n \) and equilibrium dissociation constant \( K_d \) as in control rat fibroblasts. These results suggest that the growth of MCA sarcoma cells is partially dependent upon glucocorticoids. This effect of glucocorticoids, however, was not of sufficient magnitude to improve survival and prevent cachexia. We conclude that glucocorticoids appear to influence MCA sarcoma growth in the rat, and that glucocorticoid receptor blockade, perhaps in combination with other antitumor agents, merits future study in the treatment of malignant tumors.

INTRODUCTION

Steroid hormones and their receptors appear to influence the natural history of human mesenchymal tumors (1-6). For example, females with sarcomas have a better prognosis than males, and this improvement declines after the menopause (6). Additionally, pregnancy and the postpartum state alter the natural history of human mesenchymal tumors (1-6). For example, females with sarcomas have a better prognosis than males, and this improvement declines after the menopause (6). Additionally, pregnancy and the postpartum state alter the natural history of human mesenchymal tumors (1-6). For example, females with sarcomas have a better prognosis than males, and this improvement declines after the menopause (6). Similarly, tumor weight and time-integrated tumor volume were less in intact animals treated with RU 486 than in intact animals treated with placebo. The glucocorticoid receptors in the tumor cells had similar binding capacity \( R_n \) and equilibrium dissociation constant \( K_d \) as in control rat fibroblasts. These results suggest that the growth of MCA sarcoma cells is partially dependent upon glucocorticoids. This effect of glucocorticoids, however, was not of sufficient magnitude to improve survival and prevent cachexia. We conclude that glucocorticoids appear to influence MCA sarcoma growth in the rat, and that glucocorticoid receptor blockade, perhaps in combination with other antitumor agents, merits future study in the treatment of malignant tumors.

MATERIALS AND METHODS

Animals and Tumors. Rat MCA \(^2\) was induced by injection of methylcholanthrene and maintained in male Fischer 344 rats by serial transplantation. Three to 4 wk after tumor inoculation, the rat bearing MCA was killed by decapitation. Using sterile technique, the tumor was excised and cut into 2- x 2- x 2-mm pieces in 0.9% NaCl solution. The tumor fragments were subsequently inoculated s.c. into the right flank of a recipient animal by trochar. Tumor volume was determined serially 3 times a wk for 30 days by 3-dimensional measurement of the tumor, using calipers. The tumor is a locally invasive fibrosarcoma that seldom metastasizes and uniformly kills the animal 30 to 35 days following inoculation (19, 20).

Male Fischer 344 rats, weighing 120 to 150 g, were maintained on a 12-h light, 12-h dark cycle in a temperature-controlled room and were given standard rat chow and water ad libitum. Adrenalectomized rats received normal saline ad libitum.

Drugs and Chemicals. DOCA (Sigma, St Louis, MO) in mineral oil was administered at a dose of 3 mg/kg every other day s.c. This dose had been shown previously to maintain normal physiological parameters (21). RU 486 (Roussel, UCLAF, Paris, France), prepared as a fine suspension in 0.9% NaCl solution with sodium carboxymethylcellulose, was administered at a dose of 10 mg/kg/day via i.p. injection. Control animals received vehicle alone at the same frequency. \[^3H\]Dexamethasone and dexamethasone for use in receptor studies were purchased from New England Nuclear and Sigma Chemical Co., respectively.

Receptor Studies. RFB and MCA sarcoma cells were established in tissue culture at a concentration of 10\(^6\) cells/ml in RPMI-1640 with l-glutamine. Glucocorticoid receptor number and affinity were determined by a competitive binding assay (22). Approximately 10\(^6\) RFB or MCA cells were added to serial dilutions of \[^3H\]dexamethasone (1 to 40 nM) in the presence or absence of unlabeled dexamethasone (2 nM). After 18-h incubation at 22°C, cells were washed 3 times with PBS at 0°C. Ten ml ofAquasol scintillation fluid were added to 1 ml of PBS and counted at an efficiency of 30%. Results were calculated as fmol of dexamethasone bound per 10\(^6\) cells. Scatchard analysis was used to determine \( R_n \) and equilibrium dissociation constant \( K_d \).

Experimental Protocol. In Experiment 1, 88 male Fischer 344 rats were randomized into 3 groups: adrenalectomy plus NS replacement (n = 28), adrenalectomy plus DOCA replacement (DOCA) (n = 30), and sham (n = 30). NS- and DOCA-treated animals underwent bilateral adrenalectomy on Day 0, while sham rats underwent a sham adrenalectomy procedure. DOCA and NS replacement were begun immediately after adrenalectomy. Fourteen days after adrenalectomy or sham operation, half of the rats in each of the 3 groups received a s.c. implant of MCA sarcoma, and half remained NTB. Tumor volume and body weights were measured serially 3 times a wk, as described above. Following death of the tumor-bearing rats, tumors were enucleated and weighed. Host weight was calculated as the difference between the total body weight and the tumor weight. Non-tumor-bearing rats were sacrificed by decapitation on Day 37.

In Experiment II, 18 test rats were treated with RU 486, and 12 control rats received vehicle (normal saline). After 3 days of treatment MCA fragments were implanted in half the RU 486- and vehicle-treated rats. Tumor volumes and body weights were measured serially as before. At the time of death, tumors were enucleated and weighed, and host weight was calculated as in Experiment I. The adrenal glands and spleen were removed, weighed, and preserved in formalin for future study.

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2 The abbreviations used are: MCA, malignant fibrosarcoma; DOCA, deoxycorticosterone acetate; RFB, rat fibroblasts; PBS, phosphate-buffered saline; \( R_n \), binding capacity; NS, normal saline; NTB, non-tumor-bearing.
RESULTS

Receptor Studies. The $R_a$ and $K_a$ of the MCA sarcoma cells were 47.7 fmol/10⁷ cells and 0.95 nmol/liter, respectively (Fig. 1). The $R_a$ and $K_a$ for the control rat fibroblasts were 41.8 fmol/10⁷ cells and 0.82 nmol/liter, respectively, which were not statistically different from the MCA sarcoma cells.

Effect of Adrenalectomy on Tumor Growth (Experiment I, Fig. 2, Table 1). Normal saline-treated adrenalectomized rats demonstrated a marked reduction in mean serial sarcoma volume as well as the time-integrated sarcoma volume compared to sham-adrenalectomized intact animals and steroid-replaced adrenalectomized animals (DOCA) (Fig. 2, $P < 0.05$).

Tumor weight was significantly less in the normal salinetreated adrenalectomized animals compared to either the DOCA-treated adrenalectomized or sham-operated rats (Table 1, $P < 0.05$). Neither survival nor Δ host weight was significantly different among the three tumor-bearing groups. However, Δ host weight was significantly less in the tumor-bearing compared with the non-tumor-bearing groups. DOCA replacement in NTB adrenalectomized animals restored Δ host weight to the level of sham non-tumor-bearing rats (Table 1).

Effect of RU 486 on Tumor Growth (Experiment II, Fig. 3, Table 2). Treatment with the glucocorticoid receptor antagonist RU 486 significantly inhibited sarcoma growth compared to the vehicle control (Fig. 3, $P < 0.05$). Tumor weight was significantly lower, and adrenal and splenic weights were significantly greater, in the RU 486-treated rats than in vehicle-treated control animals (Table 2, $P < 0.05$). There was no significant difference in either survival or Δ host weight between RU 486- and vehicle-treated animals. Δ host weight was significantly less while adrenal and spleen weights were significantly greater in tumor-bearing than in non-tumor-bearing vehicle-treated rats (Table 2).

DISCUSSION

Glucocorticoid receptors have been demonstrated in human tumors that were not previously thought to be glucocorticoid dependent. Fifty-five % of 141 human sarcoma specimens had specific, high-affinity binding sites for glucocorticoids (9). High-grade, malignant liposarcomas had glucocorticoid receptors, while well-differentiated liposarcomas and benign lipomas did not (2). Our current study demonstrated that cultured rat MCA fibrosarcoma cells contain saturable, high-affinity receptors that are quantitatively and qualitatively similar to rat fibroblast glucocorticoid receptors. Whether or not the MCA glucocorticoid receptor complex is functional and thus able to activate to a DNA-binding form is unknown (23).

Manipulation of the glucocorticoid milieu in vivo depressed growth of the MCA fibrosarcoma. Adrenalectomy inhibited sarcoma growth and weight by 70%, and DOCA treatment restored the normal pattern of tumor growth. Glucocorticoid receptor antagonism by RU 486 also significantly reduced tumor growth and weight (by 40%).

The apparent glucocorticoid dependence of the MCA rat fibrosarcoma may be due to a direct growth-promoting or permissive effect of glucocorticoids upon the tumor cells or may be an indirect result of insufficient nourishment of the tumor in the glucocorticoid-deficient environment. Alternatively, the poor growth of the tumor in the absence of glucocorticoids may be attributable to immune suppression at physiological or mildly increased glucocorticoid concentrations. Indeed, in the rat, glucocorticoids at physiological levels have been shown to suppress the inflammatory response (24, 25). Moreover, administration of RU 486 to either tumor-bearing or non-tumor-bearing animals resulted in significant splenic hypertrophy, with proliferation of myeloid elements, and thymic enlargement.3

The observed antitumor effects of bilateral adrenalectomy and RU 486 did not result in better host weight accrual or improved survival. Although bilateral adrenalectomy was in

1 L. Laue, unpublished observation.
rats, analogous to observations in many states of chronic illness. Analysed in the tumor-bearing compared to the non-tumor-bearing treated animals, the magnitude of this weight loss was accelerated responsible for the weight loss observed in the placebo-body weight — tumor weight).

The presence of tumor caused adrenal hypertrophy in intact rats, analogous to observations in many states of chronic illness or other stress. Adrenal hypertrophy was also observed following administration of RU 486 to the non-tumor-bearing animals. This indicates successful peripheral glucocorticoid blockade, inhibition of glucocorticoid negative feedback, and increased secretion of pituitary adrenocorticotropic hormone resulting in adrenal hypertrophy. It appears that the degree of glucocorticoid blockade produced by RU 486 resulted in maximal adrenal hypertrophy. Consequently, the stress resulting from the tumor burden led to no further adrenal enlargement in the presence of RU 486 than was observed with RU 486 alone in the non-tumor-bearing rats.

We conclude that the absence or blockade of glucocorticoid action resulted in significant reduction of rat fibrosarcoma growth. However, the effect of glucocorticoid removal was not of sufficient magnitude to arrest the growth of implanted fibrosarcomas, prevent development of cachexia, or prolong survival. This lack of substantive therapeutic effect, however, does not exclude the possibility that other, less malignant tumors may respond to antiglucocorticoid therapy. These results suggest that the application of an antiglucocorticoid such as RU 486 may have some role in the treatment of glucocorticoid receptor-positive solid tumors for which standard therapy has not been successful or is not available. Whether side effects from chronic RU 486 therapy, such as development of adrenal insufficiency, will limit the usefulness of this agent in such a clinical setting remains to be determined.

### REFERENCES

GLUCOCORTICOIDS AND FIBROSARCOMA


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