Enhanced Inhibition of Mammary Carcinogenesis by Combined Treatment with N-(4-Hydroxyphenyl)retinamide and Ovariectomy¹

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ABSTRACT

Bilateral ovariectomy and dietary administration of the retinoid N-(4-hydroxyphenyl)retinamide (4-HPR) are both effective inhibitors of chemical carcinogenesis in the rat mammary gland. The present study was designed to determine whether an enhanced inhibitory effect is obtained with combined ovariec-
tomy and 4-HPR administration, compared to either treatment alone. In separate experiments, 50-day-old virgin female Sprague-Dawley rats received either a single i.v. injection of 50 mg N-methyl-N-nitrosourea per kg body weight or a single intragastric dose of 20 mg 7,12-dimethylbenz(a)anthracene. The experimental design was the same in both the N-methyl-N-nitrosourea and 7,12-dimethylbenz(a)anthracene experiments: Group 1, 25 intact rats, placebo diet; Group 2, 25 intact rats, supplement of 782 mg 4-HPR per kg diet; Group 3, 50 ovariec-
tomized rats, placebo diet; Group 4, 50 ovariectomized rats, supplement of 782 mg 4-HPR per kg diet. Feeding of the 4-
HPR supplement was begun 7 days after carcinogen adminis-
tration; ovariectomy was performed 7 days post-7,12-dimeth-
ybenz(a)anthracene or 14 days post-N-methyl-N-nitrosourea. In both experiments, combined ovariectomy plus 4-HPR was significantly more active in suppressing mammary cancer induction than was either manipulation alone. 4-HPR was a more effective inhibitor of carcinogenesis in ovariecitomized rats than in intact animals. These data indicate that 4-HPR is highly effective in inhibiting ovarian hormone-independent cancers and suggest that retinoid inhibition of mammary carcinogenesis does not involve an influence on ovarian hormone action.

INTRODUCTION

Chemical carcinogenesis in the rat mammary gland is subject to modulation by a variety of dietary, pharmacological, and surgical manipulations. Administration of agents which inhibit the development and growth of cancers in the preneoplastic phase has been termed chemoprevention (16). Effective chemoprevention in an experimental mammary cancer system can be defined as a decrease in carcinoma incidence or multiplicity or as an increase in cancer latent period in a treated group when compared to an untreated control. Previous studies have demonstrated that several retinoids are effective inhibitors of mammary carcinogenesis induced in the rat by the chemical carcinogens DMBA³ (10), MNU (11), or benzo(a)pyrene (7). While most studies have used the naturally occurring compound retinyl acetate (6, 7, 10, 11), the synthetic retinoids retinyl methyl ether (1) and 4-HPR (13) also possess chemopreventive activity in the rat mammary gland. 4-HPR is particularly notable in this regard, because it does not accumulate in the liver after prolonged dietary administration (13). Accumulation of retinyl esters with resulting hepatotoxicity is a major factor limiting chronic, high-dose administration of retinyl acetate.

The DMBA- and MNU-induced mammary carcinomas are also subject to inhibition by alteration of host ovarian hormonal status; these alterations can be achieved by surgical ablation (2, 12, 19) or by administration of antiestrogens such as tamoxifen (3). Because both retinoid administration and ovariec-
tomy are effective in the inhibition of chemical carcinogen-
esis in the rat mammary gland, it is of interest to determine to what extent these 2 treatments will interact when administered together. The present study was designed to determine the cumulative effect of bilateral ovariectomy and dietary adminis-
tration of 4-HPR in the DMBA and MNU models for the exper-
imental study of breast cancer.

MATERIALS AND METHODS

Experimental Animals and Diets. Virgin female Sprague-Dawley rats 42 days old were obtained from Harian/Sprague-Dawley, Madi-
son, Wis. A total of 430 rats was used in the studies. Animals were housed in groups of 3 in polycarbonate cages on Ab-Sorb-Dri bedding (Ab-Sorb-Dri, Garfield, N.J.) in a room illuminated 14 hr each day and maintained at a temperature of 22 ± 1°. Except where indicated otherwise, animals were allowed free access to diet and drinking water throughout the studies. Basal diet for the studies was Wayne laboratory chow (Allied Mills, Chicago, Ill.). Vitamin A level in the basal diet is otherwise, animals were allowed free access to diet and drinking water throughout the studies. Basal diet for the studies was Wayne laboratory chow (Allied Mills, Chicago, Ill.). Vitamin A level in the basal diet is 2.55% retinyl acetate. Retinoid-supplemented diets contained an additional 782 mg (2 mmol) 4-HPR per kg diet. 4-HPR was synthesized by Dr. Y. Fulmer Shely, Southern Research Institute, Birmingham, Ala. In preparation for mixing into diets, 4-HPR was dissolved in absolute ethanol: triptocain (1:3; 5 g/kg diet) with 0.05 ml Tenox 20 (Eastman Chemicals, Kingsport, Tenn.) and 0.05 ml dl-α-tocopherol per kg diet added as antioxidants (13). Placebo diet contained the vehicle without added 4-HPR. Fresh batches of diet were prepared each week and were stored at 4° prior to use; all diet materials in animal cages were changed twice weekly. Analysis of samples of diets indicated complete stability of the retinoid under these conditions.

MNU Study. Mammary carcinomas were induced in rats by a single i.v. injection of MNU, as described previously in detail (5). Crystalline MNU was purchased from Ash Stevens, Inc. (Detroit, Mich.), and was dissolved in 0.85% NaCl solution immediately prior to use. The concent-
tration of the MNU solution was adjusted to 12.5 mg MNU per ml. At 50 days of age, rats were lightly anesthetized with ether and received a single i.v. injection of 50 mg MNU per kg body weight via the jugular vein. Control animals received a single injection of 0.4 ml 0.85% NaCl solution per 100 g body weight.

1 Supported in part by Contracts NO1-CR-23292 and NO1-CB-74207 from the National Cancer Institute. Presented in part at the 72nd Annual Meeting of the American Association for Cancer Research, Inc., Washington, D. C., April 27 to 30, 1981 (8).

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; MNU, N-methyl-N-nitrosourea; 4-HPR, N-(4-hydroxyphenyl)retinamide; i.g., intragas-
tric, T₀, time to 50% cancer incidence (median cancer induction time); T₂₅, time to 25% cancer incidence. Received June 16, 1981; accepted November 5, 1981.

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Seven days following MNU administration, animals were randomized into groups by weight. Two groups of 25 MNU-treated animals were left intact and were fed either the placebo diet or the 4-HPR-supplemented diet beginning at Day 7 postcarcinogen. Two groups of 50 MNU-treated rats were fed the placebo or 4-HPR-supplemented diet beginning at Day 7 post-MNU and were bilaterally ovariectomized under light ether anesthesia at 14 days postcarcinogen. Two groups (25 each) of 0.85% NaCl solution-treated controls were fed either the placebo or 4-HPR diet and were ovariectomized at the same time as the carcinogen-treated rats.

Beginning 4 weeks after MNU administration, all animals were palpated twice weekly to monitor mammary tumor appearance. Animals were weighed weekly throughout the experiment and were observed daily for any indications of toxicity. Moribund animals were killed. Due to high tumor-related mortality in the intact placebo group, the remaining animals in the 2 intact groups were killed at 225 days post-MNU, while ovariectomized animals were killed at 300 days postcarcinogen. Animals killed or found dead were necropsied promptly. Mammary tumors were removed and coded as to location, and any other abnormal tissues were removed and prepared for histological study. Tissues were fixed in 10% buffered formalin, stained with hematoxylin and eosin, and classified histopathologically.

**DMBA Study.** Crystalline DMBA was obtained from Sigma Chemical Co., St. Louis, Mo. and was dissolved in sesame oil (laboratory grade; Fisher Scientific Co., Pittsburgh, Pa.) at a concentration of 20 mg/ml. At 50 days of age, rats were inoculated with a single i.g. dose of 20 mg DMBA; vehicle controls received a single i.g. dose of 1 ml sesame oil only. All animals were starved for 18 hr prior to DMBA or sesame oil administration. Seven days following DMBA administration, animals were randomized into groups by weight. Two groups of 25 DMBA-treated rats were left intact and fed either the placebo or 4-HPR diets beginning 7 days post-DMBA, while 3 groups of 50 DMBA-treated rats were bilaterally ovariectomized and fed either the placebo or 4-HPR diets beginning 7 days postcarcinogen. Four groups of 20 sesame oil controls were assigned placebo, 4-HPR, ovariectomy:placebo, and ovariectomy:4-HPR treatment regimens.

Animal observation, palpation, weighing, and necropsy procedures were the same as described above for the MNU study; all animals were killed 300 days after DMBA administration.

**Statistical Analysis.** Tumor incidence was calculated by the life table method; statistical comparisons of tumor incidence curves were performed by using the logrank test (14). Intergroup comparisons for numbers of tumors per animal were performed with Student’s t test, based on square root-transformed data, as suggested by the method of Snedecor and Cochran (15). Comparison of mean group weights was performed by using Student’s t test. \( T_{25} \) and \( T_{30} \) values were compared by the Wilcoxon rank sum analysis (15) for the first 50 and 25% of each group bearing mammary cancers.

### RESULTS

**MNU Study.** Mammary carcinogenesis induced in rats by MNU was significantly inhibited by administration of 4-HPR, ovariectomy, or combined 4-HPR plus ovariectomy. While administration of 4-HPR alone or ovariectomy alone both resulted in a decreased tumor response in comparison to the intact, placebo-fed control, these inhibitory effects were significantly enhanced when the 2 regimens were combined.

All retinoid-treated and/or ovariectomized groups showed a reduction in mammary tumor incidence and a delay in tumor appearance compared to control (Table 1). Administration of 4-HPR alone was the least effective of the treatment protocols but nevertheless induced a significant inhibition of tumorigenesis. Although the final tumor incidence in the 4-HPR group was 92%, as compared to a 100% incidence in the placebo control group, the increased tumor latent period in the 4-HPR group was sufficient for logrank analysis to indicate a statistically significant (\( p < 0.05 \)) difference between the 2 groups. Ovariectomy alone reduced tumor incidence at 225 days post-MNU from 100% in the intact, placebo group to 18% in the ovariectomized placebo group. This ovariectomy-induced inhibition of carcinogenesis was augmented by 4-HPR administration, as the ovariectomy plus 4-HPR group showed a tumor incidence of only 2% at 225 days. The inhibition of carcinogenesis achieved by ovariectomy plus 4-HPR was significantly (\( p < 0.01 \)) greater than that of the group subjected to ovariectomy alone.

It is noteworthy that logrank analysis involves a comparison of tumor incidence curves throughout an experiment rather than data at any specific point in time. Although final tumor incidence was reduced by only 8% in the intact, 4-HPR group as compared to the intact, placebo-fed control, time to tumor appearance was increased by 4-HPR treatment (see Table 1, \( T_{25} \) and \( T_{30} \) values). It is this increased tumor latency, rather than a reduction in final cancer incidence, that is responsible for the statistically significant difference in tumor response in these 2 groups.

A trend similar to that seen with tumor incidence is evident in a comparison of the number of cancers per rat among MNU-treated groups at 225 days post-MNU. Administration of 4-HPR to intact animals reduced carcinoma multiplicity by approximately 30% compared to intact, placebo diet controls. Ovariectomy reduced the number of cancers per rat to approx-

#### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Retinoid</th>
<th>Endocrine status</th>
<th>Body wt</th>
<th>Tumor incidence (%)</th>
<th>( T_{25} ) (days)</th>
<th>( T_{30} ) (days)</th>
<th>Cumulative tumors/rat</th>
<th>Cumulative cancers/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>Placebo</td>
<td>Intact</td>
<td>289 ± 5</td>
<td>100</td>
<td>48</td>
<td>55</td>
<td>4.82 (100.0)</td>
<td>4.50 (100.0)</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>4-HPR</td>
<td>Intact</td>
<td>292 ± 7</td>
<td>92 ( ^{d} )</td>
<td>66 ( ^{e} )</td>
<td>85 ( ^{e} )</td>
<td>3.39 ( ^{d} ) (70.3)</td>
<td>3.29 ( ^{d} ) (73.1)</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>Placebo</td>
<td>Ovariectomy</td>
<td>367 ± 7</td>
<td>18 ( ^{e} )</td>
<td>274 ( ^{e} )</td>
<td>—</td>
<td>0.24 ( ^{e} ) (5.0)</td>
<td>0.24 ( ^{e} ) (5.3)</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>4-HPR</td>
<td>Ovariectomy</td>
<td>348 ± 11</td>
<td>2 ( ^{e} )</td>
<td>—</td>
<td>—</td>
<td>0.02 ( ^{e} ) (0.4)</td>
<td>0.02 ( ^{e} ) (0.4)</td>
</tr>
</tbody>
</table>

*a* Includes malignant (adenocarcinomas, papillary carcinomas) and benign (fibroadenomas, fibromas, lobular hyperplasia) mammary tumors.

*b* Mean ± S.E.

*c* Numbers in parentheses, percentage of intact placebo group (Group 1).

\( d ^{p} < 0.05 \) versus intact placebo (Group 1).

\( e ^{p} < 0.01 \) versus intact placebo (Group 1).

\( f \) Group never reached 50% cancer incidence.

\( g ^{p} < 0.01 \) versus ovariectomy placebo (Group 3).

\( h \) Group never reached 25% cancer incidence.
imately 5% of that of the intact, placebo group, while combined ovariectomy plus 4-HPR treatment reduced the number of cancers to less than 1% of that observed in the control group. 4-HPR appeared to be a more effective inhibitor of mammary carcinogenesis in ovariectomized than in intact hosts. While 4-HPR administration caused a reduction of approximately 30% in the number of cancers per rat in intact hosts (Group 2 versus Group 1), this reduction was greater than 90% when 4-HPR was administered to ovariectomized animals (Group 4 versus Group 3).

Time to first tumor and T50 values were also significantly increased by ovariectomy and/or administration of 4-HPR. The time of first tumor appearance in the intact, placebo control group was 33 days and a 50% tumor incidence was reached at 55 days; these values were increased to 43 and 85 days, respectively, by 4-HPR administration. Ovariectomy increased the time to first tumor to 99 days, while no tumors were found in the group receiving combined treatment with ovariectomy plus 4-HPR until 190 days post-MNU. By contrast, at 190 days postcarcinogen, the intact, placebo-fed group had a 100% incidence of mammary cancer.

Administration of 4-HPR had no significant influence on body weight in any experimental groups. Mean body weights in the various 4-HPR-treated groups ranged from 94 to 101% of their respective placebo-fed controls (Tables 1 and 2). Bilateral ovariectomy did have an influence on animal body weight, however, since ovariectomized animals had a significantly (p < 0.05) increased mean weight compared to intact controls.

**DMBA Study.** The patterns of mammary cancer inhibition observed in DMBA-treated animals were generally similar to the trends reported for animals receiving MNU. While both 4-HPR and bilateral ovariectomy resulted in a significant inhibition of carcinogenesis when administered alone, a synergistic interaction was noted in animals receiving both 4-HPR and ovariectomy. Administration of 4-HPR alone delayed the time of first tumor appearance from 26 days in the intact, placebo-fed group to 64 days, while the first tumor in the ovariectomized, placebo-fed group was observed at 33 days. By contrast, the first palpable mammary tumor in the ovariectomized, 4-HPR group appeared at 117 days postcarcinogen; at this time, tumor incidence in the intact control group was 74%. The same trend was seen in tumor incidence at 225 days (Table 2) and at the termination of the study at 300 days. Although a decreased mammary tumor incidence was induced by administration of 4-HPR or ovariectomy, combined treatment was significantly more effective than was either modality alone.

The combined treatment of 4-HPR and ovariectomy resulted in a greater than 98% inhibition of carcinoma multiplicity in DMBA-treated animals. While the cumulative effect of 4-HPR plus ovariectomy in reducing the number of cancers per animal was striking, the administration of 4-HPR alone had an equivalent inhibitory effect on the number of mammary cancers per rat induced by DMBA. Although the number of cancers per rat was significantly decreased in the 4-HPR group at the termination of the study (300 days), this reduction in cancer multiplicity was not statistically significant at 225 days post-DMBA (0.05 < p < 0.10) (Table 2). At earlier times in the study (Days 120 through 180), the number of cancers per animal in the intact, placebo-fed group and the intact, 4-HPR group was nearly identical. This decreased efficacy of tumor inhibition in intact animals is particularly unusual in that 4-HPR retained its synergistic chemopreventive effect in terms of number of cancers per rat when its administration was combined with ovariectomy.

Nonmammary tumors were observed in low incidence in both animals receiving MNU and those receiving DMBA. In 150 animals receiving MNU, 6 renal sarcomas, one renal carcinoma, 2 lymphosarcomas, one pancreatic carcinoma, and 3 abdominal masses of undetermined origin (2 sarcomas, one carcinoma) were observed. This low (~9%) incidence of nonmammary tumors and the susceptibility of the kidney to high-dose MNU administration are consistent with our earlier description of the single-dose MNU model (5). Rat skin was sensitive to i.g. administration of DMBA, as 3 squamous cell carcinomas, one keratoacanthoma, and one sebaceous adenoma were found in 150 animals. Other nonmammary tumors found in DMBA-treated animals were 2 abdominal masses of undetermined origin (one sarcoma, one carcinosarcoma), one

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<th>T50 (days)</th>
<th>T50 (days)</th>
<th>Cumulative tumors/rat</th>
<th>Cumulative cancers/rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>Placebo</td>
<td>Intact</td>
<td>302 ± 5d</td>
<td>91</td>
<td>64</td>
<td>62</td>
<td>5.40 ± 0.34d (100.0)c</td>
<td>3.82 (100.0)</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>4-HPR</td>
<td>Intact</td>
<td>290 ± 6d</td>
<td>85d</td>
<td>75</td>
<td>87</td>
<td>3.67 ± 0.06e (67.9)</td>
<td>2.90 (75.9)</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>Placebo</td>
<td>Ovariectomy</td>
<td>362 ± 6dy</td>
<td>26d</td>
<td>—</td>
<td>—</td>
<td>0.34 ± 0.06e (8.3)</td>
<td>0.29 ± 0.06e (7.6)</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>4-HPR</td>
<td>Ovariectomy</td>
<td>339 ± 4d</td>
<td>6d</td>
<td>—</td>
<td>—</td>
<td>0.06 ± 0.06e (1.1)</td>
<td>0.06 ± 0.06e (1.6)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage of intact placebo group (Group 1).
* Mean ± S.E.
* p < 0.05 versus intact placebo (Group 1).
* p < 0.01 versus intact placebo (Group 1).
* Group never reached 50% cancer incidence.
* p < 0.01 versus ovariectomy placebo (Group 3).
* Group never reached 25% cancer incidence.
hemangioma, and one hemangiosarcoma. One lipoma was the only tumor found in non-carcinogen-treated groups. No metastatic mammary lesions were found in any animals given either MNU or DMBA.

DISCUSSION

The data from the present studies using 2 experimental models for breast cancer clearly demonstrate a synergistic interaction between 4-HPR and bilateral ovariectomy in the inhibition of rat mammary carcinogenesis. While 4-HPR and ovariectomy were effective inhibitors of carcinogenesis when administered alone, groups receiving combined 4-HPR treatment and ovariectomy showed a greatly enhanced inhibition of carcinogenesis with a more than 90% reduction in cancer incidence and a greater than 96% inhibition of tumor number compared to control. Animals receiving 4-HPR plus ovariectomy also showed a significantly increased tumor latent period compared to both the control group and groups receiving either treatment alone.

That the combined effect of 4-HPR and ovariectomy is one of true synergy, as opposed to simple additivity of effects, can be determined by a comparison of the efficacy of retinoid inhibition of carcinoma multiplicity in intact and ovariectomized hosts. Administration of 4-HPR resulted in an inhibition of carcinoma multiplicity of approximately 27 and 24% in intact hosts receiving MNU and DMBA, respectively. By contrast, the percentage of inhibition of number of cancers per rat in ovariectomized hosts was 92 and 79% in the 2 studies. Thus, the retinoid was relatively more effective in inhibition of carcinogenesis in ovariectomized than in intact hosts; the same trends are seen when both malignant and benign mammary tumors are included.

This synergistic inhibition of mammary carcinogenesis does not appear to be a function of reduced activity of 4-HPR at high carcinogen doses. On the basis of the data from the present study, it could be postulated that the magnitude of the inhibition of carcinogenesis achieved by 4-HPR administration is reduced in models (i.e., intact animals) with a high tumor incidence and short latent period. In such a scheme, the chemopreventive efficacy of the compound would be "overwhelmed" by the rapid cancer response; however, this antitumor activity could be enhanced by administration of the compound in combination with another manipulation (i.e., ovariectomy) which would decrease the tumor response to a level at which the 4-HPR was maximally active. In such a situation, the appearance of a synergy would be an artifact caused by the reduced activity of the chemopreventive agent at high carcinogen doses. While this possibility cannot be discounted, previous studies in our laboratory have noted no loss in the antitumor activity of 4-HPR over a range of MNU doses between 5 and 50 mg/kg.4 Thus, it appears improbable that the enhanced activity of 4-HPR in ovariectomized animals is due to a less than optimal activity in the intact group.

The mechanisms for this apparent synergy between ovariectomy and 4-HPR are unclear, although several hypotheses for the phenomenon can be developed. As noted in the present study, ovariectomized rats increase their body weights to a level approximately 20% greater than that of intact controls (4, 9, 17); this increase is associated with a transient increase in food intake (9, 17). Thus, it can be postulated that, following surgery, ovariectomized rats consume a larger quantity of 4-HPR-supplemented diet than do intact animals, resulting in increased exposure to the chemopreventive agent and an enhanced inhibition of carcinogenesis. Although the simplicity of this hypothesis is appealing, 2 factors suggest that increased consumption of 4-HPR probably plays at most a minor role in inhibiting mammary cancer in ovariectomized rats. As stated above, the increase in food intake following ovariectomy is of relatively short duration. Landau and Zucker (4) found that food intake in ovariectomized Sprague-Dawley rats was elevated for only 40 days following surgery. After this time, food intake returned to control levels, although body weight remained elevated due to factors unrelated to diet. Similarly, Tarttelin and Gorski (17) noted a peak in food intake 25 days after ovariectomy, after which time food intake returned to control levels. In addition, the magnitude of this increase in food consumption is relatively small, ranging from approximately 10 to 20% (4, 9). Therefore, although a possible enhancement of chemoprevention mediated by an increased level of exposure to 4-HPR cannot be discounted, the fact that any such increased exposure (a) is of relatively minor magnitude and (b) occurs only for a short time suggests that other factors unrelated to food intake are primarily responsible for the enhanced cancer inhibition seen in ovariectomized rats.

Two other general mechanisms can be identified, although little data exist concerning either one. One possibility is that ovariectomized animals have a change in 4-HPR metabolism, transport, or storage with the end result being an increased level of the active metabolite reaching the mammary parenchymal target cell. Alternatively, one can hypothesize a functional change in the mammary parenchymal cell itself, which would result in increased entry of and/or an enhanced cellular response to the active 4-HPR metabolite. However, both of these hypotheses await confirmation.

Several comparisons can be made between the MNU- and DMBA-induced mammary cancer models used in these studies. Administration i.v. of 50 mg MNU per kg body weight or administration i.g. of 20 mg DMBA resulted in the induction of mammary tumors with approximately equal latency. The time to first tumor was 33 days with a T50 value of 55 days in the MNU control group versus 26 and 87 days, respectively, in the DMBA groups. At 225 days, the DMBA control group had 5.40 tumors/animal compared to 4.82 in the MNU group. By contrast, however, at 225 days postcarcinogen, over 93% of the MNU-induced tumors were cancers, compared to only 70% in the DMBA-treated animals. Since the mammary carcinoma, as opposed to the fibroadenoma, is the lesion of interest in the model, the relatively high proportion of benign lesions seen in DMBA-treated animals is a disadvantage of this model system.

A key comparison of the 2 models resulting from the present experiments is that 4-HPR appears to be somewhat less effective against DMBA-induced mammary carcinogenesis than against mammary cancers induced by MNU. Administration of 4-HPR alone resulted in a significant inhibition of the number of MNU-induced mammary cancers per rat at 225 days postcarcinogen. By contrast, the inhibition of DMBA carcinogenesis by 4-HPR was not significant at the 5% level at that time (0.05 < p < 0.10), although 4-HPR inhibition of carcinoma multiplicity in the DMBA study was statistically significant at 300 days postcarcinogen. When 4-HPR administration was
combined with ovariectomy in the DMBA experiment, however, a synergy between the 2 modifiers was observed. This somewhat anomalous behavior of 4-HPR in inhibition of DMBA carcinogenesis is presently unexplained, although it should be noted that, on a percentage of inhibition basis, the inhibition achieved by 4-HPR in DMBA-treated animals approaches that achieved by 4-HPR in DMBA-treated animals. Furthermore, a similar effect on mammary carcinogenesis was noted with the use of the retinyl acetate and 2-bromo-a-erocryptine, an inhibitor of pituitary prolactin secretion. These results are consistent with those of Welsch et al. (8) who noted that, on a percentage of inhibition basis, the inhibition achieved by 4-HPR in DMBA-treated animals approaches that achieved by 4-HPR in DMBA-treated animals. Furthermore, a similar effect on mammary carcinogenesis was noted with the use of the retinoid retinyl methyl ether, but in the case of retinyl methyl ether, the retinoid was more active in the DMBA (1) model than in the MNU system (12).

The data from these studies, which show an interaction between retinoid administration and altered host hormonal status, are consistent with those of Welsch et al. (8) who noted a synergistic inhibition of MNU-induced mammary carcinogenesis by coadministration of retinyl acetate and 2-bromo-a-ergocryptine, an inhibitor of pituitary prolactin secretion. These data also suggest that retinoid inhibition of mammary carcinogenesis is not mediated via an alteration in host metabolism of estrogen or prolactin. If the retinoids were exerting their cancer-inhibitory effect through an influence on estrogen or prolactin, no synergy would be expected when a retinoid was administered concomitantly with a modifier of estrogen or prolactin synthesis, release, or activity. Since such an interaction has been found in 2 studies, other nonhormonally mediated mechanisms of retinoid action are implied.

It is apparent from these studies that concomitant administration of 4-HPR and other modifiers of mammary carcinogenesis provides treatment regimens with greatly enhanced chemopreventive efficacy, compared to either agent alone. Further combination studies are currently in progress; through these studies, it is hoped to (a) develop nontoxic treatment regimens with increased anticancer activity and (b) provide information concerning the mechanism of action of retinoids in the inhibition of experimental carcinogenesis.

REFERENCES

Announcements

The submission of abstracts is May 1, 1983. Forms for registration, hotel reservations, and abstracts will be made available at the end of 1982. For further details, contact: Dr. J. Kieler, The Danish Cancer Society, Laboratory of Environmental Carcinogenesis, Ndr Frihavnsgade 70, DK-2100 Copenhagen Ø, Denmark. A satellite meeting on modifiers of carcinogenesis, being organized by Dr. E. Thorling, will follow the main meeting on September 22 and 23. Information concerning other activities of the EACR and application forms for membership may be obtained from: Dr. M. R. Price, Secretary of EACR, Cancer Research Campaign Laboratories, University of Nottingham, Nottingham NG7 2RD, United Kingdom.

COURSE ON GENETICS IN CLINICAL ONCOLOGY

A course, entitled "Genetics in Clinical Oncology," will be offered for the second time on October 7 to 8, 1982, by the Laboratory of Genetics, Department of Pathology, Memorial Hospital for Cancer and Allied Diseases, New York, New York. The objective of this course is to provide current knowledge of genetics as it pertains to clinical oncology in such a manner that it will take on practical value. Topics to be covered include: current theory, such as "new genetics," concerning the etiology and nature of cancer; role of chromosome changes in leukemia and solid tumors; role of heredity in predisposing a person or a family to cancer; and practical matters such as genetic counseling for the cancer patient or family and indications for genetic and cytogenetic work-up.

The course has been approved for 15 credit hours in Category I of the Physicians Recognition Award of the American Medical Association. The fee for the course, including registration, reception, and lunches, is $200. For more information, write to: Dr. R. S. K. Chaganti, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021.

FOURTEENTH MEETING OF THE INTERNATIONAL SOCIETY FOR PEDIATRIC ONCOLOGY

The Fourteenth Meeting of the International Society for Pediatric Oncology will be held in Berne, Switzerland, September 21 to 25, 1982. "New Modes of Therapy and Supportive Care" will be the primary topic, covering such areas as immune modulation, clinical applications of monoclonal antibodies, bone marrow transplantation, infection and nutrition, new modes of surgery, and new modes in radiooncology. Reports on current trials will also be presented. The language of the conference will be English. For further information, contact Dr. Hans P. Wagner, %SPOG, Institute for Clinical and Experimental Cancer Research, Tiefenauaspital, 3004 Berne, Switzerland.

Errata

The following error occurred in the February 1982 article by D. McCormick et al., entitled "Enhanced Inhibition of Mammary Carcinogenesis by Combined Treatment with N-(4-Hydroxyphenyl)retinamide and Ovariectomy." On page 506, the first paragraph of "Materials and Methods," Line 14, should read, "... 4-HPR was dissolved in absolute ethanol:trioctanoin (1:3; 50 g/kg diet) with 0.5 ml Tenox 20 ... and 0.5 ml DL-alpha-tocopherol per kg diet ..."

In the article by K. N. Prasad et al., entitled "Effects of Tocopherol (Vitamin E) Acid Succinate on Morphological Alterations and Growth Inhibition in Melanoma Cells in Culture," which appeared in the February 1982 issue, Footnote 3, on page 551, should read, "The abbreviations used are: SEM, serum-free medium; [F-12 medium containing insulin (5 μg/ml), transferrin (100 μg/ml), 20 nM progesterone, 100 μM putrescine; and 30 nM sodium selenite]..."
Enhanced Inhibition of Mammary Carcinogenesis by Combined Treatment with N-(4-Hydroxyphenyl)retinamide and Ovariectomy

David L. McCormick, Rajendra G. Mehta, Carol A. Thompson, et al.


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