Effect of Retinyl Acetate on the Occurrence of Ovarian Hormone-responsive and -nonresponsive Mammary Cancers in the Rat

Henry J. Thompson, L. David Meeker, Anthony R. Tagliaferro, and Peter J. Becci

ABSTRACT

The inhibitory activity of retinyl acetate against the induction of ovarian hormone-responsive and -nonresponsive mammary gland adenocarcinomas was studied in intact and castrated female Sprague-Dawley rats. Three experiments were conducted. Mammary cancer was induced by a single p.o. administration of 7,12-dimethylbenz(a)anthracene (DMBA) at 50 days of age. Animals in Experiments 1 and 2 each received 20 mg DMBA, whereas those in Experiment 3 received 15 mg. In all experiments, animals were fed a chow diet supplemented per kg with either a placebo or 328 mg retinyl acetate starting 7 days after carcinogen treatment. In Experiment 1, rats were castrated at either 7, 60, or 90 days postcarcinogen and were killed 120 days after DMBA was given. In Experiment 2, rats were castrated 30 days after DMBA and were killed 240 days after carcinogen treatment. In Experiment 3, rats were castrated when a detected tumor attained a measurable diameter, and the hormone responsiveness of their tumors was subsequently determined. The experiment was terminated 279 days after DMBA treatment. In both intact and castrated rats, mammary tumor occurrence was inhibited by treatment with retinyl acetate. However, there were no differences in the latency to appearance time of hormone-responsive and -nonresponsive cancers in intact animals receiving either placebo or retinyl acetate. The data indicate that retinyl acetate inhibits DMBA-induced mammary tumorigenesis in either the presence or the absence of the ovaries. It appears that retinyl acetate is effective in inhibiting both ovarian hormone-responsive and -nonresponsive mammary tumors.

INTRODUCTION

Retinyl acetate has been shown to inhibit the induction of mammary cancer in the rat by either DMBA or 1-methyl-1-nitroso-2-propanol (8, 9). In those studies, the acetate ester of vitamin A was shown to prolong the latency of cancer appearance and to reduce the incidence and the average number of cancers per rat. It has also been reported that continual dietary administration of retinyl acetate is necessary to sustain inhibitory activity against mammary carcinogenesis (13). The mechanism(s) by which this inhibitory effect is exerted has not been elucidated; however, speculation has arisen concerning the level at which this inhibitory activity is exerted.

Tumorigenesis by modifying endocrine status, possibly by interfering with some aspect of ovarian or pituitary function. The inhibitory activity of either a deprivation or an uptake of ovarian and/or hypophysial hormone(s) on mammary carcinogenesis is well documented (4). However, it has been reported that retinyl acetate inhibits mammary carcinogenesis to a greater extent in the presence of the inhibitor of prolactin secretion, 2-bromo-α-ergocryptine, than can be attributed to the action of either compound alone (15). This implies a mode of action which is at least partially divorced from pituitary effects. This study was initiated to determine whether the ovarian hormones play a role in retinyl acetate inhibition of DMBA-induced mammary carcinogenesis. Further, the effect of retinyl acetate on the occurrence of both ovarian hormone-responsive and -nonresponsive tumors was evaluated.

MATERIALS AND METHODS

Three experiments were conducted. In each study, virgin female Sprague-Dawley rats obtained from Taconic Farms, Inc., Germantown, N. Y., were housed in stainless steel wire mesh-bottomed cages (4 rats/cage) in an environment-controlled room maintained at 22 ± 1 °C (S.D.) with a 12-hr light-dark cycle. Animals were provided with Purina Rodent Chow 50002 (Ralston Purina Company, Indianapolis, Ind.) and tap water ad libitum. In Experiments 1 and 2, rats received 20 mg DMBA dissolved in sesame oil via gastric intubation at 50 days of age; the dose was reduced to 15 mg in Experiment 3. In Experiment 1, 7 days after the administration of DMBA, animals were randomized into 2 groups of either 20 or 22 rats each and fed chow diet supplemented with either a placebo or 328 mg retinyl acetate per kg diet in the form of stable gelatinized beadlets. At this time, 2 groups of animals (one fed placebo and one fed retinyl acetate) were bilaterally ovariectomized. Animals were palpated for the detection of mammary tumors twice each week. At 60 days and again at 90 days after DMBA treatment, intact animals with palpable tumors were ovariecctomized, and their tumors were assessed for hormonal status by periodic measurement with vernier calipers to establish whether or not regression of the tumors occurred. The study was terminated 120 days after carcinogen administration. In Experiment 2, animals were randomized into 2 groups of 25 rats each and fed chow diet supplemented with either placebo or 328 mg retinyl acetate per kg of diet in the form of stable gelatinized beadlets. Thirty days after carcinogen administration, all animals were bilaterally ovariectomized. Animals were palpated for the detection of mammary tumors and weighed weekly. The study was terminated 240 days after carcinogen treatment.

In Experiment 3, animals were randomized into 2 groups of 48 rats each and were fed chow diet supplemented with either a placebo or 328 mg retinyl acetate per kg of diet in the form of stable gelatinized beadlets beginning 7 days after DMBA administration. Animals were palpated for the detection of mammary tumors and weighed weekly. Upon detection, tumor growth was monitored carefully. When a tumor reached a measurable diameter, the animal bearing the tumor was bilaterally ovariectomized. Tumors were measured for an additional 14
days following the ablation surgery. If a tumor decreased in size by greater than 50%, it was classified as ovarian hormone responsive; if its size was greater than 50% but less than 150%, it was classified as ovarian hormone nonresponsive; and if the tumor grew greater than 150% of its size at ovariectomy, it was classified as an autonomous tumor. The study was terminated at 279 days after carcinogen treatment; however, individual animals were terminated 14 days after their ovariectomy.

In each experiment, specimens of all tumors, as well as areas of tissue in which tumors had completely regressed, were removed at necropsy, fixed in 10% neutral buffered formalin, and processed for histopathological evaluation. Statistical analyses of the data were performed as follows. Tumor incidence and the proportion of responsive to nonresponsive tumors were compared using a χ² test without the overly conservative Yates correction for continuity (1). The hypothesis that retinoid treatment delayed the onset of the first tumor (tumor latency) was tested using the nonparametric one-tailed Mann-Whitney test (2) and the Cox-Mantel test for equality of incidence curves (5). The number of observed tumors in the control and retinoid treatment groups were compared using the likelihood ratio test as recommended by Snedecor and Cochran (11). 

RESULTS AND DISCUSSION

Experiment 1. The incidence and total number of mammary gland adenocarcinomas induced in rats which were ovariectomized 7 days after administration of DMBA are shown in Table 1. Retinyl acetate had marked inhibitory effect on the induction of carcinomas in the ovariectomized animals. The compound caused a significant reduction in cancer incidence from 23 to 0%. No benign fibroepithelial mammary tumors were induced in these groups. However, the combined effects of retinyl acetate treatment and castration 7 days after DMBA administration resulted in a significant difference in the rate of body weight gain in comparison to the castrated rats fed placebo diet. At the end of the study, a 21% difference in weight existed. This difference in rate of gain became apparent by Day 45 after carcinogen treatment and was considered of significant magnitude to complicate interpretation of the carcinogenesis data (14). It was therefore decided to ovariectomize the intact rats with palpable mammary tumors of a measurable diameter to determine the ratio of ovarian hormone-responsive to -nonresponsive tumors in animals fed either placebo or retinyl acetate diets. The results of this manipulation as well as the cumulative cancer incidence data prior to ovariectomy are shown in Table 2. The dietary administration of retinyl acetate to noncastrated rats reduced cancer incidence and the average number of tumors per rat. At 14 days after ovariectomy, 65% of the palpable tumors occurring in the placebo-fed rats had regressed by more than 50% of their original volume and were judged as ovarian hormone responsive while 35% had regressed less than 50% of their volume at ovariectomy and were

### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Day of Castration</th>
<th>No. of Rats</th>
<th>Termination (Days after DMBA)</th>
<th>No. of Tumor-Bearing Rats</th>
<th>Incidence (%)</th>
<th>No. of Cancers</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Placebo</td>
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<td>22</td>
<td>120</td>
<td>5</td>
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<td>5</td>
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<tr>
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<td>0</td>
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<td>25</td>
<td>240</td>
<td>4</td>
<td>16</td>
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</table>

1. Animals in these 2 experiments were given 20 mg DMBA and fed diets as described under "Materials and Methods." 2. Significantly different from respective control (p < 0.05). 3. Significantly different from respective control (p < 0.06).

### Table 2

Effect of retinyl acetate on occurrence of ovarian hormone-responsive and -nonresponsive tumors in intact rats

Animals were treated as described under "Materials and Methods." Animals in Experiment 1 received 20 mg DMBA and were killed 120 days post-DMBA; animals in Experiment 3 received 15 mg DMBA and were killed 14 days postovariectomy.

<table>
<thead>
<tr>
<th>Cumulative incidence before ovariectomy</th>
<th>Hormonal status of tumors</th>
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<tbody>
<tr>
<td>Experiment</td>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>1</td>
<td>Placebo</td>
</tr>
<tr>
<td>1</td>
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<td>3</td>
<td>Placebo</td>
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<tr>
<td>3</td>
<td>Retinyl acetate</td>
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</tbody>
</table>

1. Animals in this part of Experiment 1 were ovariectomized only if they bore a tumor with measurable dimensions; ovariectomies were performed at either 60 or 90 days after DMBA treatment. Animals in Experiment 3 were castrated when their tumors attained measurable dimensions.
2. Of the 73 tumors palpated in Experiment 1, 67 occurred in animals which were ovariectomized. Of the 67 tumors, 57 were histologically confirmed as adenocarcinomas and were distributed as follows: hormone responsive, placebo, 30; hormone responsive, retinyl acetate, 8; hormone nonresponsive, placebo, 8; hormone nonresponsive, retinyl acetate, 0. All tumors in Experiment 3 were classified as adenocarcinomas.
3. All palpable tumors which reached a measurable diameter were subsequently classified as either ovarian hormone responsive or nonresponsive or as autonomous tumors.
4. T<sub>50</sub>, number of days after DMBA administration at which 50% of animals in the group had one or more cancers; MTA, mean time of appearance of the tumors in a given classification.
5. Percentage of tumors in a group in a given classification.
6. Numbers in parentheses, percentage of incidence.
7. Significantly different from respective control (p < 0.05).
judged as nonresponsive tumors. However, by the end of the study, in only 38 of the 51 regions in which a tumor was palpated and measured was it possible to confirm histopathologically that the tumor had been an adenocarcinoma. In the retinoid-treated group, 63% of the tumors totally regressed, whereas 37% were between 50 and 70% of their volume at ovariectomy and were therefore classified as nonresponsive. Again, by the time of necropsy, none of the hormone-responsive tumors palpated and measured could be confirmed as adenocarcinomas. This difficulty was addressed by the protocol of Experiment 3.

**Experiment 2.** This experiment was executed to ameliorate the depression of body weight gain resulting from ovariectomy plus retinyl acetate treatment. Since it has been reported that the most critical period in which the effect of caloric restriction on tumor occurrence can be manifested is during the first 30 days after DMBA administration (12), ovariectomy was not performed until that time. Furthermore, the study was extended for 240 days rather than the 120-day period used in Experiment 1 to provide additional time for the occurrence of ovarian hormone-nonresponsive tumors. This protocol allowed for the appearance of tumors in the retinyl acetate-treated group. The incidence and number of mammary gland adenocarcinomas are given in Table 1. Retinyl acetate treatment reduced tumor occurrence and prolonged the latency of tumor appearance. At the termination of the study, the retinyl acetate-treated group mean body weight was 87% of the placebo control value resulting from a 10% reduction in food intake. We have recently determined that restrictions in food intake of 10% or less do not significantly influence mammary carcinogenesis in this tumor system.4

These data both parallel and clarify the results of the 7-day ovariectomy data of Experiment 1. They suggest that retinyl acetate suppresses but does not totally inhibit the development of ovarian hormone-nonresponsive tumors.

**Experiment 3.** The feeding of retinyl acetate had no apparent differential influence on the time of occurrence of hormone-responsive or -nonresponsive tumors in comparison with placebo-fed animals (Table 2). These data suggest that retinyl acetate exerts its inhibitory effect independent of the ovarian hormone status of the tumor. Retinyl acetate treatment did, however, reduce cancer incidence and delay onset of the disease process. The cumulative cancer incidence curves of the 2 treatment groups were significantly different (p < 0.06). It is important to note that the difference in the 2 incidence curves appears in the latter part of the experimental period (after 112 days). This further supports our earlier hypothesis (13) that some animals are considerably more responsive to retinyl acetate treatment than are others. It now appears that this effect is not attributable to hormonal status of the tumors.

It is important to establish the degree to which the inhibitory activity of retinyl acetate against mammary carcinogenesis depends on alterations of the hormonal milieu of an animal. The results of this investigation clearly imply that the ovarian hormones are not directly involved in mediating the inhibition of mammary carcinogenesis by retinyl acetate. This observation parallels the preliminary report of McCormick et al. (6) in which inhibition of mammary carcinogenesis in ovariectomized rats by the nontoxic retinoid hydroxyphenylretinamide was found. Furthermore, our findings are compatible with the data of Welsch et al. (15) which suggest that retinoids inhibit mammary carcinogenesis to a greater extent than does 2-bromo-a-ergocryptine alone.

In the present series of experiments, the intention was to determine if the animal differences in sensitivity to retinoid could be explained by a predominant inhibitory effect on only ovarian hormone-nonresponsive tumors, possibly due to the presence of higher levels of retinoic acid-binding protein in those tumors in comparison to hormone-responsive tumors (7, 10). Our "ovary-intact" animal data do not support the concept of a preferential effect of retinyl acetate on nonresponsive tumors. Although there may be quantitative differences in the levels of retinoic acid-binding proteins in the 2 tumor types, those differences may be related to the differentiation process rather than indicate tumor responsiveness to retinoid treatment.

In conclusion, the results of this investigation suggest that the effect of retinyl acetate on DMBA-induced mammary tumorigenesis is one which is exerted in either the presence or the absence of the ovaries. Retinyl acetate appears to be effective in inhibiting both ovarian hormone-responsive and -nonresponsive mammary tumors.

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**REFERENCES**


4 Unpublished observation.

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Retinyl Acetate Inhibition of Mammary Cancer

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