Effects of 4-Nitroestrone 3-Methyl Ether on Dimethylbenz(a)anthracene-induced Mammary Tumors


ABSTRACT

4-Nitroestrone 3-methyl ether has been shown to be an effective growth inhibitor of certain dimethylbenz(a)anthracene-induced rat mammary tumors in intact or ovariectomized rats. When administered at optimum levels (24 mg/kg daily), this A-ring-substituted estrone displayed no toxicity, slight estrogenicity, and an antitumor activity which was comparable to that of tamoxifen and nafinoxine and was surpassed only by ovaricotomy or pharmacological doses of 17β-estradiol 3-benzoate. In addition, the appearance of mammary tumors was prevented when this estrogen derivative was administered to rats just prior to or after dimethylbenz(a)anthracene intubation. Unique to the action of the methyl ether of 4-nitroestrone on mammary tumors was the destruction of adenocarcinomas while permitting the appearance of fibroadenomas. Systemically, 4-nitroestrone 3-methyl ether brought about focal atrophy within the pituitary and ovaries while causing moderate hypertrophy of the uterus. Plasma prolactin was unaffected.

INTRODUCTION

While there has been considerable recent interest in the triphenylmethane antiestrogens as agents which inhibit hormone-dependent mammary tumors through their interaction with the estrogen receptor, there has been little interest in estrogen sulfotransferase in incubations of secretory porcine uterine mucus (5). Another target tissue, which actively sulfurylates estrogens, is the hormone-dependent mammary tumor (1, 2, 4, 7, 9, 18, 19, 22) which, unlike normal mammary epithelium, is presumed to contain high levels of this hormonally controlled enzyme (9, 22).

The DMBA3-induced rat mammary tumor offers an excellent model with which to: (a) test the effects of A-ring-substituted estrogens on hormone-dependent tumors; and (b) at the same time obtain information on the importance of estrogen sulfonylation to the growth of this neoplasia. Herein, we describe the effects of the administration of certain A-ring-substituted estrogens to rats with DMBA-induced mammary tumors. Subsequent reports will deal with the importance of estrogen sulfonylation in these tumors.

MATERIALS AND METHODS

Chemicals and Reagents. Estrone, 17β-estradiol 3-benzoate, estrone sulfate, aryI sulfatase (EC 3.1.6.1), β-glucuronidase (EC 3.2.1.31), DMBA, and 5-fluorouracil were obtained from Sigma Chemical Co. (St. Louis, Mo.). 4-Nitroestrone 3-methyl ether, 4-nitroestrone, and 2,4-dinitroestrone have been synthesized in our laboratories by published procedures (31). Tamoxifen (free base) and nafinoxine (U-11,100A) were kindly supplied by Drs. D. H. McCurdy of Stuart Pharmaceuticals (Division of ICI Americas, Inc.) and J. Babcock of the Upjohn Co. (Kalamazoo, Mich.), respectively. Adriamycin (doxorubicin-HCl) was purchased from Adria Laboratories, Inc., Columbus, Ohio.

Animals. Virgin female Sprague-Dawley rats (The Charles River Co., Wilmington, Mass.) were housed 4 to 6/cage in a light (12 hr/day)- and temperature (24°)-controlled room and given a diet of Wayne Lab-Blox laboratory chow (Allied Mills, Inc., Chicago, Ill.) and tap water ad libitum. At 50 days of age, rats were intubated with DMBA (10 mg/100 g body weight) dissolved in sesame oil (20 mg/ml). Beginning at Day 45 after intubation, all animals were weighed and palpated once per week. Tumor volumes were calculated by measuring 2 diameters with a caliper and the third dimension with a ruler, then by substituting values:

\[ \text{Volume (cu cm)} = \frac{1}{6} abc \]

where a, b, and c are the 3 different diameters of the tumor. The agreement of the in vivo tumor volume and measurements of excised tumors identified at necropsy was 95 to 99%. When about 75% of rats had palpable tumors (81 to 92 days after intubation), the animals were randomized, excluding rats with tumors larger than 2.00 ml and rats with more than 5 tumors/animal. The mean initial tumor volumes in control and treated groups ranged between 0.4 and 0.9 cu cm over the various experiments. Where necessary, ovaricotomy (ether anesthesia) was performed on the first or second day after the initiation of the study. The

1 Supported by NIH Grants USPHS CA 23079 and CA 22828 from the National Cancer Institute and in part by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. Reported in part at the 16th Annual Meeting of the American Association for Cancer Research, San Diego, Calif., May 1980 (24).

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significance of difference between treatment groups was examined by Student's t test. Estrogen derivatives (>99% pure by thin-layer chromatography) and the nonsteroidal antiestrogens were injected s.c. daily (Monday to Friday). After distribution in SSV, the steroids (20 mg/ml) were administered at 0.12 to 54 mg/kg body weight. The suspension was sonicated before use to achieve uniform distribution. Tamoxifen, nafloxidine, and 17β-estradiol 3-benzoate were dissolved in a minimum amount of 100% ethanol and added to sesame oil, and the ethanol was evaporated under a stream of nitrogen to give a final concentration of 1 mg/ml oil. 17β-Estradiol 3-benzoate was dissolved directly in sesame oil to a concentration of 2.5 mg/ml; 5-fluorouracil was dissolved in water (25 mg/ml), and Adriamycin was dissolved in 0.9% NaCl solution. The control group was given injections of SSV or sesame oil alone.

The toxicity of all antitumor agents was determined by comparing body weights of treated and control animals.

Postmortem Examination and Histopathology of Tumors. Rats were selected for colchicine injections (2 mg/kg body weight) 4 hr prior to necropsy to obtain an accurate measurement of mitotic indices of tumors. Animals were sacrificed with CO2 gas or bled to death via the abdominal aorta. Tumors and tissues were removed and preserved in 10% neutral buffered formalin for histopathological examination. Gross anomalies of abdominal, thoracic, and cranial cavities were recorded. Uteri were removed, trimmed, and weighed fresh. Ovaries, adrenals, and pituitaries were trimmed and weighed after fixation.

Histopathological observations on hematoxylin- and eosin-stained tumors and organ sections were performed, and comparisons between the control and treated groups were carried out using computer analysis. The microscopic parameters used to judge the degree of anaplasia in DMBA-induced mammary neoplasms, when treated animals were compared to controls, were: (a) the type of epithelium or mammary tissue involved; (b) the degree of encapsulation of the neoplasm; (c) the number of mitotic figures observed; (d) the extent of stroma invasion of the neoplastic epithelium; (e) the severity of lymphocytic infiltration of the neoplastic epithelium; and (f) the regressive, degenerative, or vacuolative changes in the neoplastic epithelium (3, 10, 11, 27, 29). Histological examination was performed on all mammary tumors.

RESULTS

Effect of 4-Nitroestrone 3-Methyl Ether on DMBA-induced Rat Mammary Tumors. In vitro studies had demonstrated that 2,4-dibromoestrone 3-methyl ether, 2,4-dinitroestrone, and 4-nitroestrone were superior inhibitors of the isolated estrogen sulfotransferase when compared to 4-nitroestrone 3-methyl ether (25). However, limited antitumor effects (Table 1) were demonstrated by the first 3 estrogen derivatives when administered in vivo. Accordingly, 4-nitroestrone 3-methyl ether was chosen for subsequent antitumor investigations.

Chart 1 shows the effect of different levels of 4-nitroestrone

<table>
<thead>
<tr>
<th>Estrogen analogue injected</th>
<th>Mean tumor volume (cm)</th>
<th>Mean tumor no. (no. of tumors/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.48 ± 2.09a</td>
<td>3.30 ± 2.60</td>
</tr>
<tr>
<td>2,4-Dibromoestrone 3-methyl ether</td>
<td>1.24 ± 1.07</td>
<td>4.26 ± 2.53</td>
</tr>
<tr>
<td>2,4-Dinitroestrone</td>
<td>1.14 ± 1.37</td>
<td>2.90 ± 1.67</td>
</tr>
<tr>
<td>4-Nitroestrone</td>
<td>0.75 ± 0.10</td>
<td>1.95 ± 2.60</td>
</tr>
<tr>
<td>4-Nitroestrone 3-methyl ether</td>
<td>0.05 ± 0.05a</td>
<td>1.35 ± 0.40a</td>
</tr>
</tbody>
</table>

a Mean ± S.D.  

p values for these results, when compared to those of the control tumors, were <0.05. The results for the 2,4-dibromoestrone 3-methyl ether, 2,4-dinitroestrone, and 4-nitroestrone treatments were insignificant.
As indicated by the results from the ovariec tomized group, as much as one-half of the nonregressing treated tumors could be the ovarian hormone-independent cancers (Chart 2). The decrease in volume of the regressing tumors in the treated group was as dramatic as that seen in the ovariec tomized rats (initial tumor/volume/final tumor volume ratio, 0.28 versus 0.34, respectively).

The optimum antitumor level (24 mg/kg) of 4-nitroestrone 3-methyl ether appeared to be nontoxic, since the body weights of the treated [274 ± 22 (S.D.) g] and control [256 ± 22 g] groups were not significantly different at the end of 4 weeks. The ovaries of treated rats underwent notable atrophy during administration of the nitroestrone ether (control ovaries, 78 ± 21 mg versus treated ovaries, 38 ± 11 mg).

Comparison of 4-Nitroestrone 3-Methyl Ether with Other Mammary Tumor Inhibitory Agents. The effects of different treatments on mammary tumor volume and number are shown in Chart 3. In this experiment, samples of tumors and organs were taken for histopathological observations at the time when treated tumors were responding to the various agents but before the treated group tumors had disappeared or the control group tumors became necrotic. This timing was selected so that specimens could be obtained while there was still enough tumor tissue, and the resulting histological preparations could be examined for the effects of the treatments. For this reason, 17β-estradiol 3-benzoate treatment was terminated on Day 16, and the remaining groups were treated for 22 days. A pharmacological level of 17β-estradiol 3-benzoate was most effective in bringing about the regression of DMBA-induced mammary tumors (Chart 3). However, as shown previously in Charts 1 and 2, 4-nitroestrone 3-methyl ether was again quite effective after 22 days by diminishing tumor growth and appearance in relation to the control group.

In experiments to be reported separately, it was determined that the in vivo fate of tritiated 4-nitroestrone 3-methyl ether administered s.c. included substantial (15%) demethylation. Both the ether and the demethylated compound were found to be distributed throughout the tissues of the animal and in the excreta. Since 4-nitroestrone was a major metabolite of 4-nitroestrone 3-methyl ether, it was important to test whether the antitumor effects observed in animals treated with the methylated nitroestrone might be due to the demethylated metabolite. The decision to administer the demethylated estrogen analogue at one-sixth the level of 4-nitroestrone 3-methyl ether (Chart 3; Table 2) was based on the finding that this level approximated the degree of demethylation which occurred following the injection of tritiated 3-methyl ether. The data presented in Chart 3 demonstrate that, during the period of treatment, the 4-nitroestrone-treated rats bore tumors which grew more rapidly than did the control mammary cancers. However, after 22 days, this treatment did reduce the total number of tumors relative to that of controls. During the same period, administration of 4-nitroestrone 3-methyl ether brought about a slight reduction in initial tumor size resulting in neoplasms which had only 59% of the volume of the control cancers. The methyl ether derivative was more effective than was 4-nitroestrone in reducing the number of tumors relative to both the initial and the final number of tumors in control rats. When equal levels (24 mg/kg) of both estrogen analogues were administered to rats with DMBA-induced tumors, the 4-nitroestrone 3-methyl ether again promoted the greatest antitumor effect (Table 1). The information derived from the above observations suggests that the demethylated analogue is not more active than is 4-nitroestrone 3-methyl ether in inhibiting mammary tumor growth or appearance.

In the experiment represented by Table 2, combination chemotherapy (Adriamycin plus 5-fluorouracil) elicited an 18% tumor volume decrease (relative to that of controls) and a 43% reduction in tumor number after 22 days of treatment (relative to that of controls, data not shown).

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4-Nitroestrone 3-methyl ether proved not to be mutagenic in tests with Salmonella typhimurium TA98 and TA100 performed by Dr. Ching Wang, Department of Chemical Carcinogenesis, Michigan Cancer Foundation.
Table 2
Effect of treatments on rat body and organ weights and serum prolactin levels

Table 2 summarizes the data gathered concerning the effects of various treatments on body weights, organ weights, and systemic prolactin levels. It was the object of this experiment to examine (histologically and through weight measurements) numerous tissues at the time when each antitumor agent or procedure was having its effect on the mammary tumor. Such observations yield information regarding the systemic effects that each compound elicits which could relate to the observed tumor inhibition. Tamoxifen, 4-nitroestrone, and its 3-methyl ether, 70%; Adriamycin plus 5-fluorouracil, 155%; and 17β-estradiol 3-benzoate, 51%.

<table>
<thead>
<tr>
<th>Organ wt (mg)</th>
<th>Treatment</th>
<th>Body wt (g)</th>
<th>Uterus</th>
<th>Ovaries</th>
<th>Adrenals</th>
<th>Pituitary</th>
<th>Prolactin levels * (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>286 ± 26 0</td>
<td>612 ± 120</td>
<td>72 ± 11</td>
<td>77 ± 16</td>
<td>17 ± 3</td>
<td>139 ± 26</td>
</tr>
<tr>
<td></td>
<td>Ovariectomy</td>
<td>295 ± 20</td>
<td>226 ± 65</td>
<td>60 ± 12</td>
<td>15 ± 1</td>
<td>47 ± 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-Nitroestrone 3-methyl ether</td>
<td>295 ± 52</td>
<td>850 ± 27</td>
<td>32 ± 17</td>
<td>71 ± 22</td>
<td>17 ± 3</td>
<td>220 ± 150</td>
</tr>
<tr>
<td></td>
<td>4-Nitroestrone</td>
<td>288 ± 15</td>
<td>850 ± 27</td>
<td>34 ± 17</td>
<td>62 ± 12</td>
<td>16 ± 2</td>
<td>108 ± 73</td>
</tr>
<tr>
<td></td>
<td>Tamoxifen</td>
<td>262 ± 25</td>
<td>327 ± 44</td>
<td>32 ± 16</td>
<td>58 ± 10</td>
<td>13 ± 2</td>
<td>155 ± 67</td>
</tr>
<tr>
<td></td>
<td>Tamoxifen + 4-nitroestrone 3-methyl ether</td>
<td>270 ± 17</td>
<td>398 ± 42</td>
<td>30 ± 5</td>
<td>62 ± 9</td>
<td>14 ± 1</td>
<td>194 ± 89</td>
</tr>
<tr>
<td></td>
<td>Adriamycin + 5-fluorouracil</td>
<td>271 ± 13</td>
<td>590 ± 206</td>
<td>75 ± 9</td>
<td>68 ± 7</td>
<td>17 ± 2</td>
<td>86 ± 77</td>
</tr>
<tr>
<td></td>
<td>Estradiol-benzoate</td>
<td>272 ± 24</td>
<td>1090 ± 764</td>
<td>73 ± 37</td>
<td>53 ± 7</td>
<td>36 ± 8</td>
<td>535 ± 206</td>
</tr>
</tbody>
</table>

* We are grateful to Dr. R. Gale, Department of Physiology, Wayne State University School of Medicine, Detroit, Mich., for performing the radioimmunoassays on blood serum prolactin. The procedure followed the double antibody technique using antiserum prolactin from pituitary culture media and NIH standards for the standard curve and iodination (17). Blood was obtained by heart puncture at 10 a.m. just before sacrifice. Uteri from control rats showed that blood was removed throughout the estrous cycle in the various animals.

Table 3
Histopathological characteristics of tumors in various treatment groups

Table 3 shows the histopathological characteristics of tumors in various treatment groups. There were 10 rats/group except for the 9 rats which were ovariectomized. Mammary tumors induced in rats by DMBA have been shown to consist of 2 histopathological groups, adenocarcinomas and fibroadenomas (14). Normally, the untreated mammary tumors in the experiments reported herein were made up of no more than 12% fibroadenomas with the remainder being the malignant adenocarcinomas. Mammary gland papillary adenocarcinomas induced in this study were classic and consisted of ill-defined masses of discrete areas of anaplastic cells. These masses infiltrated the entire mammary nodule and therefore were not distinguishable as either lobular or ductal carcinomas. When fibroadenomas were produced, they took on the appearance of lactating adenoma (23).

For the most part, the treatments listed in Table 2 displayed a wide range of efficiency in bringing about the disappearance of adenocarcinomas while having little effect on the fibroadenomas (Table 3). A significant variance to this pattern of response is shown by those rats treated with 4-nitroestrone 3-methyl ether. In the latter case, the disappearances of adenocarcinomas was accompanied by an increase in fibroadenomas. After 4 weeks of treatment, there had been a 56% decrease in total tumors; however, of the remaining tumors, only 21% were adenocarcino-
nomas. A similar experience was documented for those tumors treated with 4-nitroestrone 3-methyl ether reported in Table 1.

Combined Effect of 4-Nitroestrone 3-Methyl Ether with Antiestrogens on Mammary Tumor Growth. Chart 4 summarizes the data on the effects of 4-nitroestrone 3-methyl ether and tamoxifen treatments. When administered at a previously tested level, 200 μg/day (8, 16), tamoxifen reduced tumor number to 54% of that of control (105% of initial tumor number) after 22 days. However, there was no improvement evident when this level of the antiestrogen was combined with the estrone analogue. Similar effects were seen in rats treated with nafoxidine (200 g/day) alone or in combination with 4-nitroestrone 3-methyl ether (data not shown). The administration of 4-nitroestrone 3-methyl ether again resulted in effective tumor inhibition (34% of control or 65% of initial tumor number in 27 days). These data demonstrate that the nitroestrone ether (when administered at a daily level of 24 mg/kg) is an antitumor agent with effects comparable to those of tamoxifen (0.8 mg/kg) in the DMBA-induced mammary tumor system. The effect of combined therapy with these 2 agents was not additive.

Administration of Antitumor Agents Prior to Mammary Tumor Development. Treatment initiated 6 days following DMBA intubation had a dramatic effect on subsequent tumor number (Chart 5). This regimen, which involved only 20 days of injections, was terminated before the expected appearance of tumors (approximately 41 days) and gave results similar to those reported for a similar regimen using tamoxifen (15). The few mammary tumors that did appear were delayed until Day 96. Administration of 4-nitroestrone 3-methyl ether for 25 days after tumors had become established in all rats (Day 92, protocol similar to that of the previous experiments) resulted in a decrease of the number of tumors per rat to a level comparable to that remaining following the earlier initiation of treatment [6 days following DMBA intubation, 0.75 versus 0.35 tumor per rat (Chart 5A)]. The estrogen analogue appears to be equally effective on both the precancerous cells and the established tumor.

Chart 4. Effect of 4-nitroestrone 3-methyl ether and tamoxifen treatments on mammary tumor appearance in rats. Tumors were induced with DMBA as described in “Materials and Methods.” Each group contained 10 animals. Injections were performed with the estrogen analogue distributed in SSV or tamoxifen dissolved in sesame oil. x, control; ○, 4-nitroestrone 3-methyl ether, 24 mg/kg body weight; △, tamoxifen, 0.8 mg/kg; ▲, tamoxifen, 0.8 mg/kg plus 4-nitroestrone 3-methyl ether, 24 mg/kg. After 22 days of treatment with 4-nitroestrone 3-methyl ether, the tumor number differed from that of controls (p < 0.05).

Tsai and Katzenellenbogen (32) have shown antiestrogens to be effective when injected into rats for 10 days prior to the intubation of DMBA. Like tamoxifen, 4-nitroestrone 3-methyl ether was quite effective in decreasing the subsequent tumor number when administered before the carcinogen (Chart 5B). The mechanism of this precarcinogen antitumor effect is unknown at the present time.

Effect of 4-Nitroestrone 3-Methyl Ether on Ovarian Hormone-independent Rat Mammary Tumors. Following ovariec-
tomy, most DMBA-induced tumors regress immediately. How-
ever, after 3 weeks, certain tumors will still be present, and “new” tumors will appear. These neoplasms will continue to grow in the absence of ovarian hormones. To test the effect of 4-nitroestrone 3-methyl ether on the ovarian hormone-independent mammary cancer, tumor-bearing rats were given injections starting on the day following ovariectomy, and these treatments continued for the duration of the experiment. The plot of tumor number (Chart 6) shows the resurgence of mammary tumors in the ovariec-tomized rats 4 weeks following the operation. This regrowth of tumors was eliminated by tamoxifen and by 4-nitroestrone 3-methyl ether. The same experiment also contained unoperated tumor-bearing rats and intact rats treated with the estrogen analogue. Among all groups, the combination of ovar-

Antitumor Activity of Nitroestrone Methyl Ether

Chart 5. Effect of time of 4-nitroestrone 3-methyl ether or tamoxifen treatment relative to DMBA intubation on mammary tumor growth in Sprague-Dawley rats. Animals were intubated with DMBA at 51 days of age. There were 12 rats in each group. Rats were given injections (s.c. 24 mg/kg with 4-nitroestrone 3-methyl ether or 0.8 mg/kg with tamoxifen) according to the following schedule: A, injection of SSV only (x); 20 injections of 4-nitroestrone 3-methyl ether beginning 6 days following DMBA intubation (○); 25 injections of 4-nitroestrone 3-methyl ether beginning 92 days following DMBA intubation (□). B, injection of sesame oil only (x); 10 injections with 4-nitroestrone 3-methyl ether 10 days prior to DMBA intubation (○); 10 injections with tamoxifen 10 days prior to DMBA intubation (△); 20 injections of 4-nitroestrone 3-methyl ether beginning 6 days following DMBA intubation (□); 20 injections of tamoxifen beginning 6 days following DMBA intubation (◇).
the reappearance of mammary tumors in ovariectomized rats appear during the treatment with the nitroestrone. Interestingly, tumors were evident (0.35/rat). New mammary tumors did not appeared when the antitumor agent was administered before tumors (1.7/rat) remaining (0.75/rat) approached the few which administered long enough (50 days), the number of preformed were allowed to grow for 20 days regressed after 4-nitroestrone carried out varying the time (relative to DMBA intubation) of days without deleterious effects to the host.

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When administered for 10 days prior to DMBA intubation or when treatment with the nitroestrone ether. Like tamoxifen, this compound was effective in prohibiting mammary tumor formation in ovariectomized rats. Each group had 12 animals. Injection (data not presented). The compound proved to be throughout body tissues and in the excreta within 8 hr of s.c. shown significant quantities of radioactivity to be distributed to be most effective as an antitumor agent.

In these investigations, 4-nitroestrone 3-methyl ether was administered to rats via daily s.c. injection of a suspension in SSV. Experiments with the tributated estrogen analogue have shown significant quantities of radioactivity to be distributed throughout body tissues and in the excreta within 8 hr of s.c. injection (data not presented). The compound proved to be relatively innocuous, although injection of 96 mg/kg body weight did result in extreme weight loss. However, the most active antitumor level (24 mg/kg) could be injected for as many as 50 days without deleterious effects to the host.

Keeping the route of administration constant, studies were carried out varying the time (relative to DMBA intubation) of treatment with the nitroestrone ether. Like tamoxifen, this compound was effective in prohibiting mammary tumor formation when administered for 10 days prior to DMBA intubation or when injected for 20 days just following DMBA (15). Tumors which were allowed to grow for 20 days regressed after 4-nitroestrone 3-methyl ether injection commenced. In fact, if the ether was administered long enough (50 days), the number of preformed tumors (1.7/rat) remaining (0.75/rat) approached the few which appeared when the antitumor agent was administered before tumors were evident (0.35/rat). New mammary tumors did not appear during the treatment with the nitroestrone. Interestingly, the reappearance of mammary tumors in ovariectomized rats was also prevented by this estrone analogue, suggesting that 4-nitroestrone 3-methyl ether was effective against tumors which grew in animals free of ovarian steroids. These effects of the nitroestrone ether were quite similar to those observed after the administration of tamoxifen (0.8 mg/kg).

Systemically, this nitrated estrone brought about focal atrophy within the pituitary and ovaries while causing a moderate hyper-trophy of the uterus. Tamoxifen, on the other hand, produced atrophy of all 3 of the same organs. Neither of these compounds, however, affected the plasma prolactin levels. Unlike tamoxifen, 4-nitroestrone 3-methyl ether appears to possess estrogenic properties at the levels administered. The 24-mg/kg daily dose of the estrone analogue, nevertheless, was much less estrogenic than was 17β-estradiol 3-benzoate (1.6 mg/kg) (as judged by the resulting uterine vascularization and plasma prolactin levels).

Overall, it would appear from the data in Charts 1 and 3 that the administration of 4-nitroestrone 3-methyl ether to rats bearing DMBA-induced mammary tumors resulted in cessation of the appearance of new tumors, while those present continued to grow, although at a reduced rate. What has actually occurred can be determined only by segregating the tumors into the growth-related categories shown by the untreated mammary tumors in Chart 2. This experimental model is composed of neoplasms which grow continuously throughout the period of observation (84%). However, it is also possible to see spontaneous regression among certain numbers of these control mammary tumors (16%). Even ovariectomy, which is the most successful treatment for this model of hormone-dependent cancer, is unable to halt the growth of some (15%) of these cancers (Chart 2). The data in Chart 2 also demonstrate that, whereas 29% of the tumors continue to grow at nearly the rate of actively growing control neoplasms, daily injections of the nitroestrone ether caused 71% of the mammary cancers to regress to an extent (initial tumor volume/final tumor volume) comparable to that brought about by ovariectomy. The A-ring-substituted estrone is, indeed, growth inhibitory to certain DMBA-induced mammary tumors in Sprague-Dawley rats.

Unique to the action of the methyl ether of 4-nitroestrone on this neoplasm is the destruction of adenocarcinomas while permitting the appearance of fibroadenomas. These benign tumors made up approximately 12% of the control cancers and usually approximately 2 to 6 tumors in the untreated animals. While the number of fibroadenomas remains relatively constant during treatment with the other tested antitumor compounds, the histopathologically characterized fibroadenomas actually increased in those animals treated with 4-nitroestrone 3-methyl ether. The data in Table 3 show that only 20% of the tumors remaining after 22 injections of the methyl ether are malignant. It is not known whether the formation of these fibroadenomas is promoted by 4-nitroestrone 3-methyl ether or if they arise from adenocarcinomas. No other antitumor agent tested eliminated adenocarcinomas of the mammary gland so effectively.

In conclusion, a specific inhibitor of estrogen sulfotransferase, 4-nitroestrone 3-methyl ether, has been shown to be an active inhibitor of certain DMBA-induced rat mammary tumors. At levels which produced maximum inhibition (24 mg/kg), this nontoxic agent was more active than was tamoxifen (0.8 mg/kg) in inhibiting the growth of adenocarcinomas and, unlike other hormonal or chemotherapeutic agents, 4-nitroestrone 3-methyl ether promoted the appearance of fibroadenomas.
REFERENCES

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