Regression of Rat Mammary Tumors Effected by a Gonadoliberin Analog

Eugene R. DeSombre, Edwin S. Johnson, and Wilfrid F. White

INTRODUCTION

The carcinogen-induced mammary cancer in the Sprague-Dawley rat has been a valuable model for the study of hormone dependence of breast cancer as applied to the disease in man. In this breast cancer model, both ablative surgery (oophorectomy (6) and hypophysectomy (2)) and hormone additive therapy using pharmacological amounts of estrogens (11), as well as antiestrogens (4), effect regression of the hormone-dependent tumors similar to the remissions effected by these therapies in man (10).

With the discovery of the structure of gonadotropin (gonadotropin-releasing factor or luteinizing hormone/follicle-stimulating hormone-releasing hormone), designated A-43818, was evaluated for its ability to effect regression of carcinogen-induced mammary tumors in the Sprague-Dawley rat. This analog, specifically (D-leuyl6, desglycyl-NH10, prolyl ethylamide)gonadotropin, is a potent synthetic luteinizing hormone/follicle-stimulating hormone-releasing hormone at low dose levels but, at the higher dose levels used in these studies, the effect appears to be that of a potent gonadotropin antagonist. Administration of 5 or 20 μg of A-43818 per day to tumor-bearing rats was essentially as effective as ovariectomy in causing mammary tumor regression. At least 80% of the tumors in the A-43818-treated animals underwent regression; about one-half of the regressing tumors disappeared in the 6-week period of continuous treatment, and, unlike the 0.9% NaCl solution control group, no new tumors appeared during the treatment period. A subsequent 4-week period of drug withdrawal resulted in the regrowth of palpable tumors and the appearance of new tumors, most of which again regressed on further A-43818 administration.

MATERIALS AND METHODS

Mammmary tumors were induced in 50-day-old female Sprague-Dawley rats by a single intragastric feeding (7) of 20 mg of DMBA in 2 ml of sesame oil. All animals used had at least 1 tumor that had a maximum single diameter that exceeded 1.5 cm. Insofar as possible, animals were divided into groups based on number, size, and age of tumors. Experimental animals received s.c. injections of 2.5 or 10 μg of A-43818 in 100 μl of isotonic 0.9% NaCl solution, twice daily for 5 days, a single injection on the 6th day, and none on the 7th. Control animals (0.9% NaCl solution) received a similar course of 0.9% NaCl vehicle. Ovariectomy

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The abbreviation used is: DMBA, 7,12-dimethylbenz(a)anthracene.
controls were subjected to bilateral ovariectomy under ether anesthesia on experimental Day 1. Tumor volumes based on ellipsoid shapes were calculated from caliper measurements of tumor diameters (4) taken 3 times per week. All animals were treated and observed for a 6-week period to assess the response of each tumor to treatment. Tumors were classified as regressing if the calculated tumor volume decreased by at least 50% and were classified as growing when the tumor volume increased by at least 50%. Tumors changing by less than 50% were considered to be static. Animals were considered to be cured if no palpable tumors remained at the end of the observation period and to be improved if the total calculated tumor volume in an animal was less than the pretreatment tumor volume for that animal. After the 6-week treatment period, some animals were withdrawn from A-43818 treatment for 4 weeks, followed by reinitiation of treatment, and compared with other animals kept on treatment continuously.

RESULTS AND DISCUSSION

In the principal part of the study, A-43818-treated, vehicle-treated, and ovariectomized, tumor-bearing rats were observed during a 6-week treatment period. When the data were analyzed in terms of animal responses, large differences between treatment and 0.9% NaCl solution control groups were observed (Table 1). The tumor volume of the 0.9% NaCl solution-treated group tripled in 6 weeks, while the tumor volumes of the A-43818-treated and ovariectomized rats decreased. The 4 groups were compared statistically by rank sum tests and, as indicated, at 6 weeks highly significant differences were found between the 0.9% NaCl solution control group and animals treated with each dose of A-43818. There was no significant difference between any 2 of the treatment groups, suggesting that by this type of analysis, at least, treatment with both doses of A-43818 and ovariectomy was essentially equally effective. Also shown in Table 1 are responses of the individual animals. In the 0.9% NaCl solution group, 1 animal showed a marginal improvement during the experiment due to the spontaneous regression of a moderately large tumor, while all the other animals had significantly increased tumor burden after 6 weeks. Ovariectomy effected apparent cures in 4 of the animals and reduced the tumor burden in 5 of the 6 remaining animals. Treatment with A-43818 was effective in 8 of the 10 animals at each dose level, 2 or 3 of which showed apparent cures.

Since the multiple mammary tumors appearing after DMBA treatment probably resulted from multiple carcinogenic events rather than metastasis of a single tumor, it was especially meaningful in this mammary tumor model system to evaluate response to treatment on the basis of individual tumors. Such an analysis of the experiment is shown in Table 2. In the 0.9% NaCl solution-treated group, 16 of the 23 initial tumors grew and only 1 of the 4 regressing tumors was a complete remission. On the other hand, treatment with either dose level of A-43818 effected regression in at least 80% of the tumors, similar to the result seen after ovariectomy. Furthermore, in the A-43818 treatment groups and the ovariectomy group, many of the regressions were complete in 6 weeks.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose*</th>
<th>No. of animals</th>
<th>Tumor volume (initial cm/cm/final cm)</th>
<th>No. of animals*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl solution</td>
<td>10</td>
<td>10</td>
<td>41/119</td>
<td>0</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>10</td>
<td>10</td>
<td>41/8</td>
<td>4</td>
</tr>
<tr>
<td>A-43818</td>
<td>10 μg</td>
<td>10</td>
<td>79/40</td>
<td>2</td>
</tr>
<tr>
<td>A-43818</td>
<td>2.5 μg</td>
<td>10</td>
<td>51/44</td>
<td>3</td>
</tr>
</tbody>
</table>

* Given s.c. twice daily Monday to Friday and once on Saturday.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose*</th>
<th>Initial tumors</th>
<th>New tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl solution</td>
<td>23</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>23</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>A-43818</td>
<td>10 μg</td>
<td>25</td>
<td>2 (13)</td>
</tr>
<tr>
<td>A-43818</td>
<td>2.5 μg</td>
<td>27</td>
<td>2 (13)</td>
</tr>
</tbody>
</table>

* Comparing tumor volume at 6 weeks to pretreatment volumes. Growing increase by at least 50%; regressing decrease by at least 50%; static change less than 50%.
Several tumors in each group did not appreciably change in size and were designated as static. One or 2 tumors in each treatment group were completely nonresponsive as shown by continued growth, despite treatment of the host with A-43818 or ovariectomy. The differences in growth rates of this small percentage of unresponsive tumors account for the major differences in the 6-week tumor volumes for the treated groups seen in Table 1. Whereas 10 new tumors appeared during the 1st 6-week period in the vehicle control group (Table 2), no new tumors appeared in the A-43818- or ovariectomy-treated animals. This result would be consistent with the ovary-inhibiting action of A-43818 (9), since ovariectomy has previously been reported to inhibit the appearance of mammary cancer in carcinogen-treated rats (3). Treatment with A-43818 was effective in early and relatively late appearing tumors and small to large tumors (Chart 1). Some of the tumors that did not disappear completely showed dramatic regression initially, followed by a slower regression as seen for the largest tumor in Chart 1, and were suggestive of 2 cell types regarding sensitivity to the treatment.

For the 2nd part of the experiment, treatment with A-43818 was stopped for a period of 4 weeks in some of the animals at each dose level to observe the effects on tumor growth. The effect in some cases was particularly dramatic (Chart 2), resulting in rapid reinitiation of tumor growth when A-43818 was no longer given. A 2nd treatment period effected an equally dramatic 2nd remission, clearly indicating that the tumor remissions were in response to A-43818 administration.

The 4-week termination of A-43818 administration not only allowed growth resumption of still palpable tumors but it also resulted in appearance of new tumors (Table 3). In the high-dose group, interruption of treatment during Weeks 7 through 10 gave rise to an increase of 2 in the number of tumors and an even more substantial increase in total tumor volume from 4.9 to 36.9 cu cm. An additional 4 weeks of treatment with the high level of A-43818 caused a major reduction of tumor volume to 14.6 cu cm. At the same time, the group maintained continuously on A-43818 showed complete disappearance of the small, barely palpable tumors remaining after 6 weeks and produced no new tumors.

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (μg)</th>
<th>No. of animals</th>
<th>Tumor volumes*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>A-43818</td>
<td>10 C*</td>
<td>3</td>
<td>14.0 (9)</td>
</tr>
<tr>
<td>A-43818</td>
<td>10 I</td>
<td>2</td>
<td>30.0 (9)</td>
</tr>
<tr>
<td>A-43818</td>
<td>2.5 C</td>
<td>4</td>
<td>17.3 (9)</td>
</tr>
<tr>
<td>A-43818</td>
<td>2.5 I</td>
<td>2</td>
<td>7.2 (7)</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>4</td>
<td>1</td>
<td>10.4 (8)</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>6</td>
<td></td>
<td>16.2 (14)</td>
</tr>
</tbody>
</table>

* Calculated volumes in cu cm of all tumors in group, based on individual tumor measurement.

C, continuous administration, 6 days per week (see "Materials and Methods"); I, interrupted treatment, no treatment given during Weeks 7 through 10.

Numbers in parentheses, palpable tumors in the group.
A similar pattern was seen with the animals treated with the low dose level of A-43818, although these results are complicated by the presence of 2 autonomous tumors in the group given continuous treatment. Interruption of the administration of the low dose level of A-43818 during Weeks 7 through 10 gave rise to 11 new tumors and an increase in total tumor volume from 0.7 to 16.9 cu cm. The subsequent 4 weeks of treatment caused regression of all but 2 of the tumors and the disappearance of 3 tumors. The rapid growth of 1 of the 2 nonresponsive tumors, however, resulted in an overall increase in tumor volume during the final 4 weeks of the experiment.

Of the 13 new tumors that appeared during interrupted treatment in the 2 groups, 11 regressed on further treatment with A-43818. An appreciable number of new tumors also appeared during Weeks 7 through 10 in the 0.9% NaCl solution-treated group, while only 1 new tumor appeared in the group treated with A-43818 continuously and none were seen in the ovariectomized animals. This might suggest that while hormonal approaches to mammary tumor therapy can control the growth of hormone-dependent tumors, such treatments may not be effecting total destruction of all tumors cells. Therefore a period during which circulating hormones return to normal levels can result in the appearance of new tumors or reappearance of "cured" tumors in the animals.

The data presented provide strong evidence that treatment with high potency gonadoliberin analog can be an effective treatment for the hormone-dependent mammary tumors in the rat. From other evidence it is clear that when used at the high dose level, A-43818 causes inhibition of follicle-stimulating hormone and luteinizing hormone release, ovarian atrophy, and physiological effects consistent with decreased circulating estrogen (9). More recent, preliminary data (E. S. Johnson and W. F. White, unpublished data) indeed show that such treatment with A-43818 resulted in depressed serum estrogen and prolactin levels in normal rats. Thus it would appear that the physiological effects of A-43818 on mammary tumors are the results of a temporary "chemical" ovariectomy. Whether there might be any direct effect of A-43818 on normal or cancerous mammary tissue remains to be established.

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REFERENCES

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