Chemoprevention of Rat Prostate Carcinogenesis by Early and Delayed Administration of Dehydroepiandrosterone

K. V. N. Rao, William D. Johnson, Maarten C. Bosland, Ronald A. Lubet, Vernon E. Steele, Gary J. Kelloff, and David L. McCormick

ABSTRACT

Two in vivo bioassays were conducted to evaluate the efficacy of dehydroepiandrosterone (DHEA) as an inhibitor of prostate carcinogenesis in rats. Prostate adenocarcinomas were induced in male Wistar-Unilever rats by a sequential regimen of cyproterone acetate and testosterone propionate, followed by a single i.v. injection of N-methyl-N-nitrosourea (MNU) and chronic androgen stimulation. In the first experiment, DHEA (1000 or 2000 mg/kg diet) was administered continuously to rats beginning 1 week before MNU exposure. In the second experiment, continuous administration of DHEA (2000 mg/kg diet) was begun either 1 week before, 20 weeks after, or 40 weeks after MNU exposure. Controls received basal diet without added DHEA. Studies were terminated at 13 months after MNU administration, and prostate cancer incidence was determined by histopathological evaluation of step sections of accessory sex glands. In the first study, continuous dietary administration of DHEA beginning 1 week before MNU resulted in a dose-related inhibition of prostate cancer induction. In the second experiment, comparable reductions in prostate cancer incidence were observed in groups exposed to DHEA beginning 1 week before, 20 weeks after, and 40 weeks after carcinogen exposure. These data demonstrate that nontoxic doses of DHEA confer significant protection against prostate carcinogenesis in rats. The efficacy of delayed administration of DHEA suggests that the compound confers protection against later stages of prostate cancer induction and can suppress the progression of existing preneoplastic lesions to invasive disease.

INTRODUCTION

The high incidence and extended natural history of prostate cancer make it an ideal target for chemoprevention. Carcinoma of the prostate is the second most common cause of cancer death in Western male populations (1), and it is estimated that ∼18% of American men will develop prostate cancer during their lifetime (2). Prostate cancer often demonstrates an indolent clinical course, yielding a long period of preneoplasia and/or early neoplasia during which interventions directed at disease prevention, stabilization, or reversal may be implemented. Human prostate cancer also has a well-defined preneoplastic lesion, PIN (3, 4), that may be used as an intermediate marker of drug efficacy in clinical chemoprevention trials (5).

The high incidence of prostate cancer in aging men suggests that agents that inhibit or delay disease development could yield significant reductions in cancer morbidity and mortality. Because several decades may be required for PIN lesions to develop into frankly invasive carcinoma (5, 6, 7), the natural history of prostate cancer affords an extended opportunity for chemopreventive intervention.

MATERIALS AND METHODS

Animals and Animal Husbandry. Before initiation of the program, the study protocols were reviewed and approved by the IIT Research Institute Animal Care and Use Committee. All aspects of the study involving animal care, use, and welfare were performed in compliance with United States Department of Agriculture regulations and the NIH Guide for the Care and Use of Laboratory Animals.

Male Wistar-Unilever (HsdCpb:WU) rats (7–8 weeks of age at the time of receipt) were purchased from virus-free colonies maintained at Harlan/Sprague Dawley (Indianapolis, IN). After a 1-week quarantine period, rats were assigned to experimental groups using a randomization process designed to ensure a comparable initial body weight in each group within a study. Rats were housed in pairs on hardwood bedding in polycarbonate cages in a temperature-controlled room maintained on a 12-h light/dark cycle. At all times during the studies, rats were permitted free access to drinking water (supplied by automatic watering system) and Teklad 4% fat rat/mouse chow (Teklad Test Diets, Madison, WI), with or without supplemental DHEA. Animal cages, food, and bedding materials were changed twice weekly. Animals were observed a minimum of once daily to monitor their general health status and received a weekly clinical examination and body weight measurement.

Antiandrogen/Androgen Pretreatment. After release from quarantine, all rats received daily oral (gavage) administration of 50 mg of cyproterone acetate (Berlex Laboratories, Montville, NJ) per kg body weight for 21 consecutive days. One day after the final dose of cyproterone acetate, rats received daily s.c. injections of 100 mg of testosterone propionate (Sigma Chemical Co., St. Louis, MO) per kg body weight for 3 days. This sequence of antiandrogen treatment followed by androgen treatment synchronizes and stimulates cell proliferation in the prostate and thereby maximizes neoplastic development in response to a single dose of chemical carcinogen (17, 20).

Carcinogen Administration and Androgen Promotion. Sixty h after the first dose of testosterone propionate, rats in designated groups in each study received basal diet without added DHEA. Studies were terminated at 13 months after MNU administration, and prostate cancer incidence was determined by histopathological evaluation of step sections of accessory sex glands. In the first study, continuous dietary administration of DHEA beginning 1 week before MNU resulted in a dose-related inhibition of prostate cancer induction. In the second experiment, comparable reductions in prostate cancer incidence were observed in groups exposed to DHEA beginning 1 week before, 20 weeks after, and 40 weeks after carcinogen exposure. These data demonstrate that nontoxic doses of DHEA confer significant protection against prostate carcinogenesis in rats. The efficacy of delayed administration of DHEA suggests that the compound confers protection against later stages of prostate cancer induction and can suppress the progression of existing preneoplastic lesions to invasive disease.

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The abbreviations used are: PIN, prostatic intraepithelial neoplasia; MNU, N-methyl-N-nitrosourea; DHEA, dehydroepiandrosterone.

Administration of effective preventive drugs during this period of preneoplasia could be used to inhibit, reverse, or stabilize lesions before their progression into invasive prostate cancer. Under optimal circumstances, administration of a chemopreventive drug to men with existing preneoplastic or early neoplastic prostate lesions would reverse or stabilize these lesions and thereby prevent their progression into clinically significant disease.

When administered in pharmacological doses, the adrenal steroid, DHEA, can inhibit experimental carcinogenesis in a variety of target tissues, including mammary gland (8, 9, 10), skin (11, 12), lung (13), liver (14), and thyroid (14). DHEA also inhibits the growth of both human and rat prostate cancer cells in vitro (15) and has been shown to suppress the growth of transplantable prostate adenocarcinomas in rats (16). On the basis of its broad range of anticarcinogenic activity in animal model systems and data suggesting antiproliferative activity in transformed prostate cells, DHEA is a logical candidate for evaluation as an inhibitor of prostate cancer induction in vivo. The present studies were conducted to characterize the chemopreventive activity of DHEA in the prostate, using an animal model for hormone-dependent prostate cancer (17) that has previously been applied to such evaluations (18, 19).

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received a single i.v. injection of MNU (Ash-Stevens, Detroit, MI) in sterile saline (pH 5.0). In study 1, rats received 50 mg of MNU per kg body weight; in study 2, rats received 30 mg of MNU per kg body weight.

Two weeks after MNU administration, all rats received s.c. implants of two silastic tubes (Dow-Corning, Midland, MI) containing 40 mg of crystalline testosterone (Sigma). Previous studies have demonstrated 2–3-fold increases in serum testosterone in animals receiving implants of silastic tubes containing testosterone (17, 21). Silastic tubes containing testosterone were replaced at 6-month intervals throughout the study.

Experimental Design and Chemopreventive Agent Administration. The goal of study 1 was to determine whether dietary administration of DHEA confers protection against neoplastic development in the rat prostate. In this group, a total of 35 rats received dietary exposure to DHEA at 1000 or 2000 mg/kg diet beginning 1 week before MNU administration and continuing until the termination of the study; a control group of 35 MNU-treated rats received basal diet without supplemental DHEA. Doses of DHEA used in this study were selected on the basis of their chemopreventive efficacy in other in vivo carcinogenesis models and overall lack of toxicity in previous diet tolerance and chemoprevention studies conducted in our laboratory (6).

Study 2 was designed to determine whether DHEA maintains its chemopreventive efficacy when administration is initiated during the period of tumor promotion and progression. In study 2, DHEA was administered to 2000 mg/kg diet to groups of 30 rats beginning either 1 week before, 20 weeks after, or 40 weeks after MNU administration (weeks −1, 20, or 40). In all groups, exposure to DHEA was continuous until study termination. A dietary control group (30 rats) received basal diet without added DHEA.

In both studies, the concentration of DHEA in formulated test diets was measured monthly by high-performance liquid chromatography, using methods developed in our laboratory. Briefly, DHEA was extracted from the diet with 100% methanol (10 ml/g diet); 10-μl aliquots of the clarified extract were injected directly onto a Whatman Partisil 10 ODS-3 column. DHEA was eluted with 100% methanol (10 ml/g diet); 10-

Influence of Carcinogen Dose on Prostate Cancer Incidence. Consistent with our previous results (18, 19), accessory sex gland adenocarcinomas were induced in high incidence in Wistar-Unilever rats receiving sequential exposure to cyproterone acetate, testosterone propionate, and MNU, followed by chronic exposure to testosterone. In study 1, the total incidence of accessory sex gland cancers in dietary control animals receiving a single dose of 50 mg of MNU/kg body weight was 84% (Table 1). Tumor induction demonstrated specificity for the prostate: 14 of 32 rats (44%) in the dietary control group developed neoplastic lesions that were clearly confined to the dorsolateral + anterior prostate, whereas only 5 of 32 rats (16%) in this group developed cancers that were clearly confined to the seminal vesicle. Because of their large size and/or infiltrative growth patterns, malignancies in remaining cancer-bearing animals could not be assigned to a precise site of origin within the accessory sex glands.

In study 2, dietary controls receiving a single dose of 30 mg of MNU/kg body weight demonstrated an 80% incidence of accessory sex gland cancer. In comparison with the first study, a much larger fraction of tumors induced by this lower carcinoma dose was microscopic (70% in study 2 versus 31% in study 1). Neoplastic development in study 2 was also more highly specific for the prostate; whereas 18 of 30 rats (60%) in the dietary control group in study 2 developed neoplasms that were clearly confined to the dorsolateral or anterior prostate, only 2 of 30 rats (7%) in this group developed cancers that were confined to the seminal vesicle. The incidence ratio of ~9:1 for prostate versus seminal vesicle cancers in study 2 is far greater than the 3:1 ratio of prostate/seminal vesicle cancers seen in the first study.

Despite differences in carcinogen dose (50 mg of MNU/kg body weight in study 1 versus 30 mg of MNU/kg body weight in study 2), the total incidence of accessory sex gland malignancies in dietary control animals was comparable in the two studies. It appears that an inverse relationship between carcinogen dose and animal survival may be at least partially responsible for this effect. Significant mortality was first seen at month 7 in groups receiving 50 mg of MNU/kg body weight (Fig. 1), and survival in these groups between months 7 and 13 decreased approximately linearly with time. By contrast, no mortality was observed in any group exposed to 30 mg of MNU/kg body weight until 10 months after carcinogen administration (Fig. 1); survival at 13 months in all groups receiving the lower carcinogen dose was >80%, whereas survival at this time point in groups receiving the 50-mg/kg dose of MNU ranged from 34 to 54%.

In addition to the improved specificity of prostate cancer induction (defined as the ratio of prostate to seminal vesicle cancer), rats receiving the lower dose of MNU demonstrated improved overall health and developed fewer incidental lesions (primarily tumors of the Zymbal’s gland) in nontarget tissues in response to the carcinogen. In consideration of the observed increases in animal survival, reductions in the incidence of tumors in sites other than the accessory sex glands, and increases in the specificity of prostate cancer induction, it appears that the MNU/Wistar-Unilever rat prostate cancer model system is optimized for chemoprevention studies by the use of a regimen involving the lower (30 mg/kg body weight) dose of MNU.
Influence of DHEA on Prostate Carcinogenesis (Study 1). Dietary administration of DHEA at dose levels of 1000 and 2000 mg/kg diet beginning 1 week before carcinogen administration conferred statistically significant protection against the induction of prostate cancers by MNU (Table 1). This protection was conferred without the induction of gross or organ-specific toxicity and without suppression of body weight gain. The chemopreventive efficacy of DHEA in the rat prostate was dose related.

Dietary administration of DHEA beginning at week 2 reduced the incidence of cancers originating in the dorsolateral prostate region and the total incidence of malignancy in all accessory glands. The 1000-mg/kg diet and 2000-mg/kg diet doses of DHEA reduced the incidence of macroscopic + microscopic cancer in the dorsolateral prostate region from 53% in dietary controls to 29 and 22%, respectively; the linear trend for increasing chemopreventive efficacy with increasing DHEA dose was highly significant (P = 0.0086). The chemopreventive activity of DHEA appears to be effected primarily by suppressing the progression from microscopic to macroscopic malignancy; whereas the incidences of microscopic cancer in the control and DHEA groups were similar in both the dorsolateral and anterior prostate and in the dorsolateral prostate region, DHEA exposure resulted in a dose-related decrease in the incidence of macroscopic cancers in the dorsolateral prostate region (incidences of 26 and 19% in low and high DHEA groups, versus 47% incidence in dietary controls; P = 0.0146 for linear trend with increasing DHEA dose). This pattern was also seen in comparisons of the total incidence of accessory sex gland cancers in these groups; although neither dose of DHEA achieved a significant reduction in the total incidence of accessory sex gland malignancy (incidences of 79 and 69% in low- and high-dose DHEA groups versus 84% in dietary controls), reducing DHEA dose was highly significant (P = 0.0086). The chemopreventive activity of DHEA appears to be effected primarily by suppressing the progression from microscopic to macroscopic malignancy; whereas the incidences of microscopic cancer in the control and DHEA groups were similar in both the dorsolateral and anterior prostate and in the dorsolateral prostate region, DHEA exposure resulted in a dose-related decrease in the incidence of macroscopic cancers in the dorsolateral prostate region (incidences of 26 and 19% in low and high DHEA groups, versus 47% incidence in dietary controls; P = 0.0146 for linear trend with increasing DHEA dose). This pattern was also seen in comparisons of the total incidence of accessory sex gland cancers in these groups; although neither dose of DHEA achieved a significant reduction in the total incidence of accessory sex gland malignancy (incidences of 79 and 69% in low- and high-dose DHEA groups versus 84% in dietary controls), reduc-

Fig. 1. Survival curves for carcinogen-treated Wistar-Unilever rats fed control diet or control diet supplemented with DHEA. ▲, 50 mg of MNU/kg body weight; 2000 mg of DHEA/kg diet beginning at week −1; ●, 50 mg of MNU/kg body weight, 1000 mg of DHEA/kg diet beginning at week −1; ■, 30 mg MNU/kg body weight (control diet); ◇, 30 mg of MNU/kg body weight, 2000 mg of DHEA/kg diet beginning at week −1; ○, 30 mg of MNU/kg body weight, 2000 mg of DHEA/kg diet beginning at week 20; □, 30 mg of MNU/kg body weight, 2000 mg of DHEA/kg diet beginning at week 40.

Table 1 Influence of DHEA on prostate carcinogenesis in Wistar-Unilever rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Low DHEA</th>
<th>High DHEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA dose level (mg/kg diet)</td>
<td>0</td>
<td>1000</td>
<td>2000</td>
</tr>
<tr>
<td>Effective number of animals</td>
<td>32</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>No. (%) of rats with lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma or carcinosarcoma, all (± carcinoma in situ)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroscopic“</td>
<td>27 (84)</td>
<td>27 (79)</td>
<td>22 (69)</td>
</tr>
<tr>
<td>Microscopic“</td>
<td>17 (53)</td>
<td>10 (29) b</td>
<td>8 (25) c</td>
</tr>
<tr>
<td>Carcinoma in situ only</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dorsolateral plus anterior prostate (clearly confined to these glands; ± seminal vesicle lesions)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma (microscopic, ± carcinoma in situ)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td>14 (44)</td>
<td>15 (44)</td>
<td>15 (47)</td>
</tr>
<tr>
<td>Microscopic</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Neurofibrosarcoma</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Anterior prostate/seminal vesicle region (originating from anterior prostate or seminal vesicle)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, all</td>
<td>3 (9)</td>
<td>2 (6)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Macroscopic</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Neurofibrosarcoma</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Seminal vesicle only (clearly confined; ± carcinoma in situ in dorsolateral/anterior prostate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma (microscopic, ± carcinoma in situ)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroscopic</td>
<td>5 (16)</td>
<td>3 (9)</td>
<td>7 (22)</td>
</tr>
<tr>
<td>Carcinoma in situ only</td>
<td>0 (0)</td>
<td>2 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

“Macroscopic lesions defined as ≥4 mm; microscopic lesions defined as <4 mm.

b 0.05 < P < 0.10 versus dietary control group.

c P < 0.05 versus dietary control group.
tions in the incidence of macroscopic accessory sex gland carcinomas were seen in both DHEA dose groups (incidences of 29 and 25%, versus 53% in dietary controls; \( P = 0.0189 \) for linear trend with increasing DHEA dose).

Dietary administration of DHEA beginning at week \(-1\) had no significant effect on animal survival (Fig. 1) or group mean body weight at any time during the study. Although a small increase in survival was seen at months 12 and 13 in the group receiving DHEA at 1000 mg/kg diet, survival curves for all groups were comparable for the majority of the observation period. No evidence of compound-related toxicity was identified during clinical observations made throughout the in-life period or as a result of gross pathology findings at necropsy. Incidental lesions identified during in-life observations and the terminal necropsy included squamous cell carcinomas of the Zymbal’s gland and mesenchymal tumors originating in the kidney; these lesions were seen in relatively low incidence in all experimental groups and are common late events in animals exposed to high doses of MNU.

**Influence of Delayed Administration of DHEA on Prostate Carcinogenesis (Study 2).** Confirming the results of study 1, administration of 2000 mg of DHEA/kg diet beginning 1 week before MNU conferred statistically significant protection against cancer induction in the dorsolateral + anterior prostate and in all accessory sex glands combined (Table 2). The incidence of cancer confined to the dorsolateral and/or anterior prostate was reduced from 60% in the dietary control group to 28% in rats receiving DHEA beginning at week \(-1\) \((P < 0.05)\). Dietary administration of DHEA beginning at week \(-1\) also reduced total cancer incidence in the accessory sex glands from 80% in dietary controls to 48% \((P < 0.05)\). Nearly all of the lesions identified in the dorsolateral + anterior prostate were microscopic; similar chemopreventive efficacy was demonstrated by DHEA in these tissues when either invasive cancer only or invasive cancer + carcinoma *in situ* were considered as end points.

DHEA retained essentially all of its chemopreventive activity when its initial administration was delayed until 20 weeks after carcinogen exposure (Table 2). Administration of DHEA beginning at week 20 reduced the total incidence of cancer in the accessory sex glands from 80% in the dietary control group to 50% \((P < 0.05)\); similarly, the total incidence of microscopic invasive carcinoma of the accessory sex glands was reduced from 70% in dietary controls to 43% in the group receiving DHEA beginning at week 20 \((P < 0.05)\). Interestingly, animals receiving DHEA beginning at week 20 demonstrated a small (but nonsignificant) increase in the incidence of carcinoma *in situ*. These data suggest that the primary action of DHEA is not at the earliest stages of preneoplastic development in the prostate (e.g., suppression of carcinoma *in situ*), but that delayed administration of the drug acts to suppress the transition from early (*in situ*) lesions to invasive carcinoma.

When analysis is limited to malignant lesions that were clearly confined to the dorsolateral + anterior prostate, the DHEA 20-week regimen reduced the incidence of invasive carcinoma from 60% in dietary controls to 40%. This effect was of marginal statistical significance \((0.05 < P < 0.10)\).

Although clear evidence of chemopreventive efficacy was also seen in rats whose initial exposure to DHEA was delayed until 40 weeks after MNU administration, the pattern of protection was slightly different. Dietary administration of DHEA beginning at week 40 reduced the incidence of invasive carcinoma that was clearly confined to the dorsolateral + anterior prostate from 60% in the dietary control group to 30% \((P < 0.05)\). When all accessory sex glands are considered, reductions in both the total incidence of cancer and the incidence of microscopic cancer induced by exposure to DHEA beginning at week 40 were of marginal statistical significance \((0.05 < P < 0.10)\).

Consistent with the results of study 1, chronic administration of DHEA at a level of 2000 mg/kg diet induced no toxicity that was identifiable by clinical observations, alterations in body weight gain, or gross pathology. Survival curves were similar in all MNU-treated groups (Fig. 1) in this study, and group mean body weights were comparable throughout the study.

The reduction in carcinogen dose from 50 mg/kg body weight in study 1 to 30 mg/kg body weight in study 2 decreased the incidence of malignant lesions in nontarget tissues (Zymbal’s gland and kidney) to near zero. Animal survival and overall animal health in study 2 were also improved compared to that in study 1.

**DISCUSSION**

The results of the present studies demonstrate that dietary supplementation with DHEA beginning 1 week before carcinogen administration can suppress the induction of hormone-dependent prostate cancer. The dosage regimen reduced the incidence of invasion from 60% in dietary controls to 40%, which was statistically significant \((P < 0.05)\). The delayed administration of DHEA at 20 weeks after carcinogen exposure also demonstrated a significant chemopreventive effect. However, the ability of DHEA to suppress the incidence of carcinoma *in situ* was less pronounced. These findings suggest that DHEA works to delay the progression of preneoplastic lesions rather than preventing their inception.

**Table 2. Influence of delayed administration of DHEA on prostate carcinogenesis in Wistar-Unilever rats**

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>First week of DHEA exposure</td>
<td>None</td>
<td>(-1)</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Effective number of animals</td>
<td>30</td>
<td>29</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

All accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle)

<table>
<thead>
<tr>
<th>Adenocarcinoma/carcinosarcoma ((* \pm ) carcinoma <em>in situ</em>)</th>
<th>24 (80)</th>
<th>14 (48)⁵</th>
<th>15 (50)⁵</th>
<th>18 (60)⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic⁴</td>
<td>3 (10)</td>
<td>2 (7)</td>
<td>2 (7)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Microscopic⁴</td>
<td>21 (70)</td>
<td>12 (41)⁵</td>
<td>13 (43)⁵</td>
<td>15 (50)⁵</td>
</tr>
<tr>
<td>Carcinoma <em>in situ</em> only</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>4 (13)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Dorsolateral/anterior prostate (clearly confined to these glands; (* \pm ) seminal vesicle lesions)</td>
<td>18 (60)</td>
<td>8 (28)⁵</td>
<td>12 (40)⁵</td>
<td>9 (30)⁵</td>
</tr>
<tr>
<td>Carcinoma <em>in situ</em> only</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>4 (13)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Dorsolateral prostate region (originating from dorsolateral or anterior prostate or seminal vesicle)</td>
<td>4 (13)</td>
<td>4 (13)</td>
<td>1 (3)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Adenocarcinoma/carcinosarcoma</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>1 (3)</td>
<td>2 (6)</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Seminal vesicle only (clearly confined; (* \pm ) carcinoma <em>in situ</em> in dorsolateral/anterior prostate)</td>
<td>2 (7)</td>
<td>3 (10)</td>
<td>2 (7)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Adenocarcinoma ((* \pm ) carcinoma <em>in situ</em>)</td>
<td>0</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>0</td>
<td>0</td>
<td>1 (3)</td>
<td>0</td>
</tr>
</tbody>
</table>

⁴ Macroscopic lesions defined as \( \geq 4 \) mm; microscopic lesions defined as \(<4 \) mm.

⁵ \( P < 0.05 \) versus group 1.

⁶ 0.05 \( < P < 0.10 \) versus group 1.
cancer in the Wistar-Unilever rat. Furthermore, our results demonstrate that chemopreventive activity is maintained when the initiation of DHEA exposure is delayed until 20 or 40 weeks after carcinogen insult. The finding that DHEA retains most or all of its chemopreventive activity when its administration is begun long after carcinogen exposure suggests that the compound is an effective inhibitor of the latter stages of prostate carcinogenesis, i.e., the transition from pre-invasive to invasive malignancy and the transition from microscopic to macroscopic carcinoma. This hypothesis is also supported by our observation from study 1 that the inhibition of prostate cancer induction by DHEA is more apparent in its effect on the incidence of macroscopic prostate cancers than on the incidence of small cancers or in situ lesions.

The efficacy of delayed administration of DHEA in the rat prostate has clear implications for the potential clinical application of this or related compounds for prostate cancer prevention. In the MNU/Wistar-Unilever rat model system, microscopic invasive carcinomas are first seen in the accessory sex glands at ~10 months after carcinogen administration, a time similar to that at which DHEA administration was initiated in the 40-week group. On this basis, it is reasonable to assume that advanced prostatic epitheliosis and/or early neoplastic lesions were present in rats in the DHEA 40-week group at the beginning of their period of drug exposure. The chemopreventive efficacy of the DHEA 40-week regimen suggests that DHEA suppressed the progression of these existing early lesions into advanced cancers. It would be expected that prostatic epithelial lesions present at 20 weeks postcarcinogen would be less well developed than are those at week 40; however, a similar argument can be made regarding the efficacy of DHEA administered to rats beginning 20 weeks after carcinogen administration.

In view of the high incidence of prostatic epithelial lesions in the aging American male population (3, 4, 6, 7), the efficacy of delayed administration of DHEA in prostate cancer chemoprevention in rats suggests that this agent (or an analogue) may provide an effective means for prostate cancer prevention in humans. Prenecastic prostatic lesions such as PIN are commonly observed in otherwise asymptomatic men during the fourth or fifth decade of life (3, 7); these lesions often require two to three decades to develop into clinically important prostate cancers. Our rodent data suggest that initiation of DHEA exposure in the presence of preneoplastic lesions can result in significant reductions in the incidence of invasive disease; although as yet unsupported by clinical data, our results have potentially direct application to human prostate cancer prevention.

One potential limitation to the present data are the unknown adequacy of rodent models as predictors of human responses to DHEA administration. Unlike rodents, humans and other primates have relatively high circulating levels of DHEA. As such, the significant protective effects observed when DHEA is administered to rodents (having lower endogenous levels of the hormone) may be less apparent or lost when DHEA is administered to humans.

Undesirable side effects may also preclude the use of DHEA 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 (24, 25). 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. Because these androgens may also be converted to estrogens, it is possible that DHEA may also have estrogenic activity. Indeed, elevation of testosterone levels in male mice (26) and increased uterine weights in immature female rats (27) have both been observed after administration of high doses of DHEA. Whether biologically significant androgenic effects are induced by levels of DHEA that are required to suppress cancer induction in humans is presently unknown.

Hormonal activity and other side effects may limit or preclude clinical utilization of DHEA for chemoprevention of prostate cancer. In the effort to reduce possible toxicities associated with DHEA exposure, a number of analogues of DHEA have been synthesized and evaluated for biological activity. One fluorinated analogue (DHEA analogue 8354; 16a-fluoro-5-androsten-17-one; fluasterone) is non-androgenic and nonestrogenic when tested in short-term bioassays in rodents (28) and has been reported to induce less hepatomegaly and peroxisome proliferation than does DHEA. This analogue has significant chemopreventive activity in animal model systems (29, 30) and is currently being evaluated in our laboratory for efficacy in prostate cancer chemoprevention. Should DHEA itself prove to be unsuitable for use in clinical prostate cancer chemoprevention, one or more DHEA analogues may be found to demonstrate useful chemopreventive activity without the undesirable hormonal effects of the parent compound.

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