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/promotion (~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/promotion, 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/promotion. 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, postcarcinogen 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

DHEA1 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(Aβ) 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, postcarcinogen 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
DEHYDROEPIANDROSTERONE ANTICARCINOGENESIS

gland carcinogenesis in female rats. The potential clinical sig-
ificance 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 disposi-
tion (2, 10). Previous work demonstrated that the chemopre-
ventive 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 syn-
thetic 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 chemo-
prevention 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.

Diet 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, Schaff-
house, Switzerland. The required vehicle for 4-HPR consisted of 12.5
g/mL of ethanol, 36.9 g of corn oil, and 0.6 g of Tenox 20 (20% tertiary
ammonium pyrophosphate) (Teklad, Madison, WI) per kg diet. Control animals (carcinogen
vehicle groups) in the study with DHEA received a
basal diet containing the 4-HPR vehicle. 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). Fresh batches
of diet were prepared weekly (high doses) or every 3 weeks (low doses),
as more animals received the high-dose diets, and stored at −20°C
before use.

As analyzed by high-pressure liquid chromatography (23), 4-HPR
was completely stable under the storage conditions used and when diet
supplemented with 4-HPR was left at room temperature for 4 days.
The stability and content of DHEA and DHEA analogue 8354 in the
basal diet were assessed by high-pressure liquid chromatography using
extraction and chromatographic methodology developed in our labo-
atory. Each steroid was extracted from the diet with 100% methanol
(50 mL/5 g of diet) by occasionally swirling the mixture over a period of
2 h at ambient temperature. After gravity filtration to remove particulate material, the volume of each filtrate was adjusted as neces-
sary to 50 mL, and 10-μL aliquots were injected directly onto a Whatman
Partisol ODS-2 column. The steroids were eluted from the column
using 100% methanol at a flow rate of 1.2 mL/min. The DHEA peak
was visualized by UV absorption spectroscopy at a wavelength of 210
nm; DHEA analogue 8354 was detected at 215 nm. Standard curves
were prepared by spiking 5 g of basal diet with known amounts of
material, extracting and quantitating as described above. Recoveries of
DHEA were generally ≥90%; recoveries of DHEA analogue 8354 were
generally ≥84%. As determined by the described method, both steroids
were stable for 4 days at room temperature when mixed in the basal
diet at the levels used in the studies. All diet materials were replaced
twice weekly.

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 rats each. Provision of diets supplemented with steroid plus retinoid
was 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, respect-
ively); 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 abnor-
ma
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 statistically significant intergroup differences in means (26). Differences in means. An appropriate discrimination test for making unplanned comparisons (least significant difference method) was used to define statistically significant intergroup differences in means. A non-significant discrimination test for making unplanned comparisons was used 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 (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 tumorigene sis 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. Treatment with DHEA over the in-life phase of the study (−1 week to end) significantly reduced mammary cancer incidence to 48% (0.2% dose) and 68% (0.1% dose). Mean (±SEM) cancer multiplicity was 0.9 ± 0.2 (0.2% dose) and 1.4 ± 0.2 (0.1% dose) cancers/rat. However, statistical comparison of the tumor data for the two groups that received DHEA from −1 week to end at 0.1% or 0.2% showed no significant intergroup differences in cancer incidence or mean multiplicity. Finally, log rank analysis of cancer incidence curves showed significant lengthening of cancer latency in groups treated with DHEA during the +1 week to end or −1 week to end periods, relative to those treated during the −1 week to +1 week period or the controls.

As shown in Table 2, combination treatment with DHEA plus 4-HPR was most effective when given from −1 week to end of the study. Cancer incidence and multiplicity also were significantly lower in the groups treated with the combination during the +1 week to end period relative to the controls. Furthermore, cancer multiplicity in the rats that received DHEA plus 4-HPR during the −1 week to +1 week period 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 end 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 ranged from 72–100%. During the experiment, only 4 rats died in a cancer-free state. In the control groups that received carcinogen vehicle and diet containing DHEA plus or minus 4-HPR, grossly visible evidence of steroid-induced pathology was not observed at necropsy.

Fig. 1 shows that the mean body weight of rats treated with dietary DHEA at 0.2% (w/w) was approximately 18% lower ($P < 0.05$) than that of placebo-fed controls within 6 days of receiving MNU injections. However, the rate of weight gain in that group recovered within the subsequent 7–14 days after carcinogen exposure, and was comparable with that of the carcinogen controls for at least 60 additional days. At termination of the study, although the gross mean body weight of the MNU-treated group on the diet containing 0.2% DHEA

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Table 1  Effect of dietary DHEA 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 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 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 (%)</th>
<th>DHEA (%) diet, w/w</th>
<th>Cancer incidence (%)</th>
<th>Cancers/rat 6–30</th>
<th>Tumors/rat 6–30</th>
<th>Terminal survival (%)</th>
<th>Terminal total body wt (g)a</th>
<th>Terminal NCW (g)a</th>
</tr>
</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>30</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>30</td>
<td>+</td>
<td>0.1*</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>100</td>
<td>100</td>
<td>294 ± 12</td>
<td>294 ± 12</td>
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</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>0.1*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>294 ± 12</td>
<td>294 ± 12</td>
<td></td>
</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.
\[ \text{Diet given from −1 week to +1 week post-MNU.} \]
\[ \text{Diet given from +1 week post-MNU to end of study.} \]
\[ \text{Diet given from −1 week post-MNU or vehicle to end of study.} \]
\[ \text{Diet contained 4-HPR vehicle (see text for details).} \]
\[ \text{P < 0.05 versus respective control group.} \]
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 AlN-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.

![Graph showing mean body weight of rats over time](image)

### Table 2 Effect of dietary DHEA plus 4-HPR on MNU-induced mammary gland carcinogenesis in female Sprague-Dawley rats

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>MNU (% diet, w/w)</th>
<th>4-HPR (mmol/kg diet)</th>
<th>Cancer incidence</th>
<th>Tumors/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>+</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>
</tr>
<tr>
<td>25</td>
<td>+</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>
</tr>
<tr>
<td>24</td>
<td>+</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>
</tr>
<tr>
<td>25</td>
<td>+</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>
</tr>
<tr>
<td>30</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>
</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>0.2*</td>
<td>1.0</td>
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<td>75 ± 6</td>
<td>75 ± 6</td>
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</tr>
<tr>
<td>10</td>
<td>Vehicle</td>
<td>—</td>
<td>100*</td>
<td>275 ± 6</td>
<td>275 ± 6</td>
<td>294 ± 12</td>
<td></td>
</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 post-MNU or vehicle to end of study.
‡ Diet contained 4-HPR vehicle (see text for details).

DHEA analogue 8354 significantly inhibited the incidence of mammary carcinomas in the female rat. In addition, overt signs of agent-mediated toxicity were observed in animals that received the compound.

**DISCUSSION**

The data herein reported are, to our knowledge, the first to show significant inhibitory activity of the adrenal-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...
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 semipurified 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 asterisks in each column of data denote statistically significant intergroup differences.

### 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 (% diet, w/w)</th>
<th>Cancer incidence (%)</th>
<th>Cancers/rat*</th>
<th>Tumors/rat**</th>
<th>Terminal survival (%)</th>
<th>Terminal total body wt (g)*</th>
<th>Terminal NCW (g)**</th>
</tr>
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<td>11.5 ± 1.0*</td>
<td>65</td>
<td>276 ± 7</td>
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</tr>
<tr>
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<td>0.4%</td>
<td>95</td>
<td>4.1 ± 0.6**</td>
<td>4.2 ± 0.6**</td>
<td>55</td>
<td>254 ± 12</td>
<td>239 ± 14</td>
</tr>
<tr>
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<td>0.7%</td>
<td>100</td>
<td>4.8 ± 1.1**</td>
<td>4.8 ± 1.0**</td>
<td>80</td>
<td>272 ± 5</td>
<td>259 ± 6</td>
</tr>
<tr>
<td>20</td>
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<td>1.0%</td>
<td>100</td>
<td>5.4 ± 0.8**</td>
<td>5.8 ± 0.8**</td>
<td>50</td>
<td>265 ± 5</td>
<td>255 ± 6</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>1.4%</td>
<td>100</td>
<td>12.2 ± 1.0*</td>
<td>12.6 ± 0.9*</td>
<td>70</td>
<td>266 ± 7</td>
<td>240 ± 6</td>
</tr>
<tr>
<td>20</td>
<td>Vehicle</td>
<td>0.2%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>70</td>
<td>266 ± 5</td>
<td>266 ± 5</td>
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<tr>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>305 ± 10**</td>
<td>305 ± 10</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.
* Diet given from -1 week to +1 week post-MNU.
** Diet given from +1 week post-MNU to end of study.

### Notes:
- 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 the 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.
- The observation, therefore, it is not possible to identify alteration of caloric shunt (34). This metabolic pathway is the primary extramitochondrial source of NADPH, which is required in a number of biochemical processes including xenobiotic catabolism by mixed function oxidase, and the biosynthesis of fatty acids and steroid hormones (34). It is also the primary pathway by which hexose phosphates are converted to the ribose and deoxyribose phosphates required for de novo biosynthesis of nucleotides and nucleic acids (34). Thus, it is apparent that treatment of rats with either DHEA or its analogue could have profound effects on a metabolically active population of target cells of the young rat mammary gland, with several possible loci at which tumor promotion could be blocked or suppressed, perhaps as a function of the inhibition of G6PDH (35).
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 was 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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REFERENCES

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

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