Exceptional Chemopreventive Activity of Low-Dose Dehydroepiandrosterone in the Rat Mammary Gland

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Abstract

To determine if the chemopreventive activity of dehydroepiandrosterone (DHEA) in the rat mammary gland can be dissociated from its toxicity, two studies were conducted in which low doses of DHEA were administered alone and in combination with other agents to rats treated with N-methyl-N-nitrosourea. Beginning 1 week prior to administration of 35 mg N-methyl-N-nitrosourea per kg body weight, groups of 20 female Sprague-Dawley rats were fed AIN-76A diet supplemented with DHEA alone (800 or 400 mg/kg diet), DHEA + tamoxifen (80 or 40 μg/kg diet), DHEA + carbenoxolone (3500 or 1750 mg/kg diet), or DHEA + tamoxifen + carbenoxolone. When administered alone at either 800 or 400 mg/kg diet, DHEA reduced mammary cancer incidence from >70% in dietary controls to 0%; mammary cancer incidence in all DHEA combination regimens was also ≤5%. The dose levels of DHEA used induced no toxicity or alteration in body weight gain. These results indicate that dietary supplementation with low doses of DHEA has chemopreventive efficacy greater than or equal to that of endocrine ablation. This protection may be mediated by the induction of differentiation in the mammary parenchyma.

Introduction

DHEA is an adrenal 17-ketosteroid that is a biosynthetic precursor to testosterone and 17β-estradiol. DHEA sulfate is the most abundant steroid in the circulation of women and shows age-related declines over the lifetime of animals and humans. The administration of pharmacological doses of DHEA can inhibit carcinogenesis in a variety of target organs in animal model systems. Effective chemoprevention by DHEA has been demonstrated in mouse mammary gland (3, 4), mouse skin (5, 6), mouse colon (7), mouse lung (8), rat liver (9), rat thyroid (9), and rat prostate (10). In most cases, chemopreventive efficacy has been achieved by relatively high dose levels of DHEA, ranging from 0.2 to 1.0% in the diet (2,000 to 10,000 mg/kg diet).

Although DHEA has significant anticarcinogenic activity in animal model systems, undesirable side effects may preclude the use of the drug at high doses for clinical cancer prevention. High dose administration of DHEA is hepatocarcinogenic in rats, an effect that appears to be mediated by its activity as a peroxisome proliferator (11, 12). This effect is clearly dose-related and does not occur at lower levels of DHEA exposure. Because peroxisome proliferation appears to be a rodent-specific phenomenon, the hepatocarcinogenicity of DHEA in rats may have limited applicability to humans. More problematic, however, is the androgenicity of high doses of DHEA; as a result of its metabolism to 4-androstene-3,17-dione and testosterone, DHEA may exert undesirable androgenic effects upon long-term, high-dose exposure.

In view of the significant chemopreventive efficacy of DHEA and the clear limitations associated with its administration at high doses, we felt it appropriate to evaluate the chemopreventive efficacy of lower DHEA doses. The specific goals of the present studies were to evaluate the efficacy of low dose DHEA administration as a component of combination chemoprevention regimens and to relate chemopreventive efficacy to effects on body weight, food consumption, and other indices of toxicity. The unexpected results of these studies demonstrated near-total inhibition of rat mammary carcinogenesis by DHEA doses ranging up to 25-fold lower than have been examined previously.

Materials and Methods

Virgin female Sprague-Dawley rats were received at 35 days of age from virus-free barriers at Harlan/Sprague-Dawley (Indianapolis, IN) and were held in quarantine for 1 week prior to randomization into a study. Rats were housed in polycarbonate shoebox cages (2 to 3 per cage) in a windowless room illuminated for 12 h each day and maintained at 22°C ± 1°C and at 30 to 70% relative humidity. At 43 days of age, rats were assorted into experimental groups using a constrained randomization procedure that blocks for body weight. This process, in which group assignment of study animals is performed at randomization, ensures that initial body weights in all experimental groups have comparable means, SDs, and ranges. The administration of chemopreventive agents was begun immediately after randomization and was continued until the end of the studies. Two experiments were conducted in which DHEA doses of 800 and 400 mg/kg diet were evaluated for anticarcinogenic efficacy. At age 50 days, all rats received a single i.p. injection of 35 mg MNU (Ash-Stevens, Detroit, MI) per kg body weight (in acidified saline, pH 5.0) or acidified saline vehicle only. Rats were observed daily and weighed weekly throughout the studies. Beginning 4 weeks after MNU administration, rats were palpated weekly to monitor mammary tumor appearance. At designated intervals throughout the study, blood samples were taken from randomly identified animals in each study group for analysis of serum DHEA levels via RIA. All rats, whether dying intercurrently or surviving until the termination of each study at 180 days post-carcinogen, received a limited gross necropsy focusing on the mammary glands. At necropsy, all gross palpable and non-palpable mammary lesions were excised, fixed in 10% neutral buffered formalin, prepared histologically by routine methods, and classified histopathologically according to the criteria of Young and Hallowes (13). The data presented are limited to histologically confirmed mammary cancers; benign tumors and other mammary lesions were excluded from the data analysis. Comparisons of cancer incidence curves for treated and control groups were made using life table analysis and the log-rank test. Tumor multiplicity data were compared using Armitage’s test for trend in proportions. Body weight data were compared by unpaired t-tests.

Results

Administration of DHEA at both the 800 mg/kg diet and 400 mg/kg diet dose levels demonstrated exceptional potency in mammary cancer chemoprevention. At 800 mg/kg diet, DHEA reduced mammary cancer response from the 70% incidence and 2.5 cancers/rat seen in...
dietary controls to 0% (Table 1). Administration of DHEA at 400
mg/kg diet also conferred complete protection against mammary
carcinogenesis; in the low-dose study, DHEA reduced mammary
cancer incidence and multiplicity from 85% and 2.60 cancers/rat in
the dietary control group to 0% (Table 2). The potent anticarcinogenic
activity of DHEA alone was such that no additive or synergistic
inhibition could be attained by coadministration of other agents used
in the study; mammary cancer response in groups of rats exposed to
DHEA in combination with tamoxifen, carbenoxolone, or tamox
ifen + carbenoxolone was reduced to an extent that was comparable
to that achieved by DHEA alone (Tables 1 and 2). Mean body weights
and body weight gains in groups exposed to DHEA administered
either alone or in combination with other agents did not differ from
those of dietary controls at any time during the study.

Dietary administration of DHEA resulted in a dose-related increase
in serum DHEA levels. In dietary controls not fed supplemental
DHEA, mean serum DHEA concentration was approximately 1 ng/ml
(Table 3), a level that reflects endogenous biosynthesis of the steroid.
After 1 week of dietary supplementation with 800 mg DHEA per kg
diet, serum DHEA increased to approximately 75 ng/ml and remained
at this level throughout the 25-week study period. A smaller, more
slowly developing increase was seen in animals receiving DHEA
supplementation at 400 mg/kg diet; serum DHEA levels in groups fed
the lower dose of DHEA increased throughout the 25-week exposure
period, reaching a maximum of approximately 65 ng/ml.

In a separate 13-week study, the influence of graded doses of
DHEA on body weight, food consumption, and mammary lobuloalveolar
development was evaluated; these data are presented in Table 4.
Dietary administration of DHEA at dose levels up to 4000 mg/kg
diet induced no gross toxicity and had no effect on body weight or
food consumption in female Sprague-Dawley rats. On this basis, it is
concluded that the threshold toxic dose for dietary administration of
DHEA is at least 10-fold higher than the 400 mg/kg diet dose level
used in the present study. Because the 400 mg/kg diet dose of DHEA
conferred complete protection against mammary cancer induction, a
large "margin of safety" between effective and toxic doses of DHEA
is evident for this end point.

Dietary administration of DHEA at doses of up to 4000 mg/kg diet
induced a massive, dose-related proliferative response in the mammary
gland of young virgin female rats (Table 4). In animals demon-
strating complete responses to DHEA, essentially all immature
mammary structures (undifferentiated ducts, alveolar buds, and ter-
ninal end buds) developed into fully differentiated lobuloalveolar
structures. By contrast, mammary glands obtained from age-matched
dietary control rats demonstrated the expected range of mammary
gland development (14), because their mammary glands included both
undifferentiated and differentiated structures.

Discussion

The results of these studies demonstrate that, when administered
continuously at low doses beginning prior to carcinogen administration,
DHEA confers essentially complete protection against neoplastic
development in the rat mammary gland. In studies conducted over the past 15
years in our laboratory, the total inhibition of mammary cancer response
in MNU-treated animals is an unprecedented finding. On this basis, the
chemopreventive activity of low-dose DHEA in the rat mammary gland
is greater than that of more than 100 chemical agents we have evaluated
for chemopreventive efficacy in this model system; the activity of DHEA
is greater than or equivalent to the anticarcinogenic activity of bilateral
ovariectomy (15). DHEA chemopreventive activity of rat mammary
carcinogenesis was not associated with gross toxicity, alterations in food
intake, or suppression of body weight gain. However, the specific mech-
anism through which DHEA acts to suppress mammary cancer induc-
ction in the rat have not been identified.

DHEA exhibits a spectrum of biological activities that are consist-
ent with its activity as an inhibitor of both the initiation and promo-
tion/progression phases of carcinogenesis. Through its activity as a
non-competitive inhibitor of glucose-6-phosphate dehydrogenase
(16), DHEA may suppress the production of NADPH, with resulting
effects on carcinogen activation and the generation of reactive oxygen
species. Supporting this proposed mechanism of action are reports that
DHEA alters the activation and/or inhibits the DNA binding of several
classes of procarcinogens in vivo (17–19) and suppresses superoxide
production in neutrophils in vitro (20).

DHEA also has biological effects that are consistent with its activity
as an inhibitor of the promotion/progression phases of tumorigen-
esis. DHEA can suppress cellular DNA synthesis, presumably through

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose level (mg/kg diet)</th>
<th>Body weight (g; mean ± SD)</th>
<th>Terminal cancer incidence (%)</th>
<th>Mean cancers/rat</th>
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<td>Control</td>
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<tr>
<td>DHEA</td>
<td>800</td>
<td>269 ± 19</td>
<td>0†</td>
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<td>274 ± 19</td>
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<td>275 ± 19</td>
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<tr>
<td>DHEA + tamoxifen + carbenoxolone</td>
<td>800 + 0.08 + 3500</td>
<td>269 ± 20</td>
<td>0†</td>
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"P < 0.01 versus Control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose level (mg/kg diet)</th>
<th>Body weight (g; mean ± SD)</th>
<th>Terminal cancer incidence (%)</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>DHEA</td>
<td>400</td>
<td>285 ± 14</td>
<td>0†</td>
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<tr>
<td>DHEA + tamoxifen</td>
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<td>281 ± 19</td>
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<td>DHEA + carbenoxolone</td>
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<td>282 ± 18</td>
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<td>DHEA + tamoxifen + carbenoxolone</td>
<td>400 + 0.04 + 1750</td>
<td>277 ± 21</td>
<td>0†</td>
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"P < 0.01 versus Control.

<table>
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<tr>
<th>DHEA dose (mg/kg diet)</th>
<th>MNU or saline</th>
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<th>9</th>
<th>17</th>
<th>25</th>
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<tr>
<td>800</td>
<td>MNU</td>
<td>75.0 ± 24.7*</td>
<td>76.0 ± 17.7</td>
<td>62.2 ± 15.7</td>
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<tr>
<td>400</td>
<td>MNU</td>
<td>40.4 ± 16.5</td>
<td>44.4 ± 19.8</td>
<td>53.0 ± 13.6</td>
<td>64.4 ± 20.3</td>
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<td>1.4</td>
<td>1.4</td>
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<td>Saline</td>
<td>47.2 ± 15.5</td>
<td>40.4 ± 25.2</td>
<td>49.4 ± 20.9</td>
<td>75.6 ± 20.4</td>
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<tr>
<td>400</td>
<td>Saline</td>
<td>27.8 ± 13.6</td>
<td>37.0 ± 19.0</td>
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* Data are expressed as ng/ml; mean ± SD.

1725
a reduction in available NADPH and pentose phosphates (21). DHEA also inhibits increases in epidermal DNA synthesis and prostaglandin E₂ biosynthesis induced by the tumor promoter, 12-O-tetradecanoyl-phorbol-13-acetate (22), and can modulate the enzymatic phenotype of preneoplastic foci in rat liver (9). Based on its role as a steroid precursor, the chemopreventive activity of DHEA in one or more target organs may also be related to endocrine influences. It appears unlikely, however, that the chemopreventive activity of DHEA in the rat mammary gland is simply a function of androgenicity; in studies previously presented in abstract form, we have found that DHEA also has significant activity as an inhibitor of prostate carcinogenesis induced in rats by a regimen of MNU + testosterone (10). On the basis of the androgen sensitivity of the prostate model system used, an enhancement rather than an inhibition of tumorigenesis would be expected if DHEA were acting simply as an androgen.

Perhaps most germane to its mechanism of chemoprevention in the breast is the activity of DHEA as a modifier of mammary gland differentiation. In the present studies, we found that DHEA induces massive lobuloalveolar proliferation and differentiation in the immature rat mammary gland, resulting in the differentiation and proliferation of immature parenchymal structures (ducts and terminal end buds) into fully developed lobuloalveolar structures. Russo and Russo (14, 23, 24) have demonstrated that the differentiation state of the mammary parenchyma is a key determinant of glandular sensitivity to carcinogenesis; whereas immature mammary glands contain numerous parenchymal structures (i.e., terminal end buds) that are highly sensitive to carcinogenic insult, fully differentiated mammary glands contain fewer of these structures, and are, therefore, relatively resistant to neoplastic development. The potentiating activity of DHEA as an inducer of mammary gland differentiation suggests that such a mechanism may underlie its chemopreventive activity. In this regard, it is clear from these data that the effects of DHEA in the rat mammary gland are not simply antiproliferative; our results suggest that the compound alters differentiation pathways in the mammary parenchyma, with effects on both breast epithelial cell proliferation and epithelial cell sensitivity to carcinogenic insult.

Acknowledgments

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References


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