Modulation of Methylnitrosourea-induced Breast Cancer in Sprague Dawley Rats by Dehydroepiandrostosterone: Dose-dependent Inhibition, Effects of Limited Exposure, Effects on Peroxosomal Enzymes, and Lack of Effects on Levels of Ha-Ras Mutations

Ronald A. Lubet, Gary B. Gordon, Russell A. Prough, Xiang-Dong Lei, Ming You, Yian Wang, Clinton J. Grubbs, Vernon E. Steele, Gary J. Kelloff, and Richard D. Moon

ABSTRACT

Dehydroepiandrostosterone (DHEA), the major steroid precursor of androgens and estrogens produced in peripheral tissues in primates, is an effective chemopreventive agent in the N-methyl-N-nitrosourea (MNU)-induced rat mammary tumor model. Dietary DHEA (5–600 ppm; 600 mg/kg diet) was administered beginning 1 week before MNU and administered continually throughout the duration of the experiment. The highest dose of DHEA (600 ppm) significantly decreased tumor incidence from 45% and increased tumor latency and decreased tumor multiplicity from 4.1 to 0.5 tumors/rat. Lower doses of DHEA (5, 24, and 120 ppm) were also effective, decreasing tumor multiplicity by 28, 40, and 55%, respectively, increasing tumor latency in a dose-dependent manner but only minimally affecting final tumor incidence. DHEA in the diet caused a dose-dependent increase in serum levels of DHEA. The 120-ppm dietary dose of DHEA resulted in serum levels of DHEA of ~42 pmol/ml levels, similar to those seen in young humans. When we examined whole mounts of mammary glands derived from rats exposed to higher levels of DHEA (600 ppm), we observed a striking increase in lobular development. The doses of DHEA used in these studies (≤600 ppm) had minimal effects on the induction of fatty acid CoA synthetase, a peroxisome-associated enzyme. In contrast, a dose of 2000 ppm substantially increased levels of peroxisome-associated fatty acid CoA synthetase. The varied and striking efficacy of DHEA was achieved in the absence of any significant effect on body weight gain in the treated rats. Furthermore, tumors from rats treated with MNU alone or rats treated with MNU plus DHEA were examined for the presence of mutations in the Ha-Ras oncogene. There was a slight decrease in the percentage of tumors bearing Ha-Ras mutations in tumors derived from MNU-control rats as contrasted with tumors from MNU-DHEA (120 and 600 ppm)-treated rats. Based on the striking chemopreventive efficacy of continual exposure to DHEA, we examined the effects of more limited exposure to DHEA. Rats were treated with DHEA for a period of 7 weeks immediately before and after MNU injection. Rats were then placed on the control diet for the ensuing 15 weeks. Even this limited exposure to DHEA for a period of 7 weeks profoundly decreased final tumor incidence and multiplicity. Additionally, we examined the effects of intermittent dosing with DHEA. Rats were treated alternatively at 3-week intervals either with diet containing DHEA or with control diet. It was found that this intermittent dosing with DHEA also substantially inhibited the formation of mammary tumors.

INTRODUCTION

DHEA, which is produced at high levels in the adrenals of primates, is the primary steroid precursor to androstenedione. DHEA is readily converted to androstenedione, which is itself converted into androgens and estrogens in peripheral tissues (1, 2). Interestingly, serum levels of DHEA and its sulfate conjugate decrease markedly throughout the life span of a human (3). There is epidemiological evidence in humans associating higher levels of DHEA/DHEA sulfate with decreased incidence of breast cancer in premenopausal females (4). However, further epidemiological studies on breast cancer in postmenopausal individuals do not support this association between high incidence of DHEA/DHEA sulfate and low levels of breast cancer (5, 6).

DHEA has proven to be an effective chemopreventive agent in a variety of mouse tumor models including the 7,12-dimethylbenzanthracene/12-O-tetradecanoylphorbol-13-acetate-induced mouse skin model (7); the 1,2-dimethylhydrazine-induced mouse colon model (8); the P-53 transgenic mouse lymphoma model (9); and the 7,12-dimethylbenzanthracene and urethane-induced mouse lung model (10). In addition, DHEA has been shown previously to have chemopreventive activity in a variety of carcinogen-induced mammary cancer models in rats: (a) the 7,12-dimethylbenzanthracene-induced rat mammary tumor model (11); (b) the MNU-induced rat mammary tumor model (12). In a recent set of experiments using the MNU-induced model, we found that even relatively low-dose levels of DHEA (400 or 800 ppm in diet) had striking chemopreventive efficacy (13); and (c) the radiation-induced, estrogen-enhanced rat mammary tumor model (14).

A wide variety of potential mechanisms have been postulated to explain the chemopreventive efficacy of DHEA including effects on cellular proliferation, effects on body weight, peroxisomal proliferation, effects on Ras farnesylation, effect on the immune response, and others (reviewed in Ref. 15). Furthermore, the striking efficacy of DHEA, even at relatively low dietary doses (13), allows one to address various questions regarding optimal treatment with DHEA in a chemopreventive setting. We therefore performed various studies to address questions dealing both with optimal dosing of DHEA as well as a potential mechanism of action of DHEA. Specific questions included: (a) What are the lowest doses levels of DHEA that effect MNU-induced rat mammary tumorigenesis? (b) Do the preventive effects of DHEA relate to altered serum levels of DHEA? (c) Does DHEA induce peroxisomal enzymes at effective preventive doses? (d) Does DHEA treatment alter the percentage of breast tumors bearing a mutation in the Ha-Ras oncogene? (e) Does DHEA alter breast morphology and lobule development? (f) Is limited treatment with DHEA sufficient to cause substantial long-term effects on mammary tumor induction by DHEA? and (g) Is intermittent dosing with DHEA effective in decreasing final tumor multiplicity?

Received 9/12/97; accepted 1/5/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 These studies were supported in part by National Cancer Institute Contracts NO1-CN-35535-02 and NO1-CN-35535-03 from the National Cancer Institute (to R. D. M.). These studies were further supported by NIH Grant CA43839 (to R. A. P.). X. D. L. was supported by the Humana Endowment for Excellence, James Graham Brown Cancer Center, University of Louisville School of Medicine.
2 To whom requests for reprints should be addressed, at National Cancer Institute-DCPC, Chemoprevention Branch, EPN-201, 9000 Rockville Pike, Bethesda, MD 20892.

The abbreviations used are: DHEA, dehydroepiandrosterone; MNU, N-methyl-N-nitrosourea; dNTP, deoxynucleotide triphosphate; Faco, fatty acid CoA synthetase.
MATERIALS AND METHODS

Rats. Virgin, female Sprague Dawley rats were received from Harlan/ Sprague Dawley at 35 days of age. After a quarantine period of 1 week, animals were randomized by weight into various treatment groups. The standard diet used was Teklad 4% Rat/Mouse Chow. DHEA was added to the diet at the doses indicated in the individual figures.

Carcinogen Treatment (MNU). The basic model used here was developed by Guillon et al. (16) and revised by Moon and coworkers (17). When the rats were 50 days of age, a single dose of MNU was administered i.v. in acidified saline (pH 5.0) at a dose of 50 mg/kg body weight.

Dose Response to DHEA. Rats were placed into groups receiving 0, 5, 24, 120, or 600 ppm DHEA in the diet. Rats were administered DHEA in the diet beginning on day 43, 1 week before the day that they were administered MNU (day 50), and remained on the DHEA diet until the time of sacrifice (102 days after MNU injection; Fig. 1 and Table 1). DHEA was obtained from Sigma Chemical Co. The dietary concentrations of DHEA were determined at three time points during the course of the experiment and was found to be within 5% of the nominal dose. The effects of DHEA on the latency of palpable tumors were determined using log-rank analysis (18). Tumor multiplicity was compared using Armitage analysis (19).

Necropsy. Rats that were moribund during the study were sacrificed by CO2 asphyxiation. At the termination of the experiment (102 days after MNU treatment), all animals were anesthetized, and blood was collected for serum preparation and determination of DHEA levels (see below). Animals were sacrificed by asphyxiation, and all mammary masses were located and matched with palpation data as to location. Tumors from each group were weighed to determine a mean tumor weight. Tumors from each of the groups were frozen to isolate DNA and examined for Ha-Ras mutations (see below).

Serum Levels of DHEA. At the end of the initial experiment examining the chemopreventive efficacy of various doses of DHEA, serum was taken from at least 12 individual rats in each of the MNU/DHEA treatment groups (Table 2). The sera from these rats were used to determine serum levels of DHEA using methods published previously (20).

Effects of DHEA Treatment on Ha-Ras Mutations: DNA Isolation. In general, the methods used here were similar to those used previously by Wang et al. (21). Pieces of tumors were either processed for histopathology or frozen in liquid nitrogen and subsequently used to determine the presence or absence of mutations in the Ha-Ras oncogene. High molecular weight DNA was isolated from tumors obtained from rats treated with MNU alone or MNU plus DHEA using Pronase-SDS lysis. Following phenol-chloroform extraction and ethanol precipitation, the DNA samples were treated with RNase and additional phenol-chloroform extractions and ethanol precipitation. The size of the DNA was checked in a 0.7% agarose gel.

DNA Amplification. Amplification reactions were carried out in a 100-μl reaction mixture containing 1 μg of genomic DNA in 50 mM KCl, 10 mM Tris (pH 8.4), 2.5 mM MgCl2, each primer at 1 μM, each dNTP (dATP, dCTP, dGTP, and dTTP) at 200 μM, gelatin at 200 μg/ml, and 2 units of Taq polymerase (Perkin-Elmer). The samples were overlaid with several drops of paraffin oil to prevent evaporation and subjected to 25 cycles of amplification as follows. The samples were heated for 95°C for 1 min, cooled to 50°C for 2 min, and incubated at 72°C for 3 min. The primers used to generate products for the PCR analysis were: RHU1, 5'-GACAGGAAGCTCCTGGTGTGGC-3'; and RHL1, 5'-CAGAGCTCACCTCTATAGT-3'.

Direct Sequencing of Amplified DNAs. PCR-amplified DNA was desalted, and excess oligonucleotides and dNTPs were removed by spin-dialysis using a Centrifucal 30 (Amicon, Danvers, ME), concentrating the volume from 100 to 40 μl. Sequencing primers (2 pmol), end-labeled with [γ-32P]ATP and T4 polynucleotide kinase, were annealed to one-tenth of the amplified Ha-Ras DNA by heat denaturing the strands at 95°C for 5 min in 10 μM Tris (pH 7.5), 20 mM MgCl2, and 50 mM NaCl. The sequence of the sequencing primer is 5'-TCTCTGGTTTGGCAACCTCG-3'. The reaction was spun briefly to collect the condensate, equilibrated at 37°C for 2 min, and divided into four tubes containing 3 μl of 80 μM dNTPs and 8 μM ddNTPs. Five units of T7 DNA polymerase (Sequenase; USB, Cleveland, OH) were added to each tube, and the reaction was allowed to proceed for 10 min at 37°C. Samples were heated to 65°C for 5 min in formamide-dye-stop mix and subjected to electrophoresis on 8% acrylamide gel. Gels were dried and exposed to X-ray film for 24 h (21).

DHEA Effects on Peroxisomal Enzymes. Liver tissue was obtained from animals in experiment I. Specifically, female rats were treated with DHEA until 43 days of age, 7 days before dosing with MNU, until day 152 of age. Alternatively, rats were treated with DHEA for a period of 28 days in the absence of MNU. At the time that individual rats were sacrificed by CO2 asphyxiation, livers were removed and quickly frozen in liquid nitrogen. KCN-insensitive peroxisomal β-oxidation of fatty acids was determined with post-5000 rpm liver supernatants by measuring NADH produced from the oxidation of the C-3 hydroxy group of palmitoyl CoA catalyzed by 3-hydroxy-yl-CoA-dehydrogenase, the rate-limiting step in β-oxidation using the methods described by Wu et al. (22).

Limited Exposure to DHEA and Mammary Gland Morphology. Rats, 50 days of age, were exposed to DHEA for 28 days. At that time, the animals were sacrificed by CO2 asphyxiation. Mammary glands were removed, and abdominal inguinal glands were processed for whole mounts using methodologies described previously.

Limited Exposure to DHEA and Chemopreventive Effects. Certain groups of rats received continual administration of DHEA (600 or 2000 ppm) beginning 1 week before MNU administration and then continually thereafter (Fig. 5). Alternatively, rats were administered DHEA continually from 7 days before MNU administration until 42 days after MNU (7 weeks) administration, after which animals were placed on standard Teklad basal diet for the remainder of the experiment. Using the methods described in experiment I, rats were initiated with MNU on day 50. The experiment was followed for 135 days after MNU on the rationale that the limited exposure regimen used might only serve to increase the latency of tumorigenesis.

Interruption Exposure to DHEA and Chemopreventive Effects. Rats received either continual administration of DHEA (600 or 2000 ppm) or were administered these chemopreventives on an intermittent basis (e.g., DHEA from 7 days before until 14 days after MNU treatment, 35-56, 77-98, and 119-135 days at other times animals received basal Teklad diet; Fig. 6). Using methods as described in experiment I, animals were initiated with MNU on day 50. The experiment was followed for 135 days after MNU on the rationale that the interruption exposure regimen might only have served to increase the latency of tumorigenesis.

RESULTS

Effects of DHEA on Body Weight. As is shown in Table 1, which is derived from the chemopreventive experiment shown in Fig. 1, doses of DHEA in the diet from 5–600 ppm had minimal effects on...
body weight gain in female Sprague Dawley rats. Similarly, weight data derived from the second set of tumor experiments (Table 2) showed minimal effects on weight gains, even after continual exposure to doses of 600 ppm or even 2000 ppm in the diet.

**Effects of Various Doses of DHEA on Tumor Incidence and Multiplicity.** DHEA caused a dose-dependent effect on MNU-induced mammary tumor incidence (Table 1). Control tumor incidence was 95%, whereas tumor incidence in animals exposed to 5, 24, 120, or 600 ppm was 90, 80, 80, and 44%, respectively. Even the lowest dose of DHEA (5 ppm) administered significantly increased tumor latency as determined by log-rank analysis (data not shown).

The effects of varying dietary concentrations of DHEA on tumor multiplicity were examined (Fig. 1 and Table 1). Whereas MNU induced 4.6 ± 0.7 (mean ± SE) tumors/rat in control rats, multiplicity was reduced 26 and 40% to 3.5 ± 0.5 and 2.6 ± 0.5 tumors/rat in animals fed 5 or 24 ppm DHEA. Feeding 120 ppm DHEA decreased multiplicity to 1.9 ± 0.4 tumors/rat, whereas a dose of 600 ppm decreased multiplicity >85% to 0.50 ± 0.14 tumors/rat.

**Serum Levels of DHEA.** DHEA levels were determined from the rats used in the initial chemoprevention experiment, which represents the serum levels achieved following 102 days of feeding a diet containing DHEA starting on day 43. In this experiment, pools of rat sera (n ≥ 3) were used for determining DHEA levels. As shown in Table 2, increasing DHEA concentrations in the diet are related to increased circulating levels of DHEA. Serum levels were similar at both time points. In animals fed a diet containing 600 ppm, the serum levels of DHEA were 100 times greater than the basal level of DHEA.

**Effects of DHEA on the Percentage of Mammary Tumors Bearing Ha-Ras Mutations.** Tumors obtained from the dose-effect study (Table 3) were used to examine the effects of DHEA treatment on the percentage of tumors containing mutations in the Ha-Ras oncogene. Tumors taken from MNU-treated rats exposed to control Teklad diet, as well as tumors derived from animals treated with MNU and continually exposed to dietary DHEA, were examined for the presence of Ha-Ras mutations using sequencing methodologies (Fig. 2). The frequency of mutations in the Ha-Ras oncogene were determined because previous studies had shown a high incidence of mutations at this locus in MNU-induced rat mammary tumors. Approximately 50% of MNU-induced control tumors or tumors derived from rats treated with 5 ppm DHEA-treated rats displayed mutations in the 12th codon of the Ha-Ras oncogene (Table 3). In contrast, a slightly lower percentage (5 of 14) of tumors derived from treatment with higher doses of DHEA displayed mutations in the Ha-Ras gene.

**Effects of DHEA on Expression of Peroxisomal Enzymes.** To determine whether doses of DHEA that were effective as a chemopreventive agent induced peroxisomal activity levels of FACO in liver were examined in Fig. 4. DHEA (2000 ppm DHEA) exposure for a period of 28 days resulted in approximately an 870% increase in FACO activity. In contrast, 600 ppm DHEA caused approximately a 180% increase. Similarly, when liver tissue derived from the tumor experiment was used, in which animals were treated with MNU on day 50 and treated with DHEA for a total of 110 days, one observed approximately a 150% increase in FACO at the 600-ppm dose and minimal effects at the lower doses of DHEA. Interestingly, levels of FACO were similar in rats exposed to DHEA for 28 or for 110 days.

**Effects of DHEA on Morphology of the Breast.** Previous data implied that DHEA might increase lobular differentiation in the breast of treated rats (14). We therefore examined the morphology of the breast, using whole mounts of the abdominal-inguinal glands, in rats exposed for 28 days to DHEA (600 ppm). The glands of DHEA-treated rats exhibit greater lobular differentiation than age-matched control rats. We observed a a relatively homogeneous level of lobular differentiation throughout the abdominal-lingual glands. In fact, based on histological appearance, the breasts of rats treated with DHEA appear similar to the breasts of pregnant rats (Fig. 3).

Based on the extraordinary efficacy of continuous treatment with DHEA in this tumor model, we examined two additional questions: (a) Could exposure to DHEA for only a limited time period from 7

---

**Table 3 Effect of DHEA treatment on the percentage of mammary tumors exhibiting mutations in the Ha-Ras oncogene**

<table>
<thead>
<tr>
<th>DHEA level</th>
<th>No. of tumors</th>
<th>Activated H-Ras</th>
<th>Codon12: GGA→GAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>30</td>
<td>15 (50%)</td>
<td>15 (50%)</td>
</tr>
<tr>
<td>5 ppm</td>
<td>9</td>
<td>5 (56%)</td>
<td>5 (56%)</td>
</tr>
<tr>
<td>120 ppm</td>
<td>7</td>
<td>2 (29%)</td>
<td>2 (29%)</td>
</tr>
<tr>
<td>600 ppm</td>
<td>7</td>
<td>3 (43%)</td>
<td>3 (43%)</td>
</tr>
</tbody>
</table>

* DHEA was administered in the diet beginning 7 days before MNU and administered continually until the end of the experiment (102 days after MNU).
* Numbers of tumors examined.

* Numbers of tumors showing activated Ha-Ras based on sequencing (see "Materials and Methods"). Numbers in parentheses are the percentage of tumors exhibiting mutations in the Ha-Ras oncogene.

* Number of tumors exhibiting specific GGA→GAA mutations in codon 12 of the Ha-Ras oncogene.
Fig. 3. Effects of administration of DHEA on mammary gland morphology and lobular differentiation. Female Sprague-Dawley rats (50 days of age) were either administered DHEA (600 ppm) in the diet or alternatively administered Teklad diet for a period of 28 days.

days before MNU injection until 6 weeks after MNU injection inhibit the outgrowth of "potential tumors," even after the rats were returned to control diet? and (b) Could an intermittent exposure to DHEA be an effective chemopreventive regimen?

Effects of Limited DHEA Exposure on MNU-induced Mammary Carcinogenesis. In the first experiment, the effects of limited treatment with DHEA, beginning 7 days before MNU injection and continuing until 42 days after MNU exposure, on tumor multiplicity was determined (Fig. 5; Table 4). Both doses of DHEA profoundly affected tumor latency as well as the final tumor incidence (Table 4). In addition, these doses of DHEA resulted in a striking decrease in tumor multiplicity of >75%. However, neither dose of DHEA using limited exposure was as effective as continual exposure to the higher dose of DHEA (2000 ppm).

Effects of Intermittent DHEA Exposure on MNU-induced Mammary Carcinogenesis. In the second experiment, rats were given 3 weeks of DHEA followed by 3 weeks of control (Teklad) diet. Continual exposure to 2000 ppm virtually ablated any tumor incidence, whereas continual exposure to 600 ppm decreased incidence by ~50% (Table 4). Although intermittent exposure to the high dose of DHEA was not as effective as continual exposure, intermittent exposure to the lower dose (600 ppm) was almost as effective as continual exposure to this dose of DHEA. In Fig. 6, one observes only a single MNU tumor in rats treated with MNU and exposed continually to 2000 ppm DHEA. Rats exposed continually to 600 ppm DHEA demonstrated a tumor multiplicity of 0.9 ± 0.3 tumors/rat, whereas control rats had a multiplicity of 5.9 ± 0.62 tumors. Interestingly, intermittent exposure to either of these two doses resulted in an 80–85% decrease in tumor multiplicity (0.9 ± 0.2 tumors/rat on the 600-ppm diet and 1.0 ± 0.18 tumors/rat on the 2000-ppm diet; Fig. 6; Table 4).

DISCUSSION

In a previous study, we showed that continual exposure to relatively high dietary doses of DHEA (1000 or 2000 ppm; Ref. 12), or even more modest doses (400 or 800 ppm; Ref. 13), were effective chemopreventive regimens in the MNU-induced mammary tumor model. In the present studies, the efficacy of a wide range of doses of DHEA (5, 24, 120, and 600 ppm) was examined (Fig. 1). The highest dose of DHEA (600 ppm) decreased tumor multiplicity by 86%, decreased tumor incidence by >50%, and greatly increased tumor latency. The lower doses (5, 24, and 120 ppm) increased tumor latency, as determined by log-rank analysis, and simultaneously decreased tumor multiplicity by 28, 40, and 55%.

Fig. 4. Effects of various dietary doses of DHEA on the levels of FACO in livers of female Sprague Dawley rats. Three different groups of rats were used. DHEA 102 days: rats were administered DHEA (600 ppm) in the diet beginning at 43 days of age and continued until 152 days of age. MNU + DHEA 102 days: rats were administered the indicated doses of DHEA in the diet beginning 7 days before MNU administration and was administered continually thereafter until 152 days of age. DHEA 28 days: rats were administered DHEA (600 or 2000 ppm) beginning at 50 days of age and continuing until 78 days of age. Methods for determining FACO activity are described in "Materials and Methods." Bors, SE.
respectively. Studies of a very high dose of DHEA (2000 ppm) demonstrated striking efficacy, decreasing tumor incidence by 95% and decreasing tumor multiplicity ≥99%. Thus, DHEA is highly effective in the rat model at very low doses. The striking efficacy of DHEA in another rat mammary tumor model (7,12-dimethylbenzanthracene induced) was reported previously by Labrie and coworkers (11, 23).

The DHEA doses used (5–600 ppm) had minimal effects on body weight gain. This observation is of considerable importance because decreases in body weight gain can severely decrease tumor development in this and many other tumor models and DHEA has been proposed to decrease body weights in some systems (24). The striking chemopreventive effects of DHEA in the rat mammary tumor model, in the absence of any effects on body weight, is in some contrast to the fact that most examples of chemopreventive efficacy observed in mice occurred at doses that resulted in substantial decreases in body weight gain (7–10). The minimal weight effects observed with DHEA may reflect compensating mechanisms. Thus, DHEA increased both estradiol levels, which might be expected to decrease body weight while simultaneously increasing androgen levels (data not shown), which might be expected to increase body weight.

As observed in Fig. 5 and Table 2, even limited exposure to DHEA (~7 to 42 days) at doses of 600 or 2000 ppm DHEA in the diet significantly decreased the final numbers of tumors that were observed. These results presumably explain in part the efficacy of DHEA when administered intermittently (3 weeks on/3 weeks off; Fig. 6). Thus, limited exposure to DHEA is sufficient to induce long-term effects in this mammary tumor model. The duration of exposure necessary to achieve chemopreventive efficacy is still not clearly defined because our experiments published previously demonstrated that exposure to relatively high doses of DHEA (1000 or 2000 ppm) for 2 weeks (1 week prior to MNU and 1 week after MNU) minimally affected final tumor incidence or multiplicity (12).

DHEA serum levels in rats exposed to DHEA for 28 days demonstrated a relatively linear increase with dose from 5–1250 ppm DHEA in the diet. In the rats used in the chemoprevention study, DHEA (5–600 ppm in diet) caused a dose-dependent increase in serum levels of DHEA. One should keep in mind that at these lower doses of DHEA (≤120 ppm), rats had two to four tumors/animal at the end of the experiment when serum levels were determined. The serum level achieved (43 pmol/ml serum) at a dose of 120 ppm in the diet is comparable to that observed in relatively young humans (3) but is significantly higher than the levels typically observed in elderly human females. Direct comparison of levels observed in rats in this study to studies published previously in humans is difficult for various reasons: (a) the methodologies routinely used for determining DHEA levels (radioimmunoassays) can be somewhat variable, making absolute comparisons problematic. Furthermore, primates in general and humans in particular have strikingly high levels of endogenous DHEA sulfate and lower levels of DHEA, whereas rodents do not synthesize this steroid. This difference in endogenous levels of DHEA makes it difficult to directly extrapolate serum levels and preventive efficacy observed in rats to the human situation. In fact, epidemiological studies in humans imply that although high levels of DHEA are associated with decreased breast cancer risk in premenopausal women (4), high levels of DHEA in postmenopausal women would appear to be associated with increased breast cancer risk (5, 6).

We next determined (Table 3) the Ha-Ras mutation pattern in tumors that grew out in the presence or absence of DHEA. Previous studies have shown that a substantial percentage of the MNU-induced rat mammary tumors have mutation in the Ha-Ras oncogenes (25, 26). Approximately 50% of the tumors from the MNU control group displayed mutations in the 12th codon of the Ha-Ras gene (Table 3). Tumors derived from rats treated with the low dose of DHEA (5 ppm) had a similar percentage of mutations in the Ha-ras gene as tumors derived from MNU control rats; in contrast, tumors derived from rats treated with higher doses of DHEA (120 or 600 ppm) had a slightly lower percentage of Ha-Ras mutations, as observed with the MNU alone group (25% or 15%, respectively).

### Table 4 Effects of limited exposure to dietary DHEA on mammary tumorigenesis

<table>
<thead>
<tr>
<th>Dose of DHEA*</th>
<th>Length of exposure†</th>
<th>Tumor incidence</th>
<th>Tumor multiplicity</th>
<th>Time (days) to first tumor</th>
<th>Final body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm DHEA</td>
<td>~7 to 135</td>
<td>100%*</td>
<td>5.9 ± 0.6</td>
<td>60 ± 4.9</td>
<td>257 ± 4.3</td>
</tr>
<tr>
<td>600 ppm DHEA</td>
<td>~7 to 135</td>
<td>45%*</td>
<td>0.9 ± 0.3*</td>
<td>77 ± 8.7*</td>
<td>262 ± 5.0</td>
</tr>
<tr>
<td>600 ppm DHEA</td>
<td>~7 to 42</td>
<td>55%*</td>
<td>1.4 ± 0.5*</td>
<td>71 ± 11*</td>
<td>259 ± 2.5</td>
</tr>
<tr>
<td>600 ppm DHEA</td>
<td>Off</td>
<td>55%*</td>
<td>1.4 ± 0.5*</td>
<td>71 ± 11*</td>
<td>259 ± 5.0</td>
</tr>
<tr>
<td>600 ppm DHEA</td>
<td>On/Off</td>
<td>0.9 ± 0.2*</td>
<td>90 ± 8.7*</td>
<td>260 ± 3.3</td>
<td>257 ± 3.4</td>
</tr>
<tr>
<td>2000 ppm DHEA</td>
<td>~7 to 42</td>
<td>5%</td>
<td>0.05*</td>
<td>92*</td>
<td>256 ± 3.3</td>
</tr>
<tr>
<td>2000 ppm DHEA</td>
<td>Off</td>
<td>1.7 ± 0.4*</td>
<td>91 ± 6.3*</td>
<td>250 ± 3.7</td>
<td>250 ± 3.7</td>
</tr>
<tr>
<td>2000 ppm DHEA</td>
<td>On/Off</td>
<td>1.0 ± 0.2*</td>
<td>86 ± 6.4*</td>
<td>250 ± 3.7</td>
<td>250 ± 3.7</td>
</tr>
</tbody>
</table>

*Administration of MNU at 50 mg/kg body weight to 50-day-old virgin female Sprague Dawley rats.
†Dose of DHEA (ppm in diet).
‡Days of exposure relative to MNU administration.
§Group means ± SE.
¶P < 0.05.
lower percentage of tumors with Ha-Ras mutations (5 of 14). This latter difference is not statistically significant. This result implies that DHEA does not strongly select for or against tumors bearing a Ha-Ras mutation and would be consistent with a Ras-independent pathway being the primary mechanism of chemoprevention.

DHEA (27, 28) and other structurally varied peroxisome proliferators all appear to induce a coordinate pleiotropic response, which is mediated by a specific nuclear receptor and which includes transcriptional activation of a wide variety of enzymes. Because certain of these enzymes can alter metabolism of various endogenous substrates, this mechanism might contribute to the preventive effects observed. The specific peroxisomal enzyme we assessed was FACC0 (22, 28). This enzyme is highly induced (9X) by high doses of DHEA (>=2000 ppm). In contrast, a dose of 600 ppm caused significant induction, ~25% of maximal, whereas lower doses of DHEA (<=120 ppm) had minimal effects. Thus, significant chemopreventive efficacy can be observed in the absence of any effects on peroxisome proliferation (120 ppm DHEA), whereas profound preventive efficacy can be obtained at a dose that has limited capacity to induce peroxisomal enzymes (600 ppm DHEA).

DHEA administration results in a variety of physiological changes that may contribute to the chemopreventive effects observed: (a) DHEA is an effective inhibitor of glucose-6-phosphate dehydrogenase, which may indirectly result in decreased levels of the ribonucleotide pools, thereby decreasing cell division (29, 30); (b) although DHEA at high doses (>=2000 ppm) induces the peroxisome-associated enzyme FACC0 (27, 28), lower but highly effective doses of DHEA minimally induced these peroxisomal enzymes; (c) previous reports showed that DHEA and its conjugated product DHEA sulfate inhibited Ras farnesylation (31). How do these results fit into our current understanding of the DHEA-induced changes associated with pregnancy or the administration of estradiol plus testosterone? These results suggest that DHEA may alter metabolism of various endogenous substrates, which might result in the induction of a coordinate pleiotropic response, which is mediated by the specific nuclear receptor and includes transcriptional activation of a wide variety of enzymes.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of D. Leavitt and C. Thomas in performing these studies.

REFERENCES


Modulation of MethylNitrosourea-induced Breast Cancer in Sprague Dawley Rats by Dehydroepiandrosterone: Dose-dependent Inhibition, Effects of Limited Exposure, Effects on Peroxisomal Enzymes, and Lack of Effects on Levels of Ha-Ras Mutations


*Cancer Res* 1998;58:921-926.

Updated version

Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/58/5/921

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.