Inhibition of Rat Mammary Gland Chemical Carcinogenesis by Dietary Dehydroepiandrosterone or a Fluorinated Analogue of Dehydroepiandrosterone

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ABSTRACT

The chemopreventive efficacy of p.o. administered dehydroepiandrosterone (DHEA), DHEA plus N-(4-hydroxyphenyl)retinamide (4-HPR), or 16α-fluoro-5-androsten-17-one (DHEA analogue 8354) was examined in rats treated with N-methyl-N-nitrosourea (MNU; 50 mg/kg body weight, i.v.) at 50 days of age. Semipurified diet (AIN-76A) containing each steroid alone, or DHEA plus 4-HPR, was administered during initiation (−1 week to +1 week post-MNU), promotion/progression (+1 week post-MNU to termination), or both phases (−1 week post-MNU to termination), or both phases (−1 week post-MNU to termination) of the carcinogenic process. Neither DHEA nor DHEA analogue 8354 (0.2%, w/w) significantly affected the initiation of mammary cancer when administered alone; however, DHEA (0.2%, w/w) plus 4-HPR (1 mmol/kg diet) significantly reduced cancer multiplicity (26%) when given during initiation. All three treatments were strongly effective when given during promotion/progression, significantly reducing mammary cancer multiplicity by 77% (DHEA), 84% (DHEA/4-HPR), and 66% (DHEA analogue 8354), relative to carcinogen controls. Cancer incidence was significantly inhibited by DHEA (33% inhibition) and DHEA/4-HPR (24% reduction) during promotion/progression. However, the most effective chemopreventive treatment encompassed both phases of carcinogenesis. Thus, under these conditions, DHEA (0.2% or 0.1%, w/w) reduced cancer incidence (52% and 32% reductions, respectively) and multiplicity (91% and 86% reductions, respectively). Further reduction in mammary cancer incidence was observed in animals that received DHEA (both doses) plus 4-HPR (1 and 0.5 mmol/kg diet, respectively). DHEA analogue 8354 (0.2% or 0.1%, w/w) given for the duration of the study reduced only cancer multiplicity (61% and 56% reductions, respectively). Tumor-related mortality was significantly lower in rats that received long-term treatment with DHEA or DHEA/4-HPR, when compared with carcinogen controls. Except for a slight, but significant, postcancerigenic decrease in the mean body weights of rats treated concomitantly with DHEA (plus or minus 4-HPR) and MNU, additional gross manifestations of steroid-induced toxicity were not observed.

INTRODUCTION

DHEA is an abundantly secreted adrenal steroid that is an intermediate in the biosynthesis of other hormones, including testosterone and estradiol-17β (1). Although a physiological role for DHEA has yet to be defined, a growing body of evidence, both epidemiological and experimental, suggests a strong inverse relationship between alteration in the serum levels or excretion of this steroid or its metabolites and a number of disease syndromes including cancer (2).

In women, plasma concentrations of DHEA (present almost exclusively as the sulfate conjugate, DHEA sulfate) show a continual decline from peak levels that occur in the second decade of life (3, 4). Conversely, the incidence of breast cancer increases with age (5). An early report by Bulbrook et al. (6) showed that preoperative urinary concentrations of 11-deoxy-17-ketosteroids were subnormal in women afflicted with primary breast cancer, and suggested that this alteration may be a predictor of risk for the subsequent appearance of the disease. Subsequent to that report, a prospective study showed a definite inverse correlation between the urinary excretion of etiocholanolone and androsterone, the two main urinary metabolites of adrenal androgens, and the development of breast cancer in women (7). More recently, it was shown that 24-h mean plasma levels of DHEA and DHEA sulfate were subnormal in women with premenopausal breast cancer, whereas postmenopausal patients had supranormal plasma levels of these compounds (8), thus providing a potential biochemical marker for the dichotomization of this disease relative to menopausal status (5).

The in vivo relevance of DHEA to the genesis of neoplastic disease has been experimentally explored in a number of animal models. Schwartz (9) has shown that p.o. administration of DHEA inhibited the appearance of spontaneous mammary cancer in female C3H(A'°) mice and reduced the incidence and multiplicity of lung tumors induced in A/J mice by either DMBA or urethan (10). In CD-1 mice, topical application of either DHEA or the synthetic derivative 3β-methylandrost-5-en-17-one significantly reduced the number and incidence of DMBA-induced skin papillomas and carcinomas (11). Dietary administration of DHEA also resulted in significant reductions in the rate of appearance and frequency of all stages (atypical hyperplasia, carcinoma in situ, invasive carcinoma) of colonic lesions induced in female BALB/c mice with 1,2-dimethylhydrazine (12). Finally, postcancerigenic dietary administration of DHEA significantly inhibited the development of thyroid tumors and the frequency of putative neoplastic precursor lesions in the livers of male F344 rats that were treated with dihydroxy-di-n-propylnitrosamine (13).

The results described above indicate that DHEA has significant chemopreventive efficacy against experimental carcinogenesis at a diverse group of target sites. However, with the exception of breast cancer, there is no clear precedent relating specific cancer risk in humans to alterations in circulating levels of this steroid or the production and excretion of its metabolites (2). For this reason, and given the lack of previous experimental data regarding chemopreventive efficacy of DHEA in mammary carcinogenesis, we have undertaken to explore the temporal specificity of DHEA as an inhibitor of MNU-induced mammary...
gland carcinogenesis in female rats. The potential clinical significance in using this breast cancer model is its similarity to the human disease in terms of histopathology (14) and response to hormonal manipulations (15–17). In addition, the use of MNU, a direct acting mammary gland carcinogen (18), to induce cancer effectively rules out complications related to DHEA-mediated effects on carcinogen metabolism and disposition (2, 10). Previous work demonstrated that the chemopreventive efficacy of retinoids (including 4-HPR) in experimental breast cancer is enhanced when they are combined with various hormonal treatment modalities (19, 20). The present study was designed to evaluate the potential steroid modulating effects of DHEA, administered alone and in combination with the synthetic retinoid 4-HPR in rat mammary carcinogenesis. Finally, the chemopreventive activity of a fluorinated derivative of DHEA, denoted DHEA analogue 8354 (21), was examined in a separate study. This analogue is a more potent inhibitor than DHEA of several biochemical processes considered central to carcinogenesis. The analogue apparently does not elicit the androgenic and estrogenic effects associated with the parent steroid (21, 22). Thus, comparison of these agents in chemoprevention studies may lead to insights as to the mechanism(s) by which these compounds suppress carcinogenesis.

MATERIALS AND METHODS

Experimental Animals. Virgin female Sprague-Dawley [Hsd: (SD) BR] rats were received from Harlan/Sprague-Dawley (Indianapolis, IN) at 28 days of age and maintained in isolation for 2 weeks. A total of 390 rats were used in the reported studies. Animals were housed in groups of 2 to 3 in polycarbonate cages containing hardwood bedding. Cage materials were replaced twice weekly. The animal rooms were illuminated for 12 h each day and maintained at a temperature of 22 ± 1°C (SE) and 50% relative humidity. Animals were allowed free access to food and water throughout each study.

Diets and Chemopreventive Agents. The basal diet for each study was modified AIN-76A semipurified diet (adjusted corn starch) TD 85449 (Teklad, Madison, WI). 4-HPR was obtained from Cilag AG, Schaffhausen, Switzerland. The required vehicle for 4-HPR consisted of 10% butylhydroquinone, 10% citric acid, 70% propylene glycol; Eastman (Bristol, NY). DHEA was purchased from Sigma Chemical Co. (St. Louis, MO). The DHEA analogue 8354 was a gift from Fort Washington Resources (Hatboro, PA). Its synthesis and chemical properties were described previously (21). Each batch of diet was prepared weekly (high doses) or every 3 weeks (low doses), as more animals received the high-dose diets, and stored at −20°C. Fresh batches of diets supplemented with steroid plus retinoid were also begun at this time according to 1 of 3 schedules, to encompass the periods of initiation (1 week before through 1 week after MNU treatment, i.e., −1 week to +1 week), promotion/progression (1 week after MNU treatment, continued to the end of the study, i.e., +1 week to the end), or both (1 week before MNU treatment, continued to the end of the study, i.e., −1 week to the end). As determined in preliminary feeding studies, DHEA was well tolerated when fed alone at 0.2% or 0.1% (w/w) of diet (6.93 and 3.47 mmol/kg diet, respectively); combination diets contained the same doses of DHEA plus 4-HPR at 1 or 0.5 mmol (391 or 195.5 mg/kg diet, respectively).

At 50 days of age, all carcinogen-treated rats received a single i.v. injection of freshly prepared MNU solution (50 mg/kg body weight) via the jugular vein as described previously (18). Crystalline MNU (Ash-Stevens, Detroit, MI) was dissolved to a concentration of 12.5 mg/ml in 0.85% NaCl solution acidified to pH ~5.0 with glacial acetic acid. Control animals received an i.v. injection of the NaCl solution only.

Commencing 4 weeks after receiving MNU, animals were palpated weekly to monitor mammary tumor appearance. The date of appearance and location of every palpable tumor were recorded. The body weights of all rats were recorded once a week for the duration of each study. All rats were observed twice daily for any indications of agent-induced toxicity. At no time during the experiments were the estrous cycles of any rats monitored.

After 180 days of chemopreventive treatment, surviving rats in all groups were sacrificed by CO2 asphyxiation. During each study, animals that appeared moribund were killed by CO2 asphyxiation. All rats that were killed or found dead were promptly given thorough postmortem examination. Mammary tumors were coded by location, removed, measured, and weighed. All tumors and any other grossly abnormal-appearing tissues were removed and fixed in 10% buffered formalin. Sections were stained with hematoxylin and eosin and classified histopathologically. Mammary tumor pathology was defined according to the criteria of Young and Hallowes (24).

DHEA Analogue 8354 Chemoprevention Study. One hundred thirty rats were randomized by weight into 7 groups at 43 days of age. Each group that received MNU (5 total) consisted of 20 animals; in addition, a control group that received steroid-supplemented diet and carcinogen vehicle also contained 20 rats. Another vehicle-treated group that received AIN-76A basal diet contained 10 animals. Dose schedules for chemopreventive treatment were identical to those described above for the DHEA/4-HPR combination study. The DHEA analogue 8354 was fed to treated rats at 0.2% or 0.1% (w/w) of diet (6.89 and 3.44 mmol/kg diet, respectively). All procedures and observations were performed as described above for the DHEA/4-HPR combination study.

Statistical Analysis. Tumor incidence curves were generated by the life table method and compared by log rank analysis (25). The statistical significance of differences between mean tumor multiplicities was assessed using one-way ANOVA. Individual tumor numbers from the DHEA/4-HPR combination study were transformed by the square root method to normalize the data before comparison by ANOVA (26). Differences in group total mean body weights at termination of the DHEA analogue 8354 study were tested for statistical significance by ANOVA, using untransformed individual weights. Due to significant unresolved heterogeneity among the group variances, total mean body weight was compared by ANOVA.

DHEA/4-HPR Combination Chemoprevention Study. Two hundred sixty rats were randomized by weight into 12 groups at 43 days of age. With the exception of the carcinogen control group, which received a diet containing 4-HPR vehicle (30 rats), the other carcinogen-treated experimental groups (8 additional) each contained 25 animals. Groups (3 total) that received MNU vehicle (0.85% NaCl solution) consisted of 10 animals each. Provisions of diets supplemented with steroid plus retinoid were made at this time according to 1 of 3 schedules, to encompass the periods of initiation (1 week before through 1 week after MNU treatment, i.e., −1 week to +1 week), promotion/progression (1 week after MNU treatment, continued to the end of the study, i.e., +1 week to the end), or both (1 week before MNU treatment, continued to the end of the study, i.e., −1 week to the end). As determined in preliminary feeding studies, DHEA was well tolerated when fed alone at 0.2% or 0.1% (w/w) of diet (6.93 and 3.47 mmol/kg diet, respectively); combination diets contained the same doses of DHEA plus 4-HPR at 1 or 0.5 mmol (391 or 195.5 mg/kg diet, respectively).

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\[4 \text{T. A. Hultin, unpublished observations.}\]
weights for the DHEA/4-HPR combination study were compared using (as appropriate) a t test for means with significant heterogeneity of variances, as suggested by Sokal and Rohlf (26). In the latter case, significance was attributed to \( P < 0.01 \). As shown in the “Results,” we also have calculated a NCW for each animal that survived to the end of each study. The NCW represents the total (gross) terminal weight of each tumor-bearing rat minus the total weight of tumor tissue (i.e., the tumor burden). This parameter (NCW) was also evaluated by ANOVA as described above to detect differences between the various means. An appropriate discrimination test for making unplanned comparisons (least significant difference method) was used to define statistically significant intergroup differences in means (26). Differences in percentage survival and cancer incidences were tested for significance by Fisher's exact test (2-tailed). In all cases, with the exception of multiple \( t \) tests, statistical significance was ascribed to a comparison only when a \( P < 0.05 \) was attained.

**RESULTS**

**DHEA/4-HPR Combination Study.** The chemopreventive activity of DHEA alone, and DHEA plus 4-HPR, were examined in a combined study using common MNU and MNU vehicle controls. However, to facilitate their presentation, the data have been separated into two Tables, with the common controls shown in each.

The data summarized in Table 1 indicate that dietary DHEA significantly inhibited MNU-induced tumorigenesis in female rats when given during the +1 week to end schedule. Thus, rats fed DHEA at 0.2\% (w/w) during this period developed cancer at an incidence of 67\% and mean (±SEM) multiplicity of 2.3 ± 0.6 cancers/rat, representing significant (\( P < 0.05 \)) reductions in these parameters relative to the controls. In contrast, treatment with 0.2% DHEA during the −1 week to +1 week period had no significant effect on mammary cancer incidence (100\%) or mean (±SEM) multiplicity (8.8 ± 1.0 cancers/rat) when compared with controls that received a diet containing 4-HPR vehicle only. This control group had a cancer incidence of 100\% and mean (±SEM) multiplicity of 10.1 ± 0.8 cancers/rat.

Table 1

| No. of rats | MNU | DHEA (% diet, w/w) | Cancer incidence (%) | Cancers/rat\(^a\) | Tumors/rat\(^a\) | Terminal survival (%) | Terminal total body wt (g)* | Terminal NCW (g)* \\
<table>
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</thead>
<tbody>
<tr>
<td>24</td>
<td>+</td>
<td>0.2%</td>
<td>100*</td>
<td>8.8 ± 1.0*</td>
<td>9.1 ± 0.9*</td>
<td>63*</td>
<td>269 ± 11*</td>
<td>254 ± 9*</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>0.2%</td>
<td>67**</td>
<td>2.3 ± 0.6**</td>
<td>2.4 ± 0.6**</td>
<td>80</td>
<td>265 ± 6*</td>
<td>261 ± 6*</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>0.2%</td>
<td>48**</td>
<td>0.9 ± 0.2**</td>
<td>1.0 ± 0.2**</td>
<td>96*</td>
<td>255 ± 5*</td>
<td>251 ± 5*</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>0.1%</td>
<td>68*</td>
<td>1.4 ± 0.2*</td>
<td>1.4 ± 0.2*</td>
<td>92*</td>
<td>261 ± 5*</td>
<td>257 ± 5*</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
<td>—</td>
<td>100*</td>
<td>10.1 ± 0.8*</td>
<td>10.7 ± 0.8*</td>
<td>57*</td>
<td>287 ± 12*</td>
<td>245 ± 10*</td>
</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>0.2%</td>
<td>100*</td>
<td>10.7 ± 0.8*</td>
<td>10.7 ± 0.8*</td>
<td>100</td>
<td>265 ± 7*</td>
<td>265 ± 7*</td>
</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>—</td>
<td>100*</td>
<td>10.7 ± 0.8*</td>
<td>10.7 ± 0.8*</td>
<td>100</td>
<td>294 ± 12*</td>
<td>294 ± 12*</td>
</tr>
</tbody>
</table>

\(^a\) Group mean average ± SEM.

\(^b\) Includes all histologically confirmed mammary carcinomas found at necropsy.

\(^c\) Includes all palpable tumors.

\(^d\) NCW represents terminal weight of tumor-bearing rat minus weight of excised tumors.

\(^e\) Diet given from —1 week post-MNU to end of study.

\(^f\) Diet given from —1 week post-MNU to —1 week pre-MNU.

\(^g\) Diet contained 4-HPR vehicle (see text for details).

\(^h\) Diet given from —1 week pre-MNU or vehicle to end of study.

\(^i\) P < 0.05 versus respective control group.

The survival data in Tables 1 and 2 show that 57\% of the carcinogen control rats lived until the end of the experiment (187 days); survival of rats that received DHEA alone ranged from 63\% (—1 week to +1 week) to 96\% (—1 week to end, 0.2\%) (Table 1). As shown in Table 2, survival of carcinogen-treated rats that received DHEA plus 4-HPR from −1 week to +1 week was significantly lower than that of the carcinogen controls. Finally, comparison of the data in Tables 1 and 2 indicates that combined treatment with DHEA plus 4-HPR during the −1 week to +1 week period was significantly more effective in reducing cancer multiplicity and incidence than was treatment with the steroid alone. When administered alone to rats at 1 mmol/kg AIN-76A diet, 4-HPR has no effect on MNU-induced tumorigenesis.

The survival data in Tables 1 and 2 show that 57% of the carcinogen control rats lived until the end of the experiment (187 days); survival of rats that received DHEA alone ranged from 63\% (—1 week to +1 week) to 96\% (—1 week to end, 0.2\%) (Table 1). As shown in Table 2, survival of carcinogen-treated rats that received DHEA plus 4-HPR from −1 week to +1 week ranged from 72–76\% diet, 4-HPR has no effect on MNU-induced tumorigenesis.

\(^*\) T. A. Ratko, C. J. Detrisac, R. G. Mehta, G. J. Kellof, and R. C. Moon, unpublished observations.
Table 2 Effect of dietary DHEA plus 4-HPR on MNU-induced mammary gland carcinogenesis in female Sprague-Dawley rats

Virgin, female Sprague-Dawley rats (50 days old) received single injections of MNU (50 mg/kg body weight) dissolved in a vehicle of 0.85% NaCl solution (pH ~5.0), or vehicle alone. Administration of AIN-76A semipurified diet (basal diet) supplemented with 4-HPR vehicle or DHEA plus 4-HPR at the indicated doses was begun as described below. The study was terminated 180 days after rats received MNU or NaCl solution. Different numbers of asterisks in each column of data denote statistically significant intergroup differences.

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>MNU (w/w)</th>
<th>DHEA (mMol/kg diet)</th>
<th>4-HPR (mMol/kg diet)</th>
<th>Cancer incidence (%)</th>
<th>Cancers/rat</th>
<th>Terminal survival (%)</th>
<th>Terminal total body wt. (g)</th>
<th>Terminal NCW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 (+)</td>
<td>0.2%</td>
<td>1.0</td>
<td>100*</td>
<td>7.5 ± 0.6**</td>
<td>7.8 ± 0.6***</td>
<td>72***</td>
<td>278 ± 8</td>
<td>255 ± 6</td>
</tr>
<tr>
<td>25 (+)</td>
<td>0.2%</td>
<td>1.0</td>
<td>76**</td>
<td>1.6 ± 0.3**</td>
<td>1.7 ± 0.3**</td>
<td>92**</td>
<td>264 ± 8</td>
<td>267 ± 4</td>
</tr>
<tr>
<td>25 (+)</td>
<td>0.2%</td>
<td>1.0</td>
<td>29***</td>
<td>0.7 ± 0.3**</td>
<td>0.7 ± 0.3**</td>
<td>92***</td>
<td>258 ± 8</td>
<td>257 ± 4</td>
</tr>
<tr>
<td>25 (+)</td>
<td>0.1%</td>
<td>0.5</td>
<td>36**</td>
<td>0.6 ± 0.2**</td>
<td>0.6 ± 0.2**</td>
<td>100**</td>
<td>263 ± 6</td>
<td>262 ± 6</td>
</tr>
<tr>
<td>30 (+)</td>
<td>0.2%</td>
<td>1.0</td>
<td>100*</td>
<td>10.1 ± 0.8*</td>
<td>10.7 ± 0.8*</td>
<td>57*</td>
<td>287 ± 12*</td>
<td>245 ± 10</td>
</tr>
<tr>
<td>10 Vehicle</td>
<td>0.2%</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>275 ± 6</td>
<td>275 ± 6</td>
</tr>
<tr>
<td>10 Vehicle</td>
<td>0.2%</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td>294 ± 12</td>
<td>294 ± 12</td>
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</tr>
</tbody>
</table>

* Group mean average ± SEM
* Includes all histologically confirmed mammary carcinomas found at necropsy.
* Includes all palpable tumors.
° NCW represents terminal weight of tumor-bearing rat minus weight of excised tumors.
* Diet given from —1 week to +1 week post-MNU.
* Diet given from —1 week post-MNU to end of study.
* Includes all palpable tumors.
* Diet given from —1 week to +1 week post-MNU.

Discussion

The data herein reported are, to our knowledge, the first to show significant inhibitory activity of the adrenal-derived steroid DHEA, or its fluorinated analogue 8354, toward the induction of mammary carcinomas in the female rat. In addition, we have shown a positive chemopreventive interaction between DHEA, and the synthetic retinoid, 4-HPR, when they were concomitantly administered to rats via the diet. The results further indicate that DHEA, or its analogue 8354, are primarily active against the promotion/progression phase of MNU-induced mammary carcinogenesis. This relationship can be easily deduced by comparing the tumor data of the appropriate carcinogen controls with those obtained in the 3 groups that received chemopreventive treatment via different feeding protocols.

A major problem in validly interpreting the results of any cancer chemoprevention study is agent-induced toxicity in the experimental animals. Among any number of toxic manifestations that may accompany treatment with various agents, weight loss, or inhibition of the rate of weight gain relative to untreated controls, particularly as a function of reduced caloric intake, can be especially confounding (27). For example, the growth and development of DMBA-induced mammary tumors in rats can be modulated by restriction of caloric intake during the presumed period of initiation, and for a short period thereafter (28), possibly as a result of alteration in serum levels of...
Table 3 Effect of dietary DHEA analogue 8354 on MNU-induced mammary gland carcinogenesis in female Sprague-Dawley rats

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>MNU</th>
<th>DHEA 8354 (g/kg diet)</th>
<th>Cancer incidence (%)</th>
<th>Cancers/rat ( \pm ) SEM</th>
<th>Tumors/rat ( \pm ) SEM</th>
<th>Terminal survival (%)</th>
<th>Terminal total body wt (g) ( \pm ) SEM</th>
<th>Terminal NCW (g) ( \pm ) SEM</th>
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<tr>
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<td>11.2 ( \pm ) 1.0 *</td>
<td>11.5 ( \pm ) 1.0 *</td>
<td>65</td>
<td>276 ( \pm ) 7</td>
<td>258 ( \pm ) 7</td>
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<tr>
<td>20</td>
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<td>0.2 ( \times ) 95</td>
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<td>4.2 ( \pm ) 0.6 *</td>
<td>55</td>
<td>254 ( \pm ) 12</td>
<td>239 ( \pm ) 14</td>
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<tr>
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<td>0.2 ( \times ) 100</td>
<td>4.8 ( \pm ) 1.1 * *</td>
<td>4.8 ( \pm ) 1.0 *</td>
<td>80</td>
<td>272 ( \pm ) 5</td>
<td>259 ( \pm ) 6</td>
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<tr>
<td>20</td>
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<td>0.1 ( \times ) 100</td>
<td>5.4 ( \pm ) 0.8 * *</td>
<td>5.8 ( \pm ) 0.8 *</td>
<td>50</td>
<td>265 ( \pm ) 5</td>
<td>255 ( \pm ) 6</td>
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</tr>
<tr>
<td>20</td>
<td>+</td>
<td>0.2 ( \times ) 100</td>
<td>12.2 ( \pm ) 1.0 * *</td>
<td>12.6 ( \pm ) 0.9 *</td>
<td>70</td>
<td>266 ( \pm ) 7</td>
<td>240 ( \pm ) 6</td>
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<td>100 ( \pm ) 10 * *</td>
<td>100 ( \pm ) 5 *</td>
<td>266 ( \pm ) 5</td>
<td>266 ( \pm ) 5</td>
<td></td>
<td>305 ( \pm ) 10</td>
</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>0.2 ( \times ) 100</td>
<td>100 ( \pm ) 10 * *</td>
<td>100 ( \pm ) 5 *</td>
<td>305 ( \pm ) 10</td>
<td></td>
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</tr>
</tbody>
</table>

\* Group mean average \( \pm \) SEM
\*\* Includes all histologically confirmed mammary carcinomas found at necropsy.
\*\* Includes all palpable tumors.
\* NCW represents terminal weight of tumor-bearing rat minus weight of excised tumors.
\* Diet given from -1 week to +1 week post-MNU.
\*\* Diet given from -1 week post-MNU to end of study.
\*\* Diet given from -1 week post-MNU or vehicle to end of study.

Virgin, female Sprague-Dawley rats (50 days old) received single injections of MNU (50 mg/kg body weight) dissolved in a vehicle of 0.85% NaCl solution (pH ~5.0), or vehicle alone. Administration of AIN-76 semi-purified diet (basal diet) supplemented with DHEA analogue 8354 at the indicated doses was begun as described below. The study was terminated 180 days after rats received MNU or NaCl solution. Different numbers of animals in each column denote statistically significant intergroup differences.

Table 3 shows the effect of dietary DHEA analogue 8354 on MNU-induced mammary gland carcinogenesis in female Sprague-Dawley rats. The table presents the number of rats, MNU dosages, DHEA 8354 dosages, cancer incidence, cancers per rat, tumors per rat, terminal survival, terminal total body weight, and terminal necropsy weight. The results indicate that the combination of DHEA and MNU significantly reduced the incidence and multiplicity of mammary tumors in rats compared to the control group. The data suggest that DHEA may have a chemopreventive effect on mammary gland carcinogenesis.

Prolactin and gonadotropins (29). In the present study, we have no data showing an effect of dietary DHEA on food consumption, therefore, it is not possible to identify alteration of caloric intake as a specific factor that could be causally related to the early transient weight loss observed in DHEA-treated rats immediately after their exposure to MNU (Fig. 1). Since transient weight loss did not occur in those groups that received DHEA and MNU vehicle, it appears that toxicity of the steroid per se was not a sole factor in that phenomenon.

Interestingly, a similar effect was documented by Schwartz et al. (30) in early transient weight loss observed in DHEA-treated rats given single injections of MNU (50 mg/kg body weight) dissolved in a vehicle of 0.85% NaCl solution (pH ~5.0), or vehicle alone. Administration of AIN-76 semi-purified diet (basal diet) supplemented with DHEA analogue 8354 at the indicated doses was begun as described below. The study was terminated 180 days after rats received MNU or NaCl solution. Different numbers of animals in each column denote statistically significant intergroup differences.
Chemical modification of the basic DHEA structure has been used as a strategy by other investigators to reduce the incidence and severity of side effects engendered in experimental animals by the parent hormone. Schwartz et al. (21) recently showed that contrary to the effects seen with DHEA, the DHEA 8354 analogue is nonestrogenic in a rat uterine weight assay, and nonandrogenic when assayed using the castrated rat seminal vesicle model. This analogue is also more potent than the parent steroid in suppressing TPA-induced increases in epidermal DNA synthesis, superoxide formation, and prostaglandin E2 synthesis (22). Our findings demonstrate no increased efficacy of the 8354 analogue over DHEA in the promotion and progression phases of mammary carcinogenesis. This suggests that the previously measured parameters that differentiate these compounds in vivo are not responsible for these observed chemopreventive effects. The similarity of the data for DHEA and the DHEA 8354 analogue in the MNU rat mammary model system thus may be related to the effects on G6PDH activity, which are similar for both compounds (21). The effects of chronic p.o. administration of DHEA or DHEA analogue 8354 on elements of rat reproductive physiology are presently unknown. A study to evaluate blood steroid hormone levels during such chronic conditions is presently underway. Such data will provide additional avenues for exploration of the chemopreventive mechanism of action of these compounds in this model of human breast cancer.

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Inhibition of Rat Mammary Gland Chemical Carcinogenesis by Dietary Dehydroepiandrosterone or a Fluorinated Analogue of Dehydroepiandrosterone

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