Regression of Prolactin-dependent Rat Mammary Carcinoma in Response to Antihormone Treatment

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SUMMARY

Rabbit antibodies to purified rat prolactin were produced and injected into female Sprague-Dawley rats bearing 7,12-dimethylbenzanthracene-induced, hormone-dependent mammary adenocarcinomas. Antihormone therapy caused a significantly increased incidence of tumor regression and a significantly decreased growth of tumors in these rats compared with controls receiving normal rabbit serum. These results provide further evidence to support the conclusion that 7,12-dimethylbenzanthracene-induced rat mammary cancer is prolactin dependent.

INTRODUCTION

A single feeding of DMBA \(^4\) induces hormone-dependent mammary adenocarcinoma in rats \((12)\). Nearly 100% regression of DMBA-induced tumors has been reported with hypophysectomy \((7)\) and bilateral oophorectomy-adrenalectomy \((27)\), each of which results in a marked decrease in serum prolactin levels \((23)\). Tumors can be reactivated in the latter animals by estrogen administration but not in the hypophysectomized rats. Administration of ovine prolactin, however, will reactivate tumor growth in hypophysectomized rats \((23)\). These and other studies have led to the conclusion that DMBA-induced rat mammary carcinoma is prolactin dependent \((23)\).

The purpose of this study was to determine whether antibodies to purified rat prolactin could induce regression of mammary tumors when injected into the animals. Unpublished observations in our laboratory indicated that antibodies to ovine prolactin would not cause tumor regression or block the prolactin activity of rat pituitary homogenates in the pigeon crop sac bioassay, indicating that there is species specificity of the prolactin. With the recent isolation and purification of rat prolactin \((18)\), it is possible to produce antisera specific to rat prolactin \((5, 14, 16, 20)\). It was hoped that injection of such an antiserum would cause inhibition of the growth or regression in the size of existing tumors.

MATERIALS AND METHODS

Tumors were induced in female Sprague-Dawley rats by intragastric administration of 20 mg of DMBA according to the method of Huggins et al. \((12)\). Prolactin was produced in rat pituitary organ cultures by the method of Meites et al. \((18)\). Separation of prolactin from the other hormones in the culture medium was accomplished by polyacrylamide vertical gel electrophoresis \((13)\), and the eluted and lyophilized prolactin was examined by disc gel electrophoresis \((21, 24)\). Antibodies to this prolactin were obtained from a female New Zealand strain rabbit by conventional methods. Subsequent booster injections consisted of highly purified prolactin \((H96B)\) kindly supplied by Ellis et al. \((5)\). This antiserum showed a single precipitation line when incubated with rat pituitary homogenate in micro-Ouchterlony plates. The antiserum did not cross-react with rat growth hormone, rat albumin, ovine prolactin, or human growth hormone. The titer of antibodies from each bleeding of the rabbit was measured by radioimmunoassay. \(^{125}\)I-Labeled rat prolactin \((100\, \text{pg})\) was added to 0.1 ml of various dilutions of rabbit antiserum to rat prolactin. This mixture was incubated at 4°C overnight. At this time, 0.1 ml of normal rabbit serum and 0.1 ml of sheep anti-rabbit \(\gamma\)-globulin were added, and the mixture was again incubated at 4°C for 24 hr. The tubes were centrifuged in a refrigerated centrifuge, and the supernatant solution was removed from the precipitate by suction. The precipitate was counted in a \(\gamma\)-scintillation counter. The titer of prolactin antiserum was determined by the dilution of antiserum that bound 50% of the radioactive prolactin.

Ten rats with 20 growing DMBA-induced rat tumors received s.c. or i.p. injections of antiserum twice daily for 36 days, \((1\, \text{ml} \text{ of } 1/12,000 \text{ titer antiserum daily for } 13 \text{ days, } 0.5 \text{ ml of } 1/32,000 \text{ titer for } 19 \text{ days, and } 0.4 \text{ ml of } 1/64,000 \text{ titer for the last } 4 \text{ days})\). Nine control rats bearing 23 tumors received injections of normal rabbit serum in the same manner. Vaginal smears (wet preparations viewed immediately) were taken daily on all rats during the experiment to follow estrous cycles, and all rats were weighed daily. During the treatment period, both the control and experimental groups of rats gained weight slowly \((7 \text{ g/rat for controls and } 8 \text{ g/rat for antiserum-treated groups})\). Tumor sizes were measured with a ruler marked in millimeters daily for a week prior to and a week following the start of injections. Thereafter, tumors were measured 3 times weekly.

After 36 days, all injections were discontinued, and the growth of the tumors was observed. Five experimental rats underwent bilateral oophorectomy through a single transdorsal...
incision, and the growth of their tumors was observed. Rats were sacrificed when the tumors grew very large and were deeply ulcerated, or they died of intercurrent infection and inanition.

Statistical evaluation of the results presented in Chart 1 was made by Student's t test. Fisher's Exact Probability for a 2 X 2 table was used for determination of the significance of the results shown in Table 1 (26).

RESULTS

The overall results in terms of growth and regression of tumors are shown in Table 1. Fifty % of antihormone-treated and 13% of control tumors regressed, while 35% of antihormone-treated and 57% of control tumors grew. Several tumors in each group remained stable. The behavior of tumors in the 2 groups differed significantly. Chart 1 shows the growth of tumors in experimental and control groups of rats. Before the start of treatment, the 2 groups did not differ significantly, but after 36 days of injections there had been a 4-fold increase in tumor area for control rats and the 2 groups differed significantly (p < 0.01). Further tumor growth in control rats could not be plotted, because most of the control rats died very shortly with large necrotic tumors. All tumors in the experimental group showed reactivation of growth following cessation of antihormone serum injections; in 5 animals, tumors decreased in size following oophorectomy.

In 5 of the 10 experimental rats, all tumors regressed during treatment. Chart 2 shows tumor growth in 1 such rat bearing 2 tumors. Variable tumor behavior was observed in 4 of the 10 rats, with some tumors growing and some remaining stable. All tumors, including the 2 that had not responded to treatment, regressed dramatically after oophorectomy. In 1 of the 10 rats, both tumors continued to grow despite treatment. This rat unfortunately died immediately following oophorectomy, and therefore it could not be ascertained whether its tumors were hormone dependent or not.

Vaginal smears showed that all antihormone-treated rats

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**Table 1**

Effect of prolactin antiserum on tumor growth

Nine rats bearing 23 DMBA-induced mammary tumors received daily injections of normal rabbit serum, and 10 rats bearing 20 DMBA-induced mammary tumors received daily injections of rabbit antiserum to rat prolactin for 36 days. Column A presents the number and percentage of tumors in each group that continued to grow in size, Column B shows the number and percentage of tumors that remained stable in size, and Column C records the number and percentage of tumors that regressed in size. A + B versus C; p = 0.01; A versus C, p < 0.025.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A. Tumors growing</th>
<th>B. Tumors stable</th>
<th>C. Tumors regressing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbit serum (9 rats, 23 tumors)</td>
<td>13</td>
<td>57</td>
<td>3</td>
</tr>
<tr>
<td>Prolactin antiserum (10 rats, 20 tumors)</td>
<td>7</td>
<td>35</td>
<td>10</td>
</tr>
</tbody>
</table>
continued to exhibit estrous cycle activity although with somewhat less regularity than controls. One experimental rat was in constant estrus for 2 weeks.

**DISCUSSION**

In 1939, Bischoff and Lyons (2), using purified bovine and sheep "mammotropins" as antigens, produced antibodies that neutralized the crop sac-stimulating activity of these mammotropins. Many investigators since have shown inhibition of hormonal effects by antihormones, both in the case of endogenous antibodies against hormones in clinical situations (1, 15, 19, 22) and in bioassays of hormones (3, 6, 9, 10, 17). In prolactin bioassays, simultaneous injection of antihormone abolishes positive response to prolactin in the rabbit lactogenic assay (25) and the pigeon crop sac assay (2,8). The results of this study indicate that administration of a highly specific antiserum to rat prolactin interferes with growth and maintenance of DMBA-induced tumors in rats, presumably by combination with and inactivation of endogenous prolactin. Maintenance of estrus indicates that the tumor regression induced by prolactin antiserum was not due to a castration effect. That spontaneous regression of DMBA-induced tumors may occur has been well documented, and the pattern of tumor growth seen in the control rats in this study is consistent with the pattern reported by others for growth of DMBA tumors (4, 11, 27, 28). Antihormone administration, however, significantly altered this pattern of growth, resulting in a reduction in tumor growth and a significantly increased incidence of tumor regression. The causative role of antiserum in these regressions is further amplified by the growth of all regressing tumors following cessation of antihormone injections.

Failure of the antiserum injections to suppress all hormone-responsive tumors may be due to a failure to inject enough antibodies to lower biologically active serum prolactin levels below the subsistence levels of all tumors in these rats. There is undoubtedly some variation in the rate of prolactin secretion in different rats, and there may be differences in the requirement for this hormone for tumor growth. Either of these factors might reduce the effectiveness of a given dose of antihormone serum in an individual rat. In addition, some tumors in the experimental group may have been hormone independent. More uniform suppression of growth of these tumors might have been achieved with higher doses of antiserum.

These findings are consistent with the concept that prolactin antiserum neutralizes the biological activity of endogenous prolactin, thereby inducing inhibition of tumor growth, and provide further evidence to support the conclusion that DMBA-induced rat mammary cancer is prolactin dependent.

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