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Effect of Glucocorticoid Replacement on Tumor Growth after Adrenalectomy in Mice

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ABSTRACT

We studied the effects of glucocorticoid replacement on tumor growth after adrenalectomy of Meth A sarcoma in mice. Tumor growth was inhibited in the adrenalectomized mice when a minimum dose of corticosterone, 0.3 mg/day, was given for replacement, and higher doses led to an even greater inhibition. Corticosterone had no effect on tumor growth in the irradiated mice. Sinecomitant immunity in the case of growth of the retransplanted excised tumor was compromised in the adrenalectomized mice. *In vivo* neutralization and immunosuppressive activities were absent in the spleen cells of the adrenalectomized mice.

It would thus appear that adrenalectomy suppresses tumor growth by mechanisms other than glucocorticoid ablation. For optimum tumor control, glucocorticoid replacement after adrenalectomy should be in excess of the minimum daily requirements.

INTRODUCTION

Adrenalectomy is palliative for patients with advanced breast cancer and a positive estrogen receptor (1, 2). In recent years, adrenalectomy can be accomplished with aminoglutethimide and hydrocortisone, as an alternate modality (3, 4). Both forms of adrenalectomy require that glucocorticoid be replaced. Although selection of patients for endocrine palliation depends on a positive estrogen receptor assay, 35 to 40% of these patients do not respond to this therapy (1, 3). Some patients with estrogen receptor-negative tumors respond well to bilateral adrenalectomy (1). Thus, the possibility that adrenalectomy affects tumor growth by mechanisms not mediated by the estrogen receptor has to be considered. There are reports of animal models in which adrenalectomy suppressed tumor growth (5–7), and one study showed definite enhancing effects of adrenal ablation on tumor growth (8). These findings were attributed to glucocorticoid ablation by mechanisms acting either through the glucocorticoid receptor (6) or host-mediated immunity (5, 7).

We studied the effects of adrenalectomy (glucocorticoid deficiency) on tumor growth in mice and whether the glucocorticoid replacement should be minimum or excess after adrenalectomy, for optimum tumor control.

MATERIALS AND METHODS

Mice. Eight- to 12-wk-old female BALB/c inbred mice, weighing 18 to 20 g, were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Ten mice in one cage were housed in a specific-pathogen-free room in the Laboratory of Animal Experiments, Kyushu University, under conditions of a room temperature of 22°C and lights on from 8:00 a.m. to 8:30 p.m.

Tumor. A methylcholanthrene-induced fibrosarcoma, Meth A sarcoma, of BALB/c origin was maintained in ascitic form by serial passages of 1×10^7 cells at weekly intervals.

Tumor Transplantation and Measurement of Tumor Size. Tumor cells were washed 3 times with HBSS[2], and viable cells were adjusted to the desired concentration by trypan blue dye exclusion staining. One-tenth ml of tumor cell suspension in HBSS was transplanted s.c. into the right flank of the mice. Tumor size was measured with a slide caliper and was expressed in terms of area in mm^2, calculated by multiplying the longest and shortest diameters.

Spleen Cells. The spleen, removed after femoral arterial exsanguination, was crushed between two glass slides and passed through two layers of gauze to remove the large fragments. After three washings of the filtrate with HBSS, viable cells were concentrated by trypan blue dye exclusion staining.

Adrenalectomy. Because of the high frequency of accessory adrenocortical tissues in mice (9), adrenalectomy included removal of the surrounding fatty tissues and renal capsules. In addition, bilateral oophorectomy was performed for the removal of ectopic glands. Control groups consisted of oophorectomized and laparotomized mice. The oophorectomized mice were also subjected to a left hemiadrenalectomy to minimize differences in operative stress. In the laparotomized mice, the bilateral adrenal glands were exposed for 15 min. The ADX mice were provided 0.85% saline *ad libitum* and every evening were given s.c. a maintenance dose of corticosterone (Sigma Chemical Co., St. Louis, MO) in sesame oil. The minimum replacement dose, 0.3 mg, was determined in experiments in which all ADX mice survived for over 14 days (10).

Plasma Corticosterone Determination. To avoid circadian variation, the mice were killed between 9 and 10 a.m. Plasma corticosterone determinations were made by radioimmunoassay (11) with the use of corticosterone antiserum (Corticosterone-3-BSA) (Cambridge Medical Diagnostics). [^{3}H]Corticosterone (85.1 Ci/mmol; Amersham, Japan) was used as a standard. The plasma of 3 mice was used for one assay, and all assays were done in triplicate.

Irradiation. Radiation was delivered using Shimadzu 250-kVp equipment, operated at 200 kVp and 20 mA with a 0.3-mm copper and 1-mm aluminum filter. The mice were exposed to 400 rads of whole-body irradiation at a rate of 14.3 rads/min, 24 h before use.

Sinecomitant Immunity. Five $\times 10^6$ viable tumor cells were transplanted s.c. into the dorsum and removed on the fourth day thereafter. Ten days after tumor resection, 1×10^6 viable tumor cells were retransplanted into the right flank of mice, and the resulting tumor size was measured (12).

Cytotoxic Activity of Spleen Cell. Cytotoxic activity of whole spleen cells was examined using an *in vivo* neutralization test (10). Spleen cells were suspended at a concentration of 4×10^8 cells/ml and the tumor cells at 2×10^7 cells/ml in HBSS. These cell suspensions were mixed in equal volumes (effector/target = 20:1). One-tenth ml of this suspension was s.c. implanted into the right flank of mice irradiated 24 h before.

Immunosuppressive Activity of Spleen Cells. Five $\times 10^6$ tumor cells were transplanted into the dorsum and removed the fourth day thereafter. These same mice were used as recipients on the ninth day after tumor resection. Two $\times 10^7$ spleen cells injected i.v. and 1×10^6 tumor cells were transplanted into the right flank on the following day. The take rate was then examined.

Statistics. Statistical analyses were conducted using analysis of variance, and the statistical package. BMDP3V, on an International Business Machines System 4381 was used for computation (13).

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[2] The abbreviations used are: HBSS, Hanks' balanced salt solution; ADX, adrenalectomized.

Table 1 *Effect of adrenalectomy on tumor growth*

Group of mice	Day of operation[a]	No. of mice	Tumor size (mm²) 10th day	15th day	20th day	25th day	30th day
ADX[b]		10	102.1 ± 5.6[c]	204.0 ± 17.4	288.8 ± 20.4	411.5 ± 21.3	ND[d]
OX	−10	10	139.3 ± 4.5	288.0 ± 13.7	447.5 ± 20.6	560.6 ± 37.6	ND
Lapa		10	174.0 ± 9.0	255.0 ± 8.6	358.6 ± 11.3	529.3 ± 9.7	ND
ADX[b]		10	92.0 ± 4.5	133.8 ± 6.4	237.5 ± 17.9	353.8 ± 21.9	ND
OX	+10	10	103.5 ± 6.9	195.7 ± 16.3	300.2 ± 25.3	470.9 ± 24.4	ND
Lapa		10	115.5 ± 9.0	161.6 ± 12.6	311.9 ± 24.6	467.7 ± 28.0	ND
ADX[e]		9		171.5 ± 13.6	280.6 ± 17.6	366.9 ± 31.3	489.2 ± 50.3
OX	+14	9		150.0 ± 12.0	266.0 ± 18.2	431.0 ± 29.3	665.4 ± 38.7
Lapa		9		158.7 ± 12.8	296.0 ± 18.5	438.5 ± 32.8	633.0 ± 27.3

[a] Operations were done before (−) or after (+) tumor transplantation.
[b] $P < 0.01$ compared to OX and Lapa.
[c] Mean ± SE.
[d] ND, not determined; OX, oophorectomized mice; Lapa, laparotomized mice.
[e] $P < 0.05$ compared to OX and Lapa.

RESULTS

Effect of Adrenalectomy on Tumor Growth. Adrenalectomy and sham-adrenalectomy were performed 10 days before and 4 days and 14 days after transplantation of 5×10^6 tumor cells. In all cases, tumor growth in the ADX mice was inhibited, compared to that seen in the sham-ADX mice (Table 1). There was no change in body weight between the groups.

By increasing the daily replacement dose of corticosterone from 0.3 to 1 mg, 2 mg, or 5 mg, growth of the tumor was even more inhibited in the mice adrenalectomized 4 days after the transplantation. Statistically significant differences were not evident in the ADX mice given daily over 1 mg of corticosterone (Fig. 1). There was no change in the body weight between the groups.

Effect of Adrenalectomy and Corticosterone Replacement on Plasma Corticosterone Concentration. Corticosterone concentrations in plasma were assessed to ensure that adrenalectomy was complete, and that replacement corticosterone concentrations were given, in the next day (Table 2). Concentrations in ADX mice receiving corticosterone increased in a dose-dependent manner: 58% at 0.3 mg; 129% at 1 mg; 164% at 3 mg; and 199% at 5 mg. In normal mice, the replacement of higher doses of corticosterone raised the level of corticosterone above the normal values.

Effect of Corticosterone on Tumor Growth. To determine the pharmacological action of replaced doses of corticosterone on tumor growth, non-ADX normal mice were used. To minimize the host-mediated effects, tumor growth was examined in mice which had been irradiated: irradiation was given 24 h prior to tumor transplantation (10). In mice with 5×10^6 transplanted tumor cells, daily injections of 0.3 mg, 1 mg, 2 mg, or 5 mg of corticosterone were given. Tumor growth was not affected by corticosterone, up to a daily dose of 5 mg (Table 3). There was no change in body weight between the groups.

Sinecomitant Immunity. Adrenalectomy or sham-adrenalectomy was performed concomitantly with tumor resection. Growth of the retransplanted tumor in the ADX mice was not affected when the mice were given 0.3 mg of corticosterone, whereas tumor growth in the sham-ADX mice was suppressed (Fig. 2). There was no change in body weight between the groups.

Cytotoxic Activity of Spleen Cells. Adrenalectomy or sham-adrenalectomy was performed together with tumor resection on the fourth day after transplantation of 5×10^6 tumor cells. Fourteen days after the tumor transplantation, spleen cells were obtained, and the cytotoxic activity (10) was examined, using

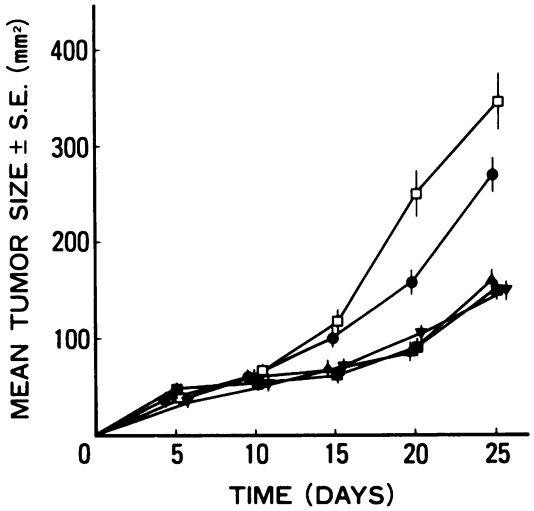

Fig. 1. Effect of replacement of corticosterone on tumor growth after adrenalectomy. Five × 10^6 tumor cells were transplanted, and adrenalectomy was performed 4 days after the transplantation. Adrenalectomized mice were given 0.3 mg (●), 1 mg (▲), 2 mg (■), or 5 mg (▼) of corticosterone daily. Laparotomized mice (□) were used for the controls. There were 10 mice per group. Tumor size increased in the 0.3 mg-maintained adrenalectomized mice, compared to those given 1 mg, 2 mg, and 5 mg ($P < 0.01$). *Bars*, SE.

Table 2 *Plasma corticosterone concentrations (μg/dl)*

Group of mice	Corticosterone replacement dose (mg) 0	0.3	1.0	2.0	5.0
Normal	6.7 ± 0.8[a]	11.4 ± 2.1	14.2 ± 3.3	17.9 ± 2.8	24.3 ± 5.7
ADX		3.9 ± 0.7	8.7 ± 1.4	11.0 ± 3.9	13.3 ± 3.1

[a] Mean ± SE.

Table 3 *Effect of corticosterone on tumor growth in nonadrenalectomized mice*

Group[a]	No. of mice	Tumor size (mm²) 5th day	10th day	15th day	20th day
		Normal mice			
Sesame oil	10	39.6 ± 3.6[b]	121.6 ± 7.8	192.4 ± 20.5	368.2 ± 24.7
0.3 mg	10	42.5 ± 1.9	117.2 ± 6.3	218.6 ± 16.4	349.1 ± 18.6
1.0 mg	10	40.8 ± 4.7	122.4 ± 12.0	203.1 ± 17.6	350.3 ± 26.8
2.0 mg	10	44.0 ± 5.2	126.1 ± 9.7	214.0 ± 9.8	357.5 ± 15.4
5.0 mg	10	39.1 ± 3.6	114.3 ± 8.1	210.2 ± 14.7	351.4 ± 16.0
		Irradiated mice			
Sesame oil	10	42.1 ± 1.7	106.3 ± 6.0	188.0 ± 13.3	352.0 ± 18.7
0.3 mg	10	41.5 ± 1.9	118.5 ± 11.2	204.3 ± 18.6	377.2 ± 17.9
1.0 mg	10	43.6 ± 2.1	125.5 ± 7.7	201.9 ± 13.3	360.6 ± 23.3
2.0 mg	10	39.3 ± 3.0	119.7 ± 9.0	197.1 ± 13.1	364.0 ± 13.2
5.0 mg	10	40.2 ± 2.7	115.4 ± 8.3	203.7 ± 15.2	358.3 ± 16.8

[a] Each dose of corticosterone was injected daily.
[b] Mean ± SE.

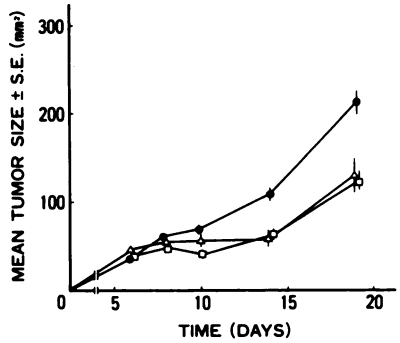

Fig. 2. Effect of adrenalectomy on sinecomitant immunity. Five × 10^6 tumor cells were transplanted, and adrenalectomy was performed 4 days after the transplantation. One × 10^6 tumor cells were retransplanted into the adrenalectomized mice 10 days after the first tumor resection (●), oophorectomy (△), and laparotomy (□). There were 10 mice per group. Tumor size increased in the adrenalectomized mice, compared to findings in the oophorectomized ($P < 0.05$) and the laparotomized ($P < 0.01$) mice. *Bars*, SE.

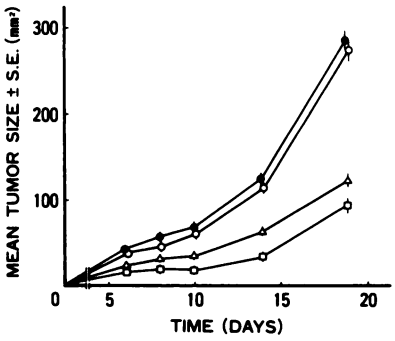

Fig. 3. Effect of adrenalectomy on cytotoxic activity of spleen cells. Two × 10^7 spleen cells were mixed with 1 × 10^6 tumor cells and transferred into the irradiated mice. The spleen cells were obtained 10 days after tumor resection of the adrenalectomized mice (●), the oophorectomized mice (△), the laparotomized mice (□), and nonimmunized mice (○). There were 10 mice per group. Tumor size increased in the adrenalectomized mice, compared to findings in the oophorectomized ($P < 0.01$) and the laparotomized ($P < 0.01$) mice. *Bars*, SE.

Table 4 *Effect of adrenalectomy on immunosuppressive activity of spleen cells*

Donor of spleen cells	Take rate		
	8th day	10th day	14th day
ADX			
+0.3 mg/day[a]	9/10	6/10	2/10
+1.0 mg/day	8/10	7/10	3/10
OX[b]	10/10	10/10	7/10
Lapa	10/10	9/10	6/10
Non-tumor bearing[c]	7/9	5/9	2/9

[a] Replacement dose of corticosterone.
[b] OX, oophorectomized mice; Lapa, laparotomized mice.
[c] One anesthesia-related death.

an *in vivo* neutralization test. Spleen cells from the ADX mice did not suppress growth of the retransplanted tumor, while cells from the sham-ADX mice did suppress the growth (Fig. 3). There was no change in body weight between the groups.

Immunosuppressive Activity of Spleen Cells. Spleen cells were obtained in the same manner as for determinations of cytotoxic activity, but without tumor resection. In the case of spleen cells from the sham-ADX mice, take of the tumor tended to increase, while those from the ADX mice did not affect the take rate (Table 4). There was no change in body weight between the groups.

DISCUSSION

We obtained evidence herein that growth of a Meth A sarcoma was inhibited in mice when adrenalectomy was done 10 days before, and 4 days after and 14 days after tumor transplantation, as was also noted by other investigators (5–7), but a regression was not evident. As the BALB/c mice we used did not survive without replacement of corticosterone, we gave a minimum dose of corticosterone daily (10). We considered that these ADX mice were glucocorticoid deficient. The plasma concentration of corticosterone decreased in the ADX mice. However, the tumor growth was further suppressed by increasing the dose, although a dose-dependent suppression was not evident in the ADX mice given over 1 mg daily. Corticosterone up to 5 mg had no evident pharmacological action on the growth of the tumor in the non-ADX mice. These results exclude the direct effect of the administered glucocorticoid, as in the case of lymphoma (14), and that of glucocorticoid ablation, as in the case of melanoma (6, 15).

Another mechanism to be considered is the host-mediated effect by adrenalectomy, such as immunological resistance (5, 7). Glucocorticoid has an immunosuppressive action (16), and adrenalectomy may enhance host immunity (17, 18). Our findings suggest that glucocorticoid ablation was not the mechanism which enhanced the immunity and inhibited the tumor growth, because the growth was further suppressed by the administration of corticosterone. However, immunological resistance might be enhanced by adrenalectomy through mechanisms other than glucocorticoid ablation. Meth A sarcoma induced a sinecomitant immunity and *in vivo* neutralization activity in the spleen cells. Unexpectedly, sinecomitant immunity was compromised, and *in vivo* neutralization activity of spleen cells was not evident in the ADX mice. Meth A sarcoma grows in the presence of immunosuppressor T-cells (19), but immunosuppressive activity was not detected in the spleen cells of the ADX mice. However, it is difficult to attribute the suppression of tumor growth to ablation of immunosuppressive activity only, because in the ADX mice given 1 mg of a replacement daily, immunosuppressive activity was not evident and growth was further suppressed. Although immunosuppressor T-cells from the spleen were reported to be cortisone resistant (20, 21), we did not exclude the effect of administered corticosterone because the ADX mice did not survive when less than 0.3 mg of corticosterone replacement were given.

Our results show that the ADX mice were immunologically incompetent against the tumor, although growth was suppressed. The incompetency might result from glucocorticoid ablation because the tumor growth was further suppressed by increasing the dose of replacement. Thus, glucocorticoid is probably necessary for antitumor resistance, at least after adrenalectomy.

Concerning adrenalectomy-induced tumor growth suppression, our observations exclude immune-based mechanisms as well as those regarding glucocorticoid ablation. As excessive doses of corticosterone also suppressed tumor growth in the ADX mice, glucocorticoid replacement should be in excess of the minimum requirements for optimum tumor control after adrenalectomy, presumably the same as in the case of aminoglutethimide and hydrocortisone.

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