Chemoprevention of rat prostate carcinogenesis by dietary 16α-fluoro-5-androsten-17-one (fluasterone), a minimally androgenic analog of dehydroepiandrosterone

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Dehydroepiandrosterone (DHEA) is a potent inhibitor of prostate carcinogenesis in rats. However, concerns related to the possible androgenicity of DHEA may preclude its use for chemoprevention of human prostate cancer. Studies were performed to compare the androgenicity of DHEA and a fluorinated DHEA analog, 16α-fluoro-5-androsten-17-one (fluasterone), and to determine the chemopreventive activity of fluasterone in the rat prostate. Comparisons of accessory sex gland weight and histology in gonadectomized male rats demonstrated that fluasterone is less androgenic than is DHEA. Fluasterone conferred significant protection against prostate carcinogenesis induced in Wistar-Unilever rats by a sequential regimen of N-methyl-N-nitrosourea + testosterone. Chronic administration of fluasterone at levels of 2000 and 1000 mg/kg diet reduced the incidence of adenocarcinoma in the dorsolateral/anterior prostate from 64% in dietary controls to 28 and 31%, respectively. Other than a dose-related suppression of body weight gain, chronic exposure to fluasterone induced no clinical evidence of toxicity; suppression of body weight gain may be either a pharmacological effect or a minimally toxic effect of the compound. These data demonstrate that a minimally androgenic analog of DHEA protects against prostate carcinogenesis induced in rats by a chemical carcinogen + androgen. The reduced androgenicity of fluasterone may obviate toxicities associated with the androgenicity of the parent compound. On this basis, fluasterone merits consideration for evaluation in clinical trials for prostate cancer prevention. The chemopreventive activity of a non-androgenic DHEA analog suggests that at least a portion of the chemopreventive activity of DHEA in the rat prostate is unrelated to hormonal effects.

Introduction

Dehydroepiandrosterone (DHEA) is a potent inhibitor of cancer induction in a wide range of in vivo experimental models for human cancer. We have previously reported that dietary administration of non-toxic dose levels of DHEA inhibits prostate carcinogenesis in Wistar-Unilever rats. In these studies, comparable protection against prostate cancer induction was achieved when DHEA administration was initiated either 1 week before administration of a chemical carcinogen, or 20 or 40 weeks after carcinogen exposure (1). In addition to its chemopreventive activity in the prostate, DHEA protects against neoplastic development in a number of other epithelial tissues. Animal carcinogenesis models in which DHEA has demonstrated significant chemopreventive activity include the rat mammary gland (2,3), mouse mammary gland (4), mouse skin (5), mouse colon (6,7), mouse lung (8), mouse lymphatic system (9), rat liver (10) and rat thyroid (10).

Although DHEA demonstrates a broad range of anticarcinogenic activity in animal models, undesirable side effects of the compound appear likely to preclude its use for cancer prevention in humans. High dose administration of DHEA is hepatocarcinogenic in rats, an effect that appears to be mediated through its activity as a peroxisome proliferator (11,12). It is important to note, however, that the effects of DHEA on peroxisome proliferation are clearly dose-related, and do not occur at lower dose levels. Furthermore, because peroxisome proliferation appears to be a rodent-specific phenomenon, the hepatocarcinogenicity of DHEA in rats is considered to have little applicability to humans (13,14).

More problematic, however, is the potential hormonal activity of high dose exposure to DHEA. As a result of its metabolism to 4-androstene-3,17-dione and subsequently to testosterone, high dose exposure to DHEA may induce significant androgenic effects. Androgens arising from DHEA may be further metabolized to estrogens, raising the possibility that DHEA may also have estrogenic activity. Indeed, elevation of plasma testosterone levels in male mice (15) and increased uterine weights in immature female rats (16) have both been observed following administration of high doses of DHEA.

Clearly, metabolism of DHEA to other hormonally active substances may pose a critical limitation to its clinical utilization for cancer prevention. To circumvent potential problems that may result from the metabolism of DHEA to androgens and/or estrogens, several substituted DHEA analogs have been synthesized whose conversion to androgens and/or estrogens is minimal. Of these compounds, 16α-fluoro-5-androsten-17-one (fluasterone; DHEA analog 8354), may provide a suitable alternative to DHEA for use as a cancer chemopreventive agent. In studies in several animal models, fluasterone appears to retain much or all of the chemopreventive activity of DHEA: fluasterone inhibits both tumor initiation and promotion in mouse skin (17), mammary carcinogenesis in rats (18) and colon carcinogenesis in rats (19). However, unlike its parent compound, fluasterone demonstrates neither significant androgenic nor estrogenic activity.
activity in short-term rodent bioassays (20), and induces less hepatomegaly and peroxisome proliferation than does DHEA.

In consideration of the potent anticarcinogenic activity of DHEA in the rat prostate (1,21), and the significant chemopreventive efficacy of fluasterone in other animal model systems, it was considered likely that fluasterone may have useful activity as an inhibitor of rat prostate carcinogenesis. In addition to providing an assessment of the chemopreventive activity of fluasterone in the rat prostate, we present the results of studies designed to determine the effects of long-term exposure to fluasterone on androgen-sensitive organ weights and histology.

Materials and methods

Before the initiation of in vivo work, study protocols were reviewed and approved by the IIT Research Institute Animal Care and Use Committee. All aspects of the program involving animal care, use and welfare were performed in full compliance with United States Public Health Service Policy on Humane Care and Use of Laboratory Animals, and in compliance with the guidelines stated in the National Research Council Guide for the Care and Use of Laboratory Animals.

Androgenicity bioassay

Animal receipt, housing and quarantine. Male Sprague-Dawley rats (21 days of age at the time of receipt) were purchased from virus-free barrier colonies at Harlan/Sprague-Dawley, Indianapolis, IN, USA. Sprague-Dawley rats were selected for use in hormone activity bioassays in consideration of their common use in both chemoprevention efficacy evaluations and in preclinical safety assessments performed to satisfy regulatory requirements. Rats were housed in pairs in suspended polycarbonate cages over hardwood bedding in a temperature-controlled room that was illuminated for 12 h each day and maintained at 22 ± 1°C and 50 ± 20% relative humidity. All rats were held in quarantine for 1 week before the start of the study.

Animal treatment. After release from quarantine, rats were randomized into groups of 15 using a constrained randomization procedure that blocks for sex and age. After release from quarantine, a total of 130 rats received daily oral (gavage) doses of cyproterone acetate (Berlex Laboratories, Wayne, NJ, USA; 50 mg/kg body weight in sesame oil) for 21 consecutive days. One day after the final dose of cyproterone acetate, rats received daily subcutaneous injections of testosterone propionate (Sigma Chemical, St Louis, MO, USA; 100 mg/kg body weight in sesame oil) for 3 days. Thirty animals each received a single intravenous injection of cyproterone acetate (Berlex Laboratories, Wayne, NJ, USA; 50 mg/kg body weight in sterile saline (pH 5.0)). On the day after MNU administration, each rat was implanted subcutaneously with two silastic capsules (30 mm × 3.2 mm OD × 1.6 mm ID), each containing 40 mg crystalline testosterone. Silastic capsules were replaced at 6 month intervals throughout the study.

In order to evaluate the influence of fluasterone on survival and body weight gain in animals that received no carcinogen, an additional cohort of 30 rats received a single intravenous injection of sterile saline (pH 5.0) only; saline-treated animals were not pre-treated with hormones, and did not receive implants of silastic capsules containing testosterone.

Fluasterone administration. One week before administration of MNU, rats were divided into experimental groups; sizes of carcinogen-treated groups ranged from 42 to 45 animals, while saline-treated groups consisted of 10 rats each. Dietary administration of fluasterone (2000 and 1000 mg/kg diet) to carcinogen- and saline-treated animals began at this time, and was continued until the completion of the study. Carcinogen- and saline-treated rats in dietary control groups received unsupplemented basal diet only (Teklad 4% Fat Rat/Mouse Diet; Harlan/Teklad, Madison, WI, USA).

In-life observations. All animals were weighed before randomization into groups, immediately before carcinogen administration, and once weekly throughout the study. Rats were observed as a minimum of once per day to evaluate general health status, and to ensure that food, bedding and water supplies in all animal cages were adequate. At these observations, intercurrent deaths were removed from the study for necropsy, and moribund animals were identified. Moribund animals and animals deemed unlikely to survive until the next scheduled observation period were removed from the animal room, euthanized and necropsied.

Postmortem procedures. Moribund animals were euthanized out of sequence; otherwise, the study was terminated 13 months after administration of MNU, and all surviving animals were euthanized by CO2 inhalation. Animal studies, whether found dead, euthanized as moribund, or euthanized at the end of the study, received a limited gross necropsy focused on the accessory sex glands. At necropsy, the accessory sex glands and urinary bladder were carefully removed en bloc from each rat, and were fixed in 10% neutral buffered formalin. After fixation, accessory sex glands from each animal were individually dissected from the tissue block and were embedded in paraffin. Six step sections were cut through the dorsolateral prostate, the anterior prostate and the seminal vesicles at intervals of 200 μm, and a single section was cut from the ventral prostate. Tissues were stained with hematoxylin and eosin, and were classified histopathologically using criteria that have been previously described in detail (24). Data presented for each group excludes animals whose tissues were lost to evaluation for reasons of cannibalism or autolysis.

All statistical comparisons were limited to invasive carcinoma only; separate statistical analyses are presented for cancer of the dorsolateral + anterior prostate, and for cancer of all accessory sex glands combined. Comparisons of prostate cancer incidence were made using $\chi^2$ analysis and Fisher’s exact test. Body weight data were compared by analysis of variance, with post hoc comparisons made using Dunnett’s test. Survival curves for treated and control groups were compared using life analysis and the log rank test (25).
Table I. Influence of fluasterone and DHEA on organ weights, histology and secretory activity in accessory sex glands

<table>
<thead>
<tr>
<th>Group</th>
<th>Gonadal status</th>
<th>Agent</th>
<th>Relative organ weight</th>
<th>Epithelial cell height</th>
<th>Epithelial cell secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Seminal vesicle</td>
<td>Ventral prostate</td>
<td>Seminal vesicle</td>
</tr>
<tr>
<td>1</td>
<td>Intact</td>
<td>None (control)</td>
<td>161 ± 35</td>
<td>186 ± 35</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>Castrate</td>
<td>None (control)</td>
<td>6 ± 2</td>
<td>8 ± 12</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>Castrate</td>
<td>Testosterone</td>
<td>302 ± 62</td>
<td>303 ± 54</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>Castrate</td>
<td>Fluasterone</td>
<td>7 ± 2</td>
<td>5 ± 2</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>Castrate</td>
<td>DHEA</td>
<td>10 ± 3</td>
<td>5 ± 2</td>
<td>2.2c</td>
</tr>
</tbody>
</table>

*mg/100 g body weight (mean ± SD).

Scale of 1.0–5.0, where 1.0 = low cuboidal epithelium and 5.0 = high columnar epithelium (mean).

*P < 0.05 versus castrated control (Group 2).

*dP < 0.01 versus castrated control (Group 2).

Results

Androgenicity bioassay

The potential androgenic activities of DHEA and fluasterone were compared using effects on the weights and histology of accessory sex glands in gonadectomized rats as biomarkers of androgen activity. As indicated in Table I, accessory sex gland weights provided little evidence of androgenicity for either DHEA or fluasterone at the doses used in the study. Mean weight of the ventral prostate (normalized to animal body weight) in sham-operated controls that were not exposed to any agent was 186 ± 35 mg/100 g body weight. Gonadectomy reduced ventral prostate weight by >90%; ventral prostate weight in gonadectomized rats was not increased by chronic exposure to either DHEA or fluasterone (Table I). In contrast, ventral prostate weight in gonadectomized rats was significantly increased by replacement therapy with testosterone; mean ventral prostate weight in this group was significantly greater than the mean ventral prostate weight measured in sham controls.

A virtually identical pattern of effects was observed in comparisons of seminal vesicle weights (Table I). In comparison to sham-operated controls, mean relative weight of the seminal vesicles was reduced by >90% by gonadectomy. DHEA or fluasterone induced only small, non-significant increases in mean seminal vesicle weight. In contrast, mean weight of the seminal vesicles in gonadectomized rats receiving testosterone was significantly greater than the mean seminal vesicle weight seen in non-gonadectomized controls.

Histopathology of the prostate and seminal vesicle provided a much more sensitive indicator of the relative androgenicity of DHEA and fluasterone. A semi-quantitative scale was established in which maximum values (5.0) were assigned for the cell height and secretory activity of accessory sex glands harvested from intact (sham-operated) rats, and minimum values (1.0) were assigned for the cell height and secretory activity of gonadectomized rats. Comparable epithelial cell height and secretory activity were seen in the ventral prostate and seminal vesicle in both intact controls and gonadectomized rats receiving testosterone replacement therapy (Table I). When compared with castrated controls receiving no other treatment, gonadectomized rats receiving DHEA or fluasterone both demonstrated significant increases in both parameters. For both endpoints in both accessory sex glands, the androgenicity of DHEA exceeded that of fluasterone, but the differences were not statistically significant.

Prostate cancer chemoprevention bioassay

Confirming our previous results (1,23), neoplastic development induced in the Wistar-Unilever rat by the regimen of MNU + testosterone is highly specific for the prostate. In the dietary control group, 82% of carcinogen-treated rats demonstrated carcinomas in the accessory sex glands (Table II). Of this 82% response, 64% were animals with small tumors that were clearly limited to the dorsolateral or anterior prostate, 11% were animals with large tumors that were clearly limited to the dorsolateral prostate complex, and 13% were animals with large tumors that were clearly limited to the seminal vesicle–anterior prostate complex; the size and invasive growth of the large tumors precluded a precise determination of their tissue of origin. Only a small fraction (4%) of tumor-bearing animals had a single carcinoma that was clearly confined to the seminal vesicle. The great predominance of incident prostate cancers over seminal vesicle cancers driving the overall cancer incidence seen in this study is consistent with observations in several previous prostate cancer chemoprevention studies conducted in our laboratory (1,22,23).

Dietary administration of fluasterone at either 2000 or 1000 mg/kg diet resulted in a statistically significant suppression of prostate cancer induction (Table II). At study termination (13 months post-carcinogen), the incidence of adenocarcinoma of the dorsolateral and/or anterior prostate was reduced from 64% in dietary controls to 28 and 31% in groups receiving the high and low doses of fluasterone, respectively (P < 0.01 for both comparisons). Similarly, administration of fluasterone reduced the total incidence of adenocarcinoma in all accessory sex glands from 82% in dietary controls to 42 and 43% (P < 0.01 for both comparisons). These data demonstrate that fluasterone confers significant protection against cancer induction in the rat prostate.

Survival was excellent in all experimental groups. At study termination, survival in MNU + testosterone-treated rats was 80% in the dietary control group and 87 and 89% in groups receiving the high and low doses of fluasterone, respectively. Survival in animals not treated with MNU + testosterone was 90% in the control group and in both groups receiving the high or low doses of fluasterone.

Clinical observations identified no pattern of gross toxicity that was associated with chronic dietary exposure to fluasterone. However, statistically significant reductions in mean body weight and body weight gain were observed in all groups exposed to the drug. At the low and high doses of fluasterone, mean terminal body weights in groups treated with MNU + testosterone were 91.4 and 84.4% of dietary
controls, respectively (Figure 1). The effect of fluasterone on mean body weight was even more pronounced in animals not receiving MNU + testosterone: in the groups treated with the low and high doses of fluasterone, mean terminal body weights were 84.4 and 75.4% of dietary controls, respectively.

Discussion

The results of the present studies demonstrate that fluasterone confers statistically significant protection against prostate carcinogenesis in the Wistar-Unilever rat model. When compared with a dietary control group, both the incidence of all accessory sex gland cancers and the incidence of cancers that are clearly limited to the dorsolateral and anterior prostate were reduced by ~50% in groups receiving dietary supplementation with fluasterone. The chemopreventive activity of fluasterone was not accompanied by any clinical evidence of systemic or organ-specific toxicity, and survival at the termination of the study in fluasterone-treated groups was equivalent to or better than was survival in dietary controls. Additional studies in gonadectomized rats demonstrated that fluasterone is only minimally androgenic, and has less androgenic activity than does its non-fluorinated parent, DHEA.

The only evidence that could be interpreted as an indicator of fluasterone toxicity in the present study was its effect on animal body weight gain. Whether the observed reductions in body weight gain reflect a pharmacological effect or a threshold toxic effect of fluasterone cannot be determined on the basis of the results of these studies, and may be impossible to determine conclusively. It is critical to note, however, that reductions in body weight gain appear to have no effect on prostate carcinogenesis in the Wistar-Unilever rat model:

![Fig. 1. Influence of dietary fluasterone administration on body weight in rats treated with MNU + testosterone.](https://academic.oup.com/carcin/article-abstract/28/2/398/2476330)

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Fluasterone 2000</th>
<th>Fluasterone 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemopreventive agent</td>
<td>Control</td>
<td>Fluasterone</td>
<td>Fluasterone</td>
</tr>
<tr>
<td>Fluasterone dose level (mg/kg diet)</td>
<td>—</td>
<td>2000</td>
<td>1000</td>
</tr>
<tr>
<td>Effective number of animals</td>
<td>45</td>
<td>43</td>
<td>42</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, all (± carcinoma in situ)</td>
<td>37 (82)</td>
<td>18 (42)</td>
<td>18 (43)</td>
</tr>
<tr>
<td>Macroscopic size</td>
<td>11 (24)</td>
<td>5 (12)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Microscopic size only</td>
<td>26 (58)</td>
<td>13 (30)</td>
<td>14 (33)</td>
</tr>
<tr>
<td>Carcinoma in situ (CIS) only</td>
<td>1 (2)</td>
<td>5 (12)</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Adenocarcinoma or carcinoma in situ</td>
<td>38 (84)</td>
<td>23 (53)</td>
<td>24 (55)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>3 (7)</td>
<td>1 (2)</td>
<td>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>29 (64)</td>
<td>12 (28)</td>
<td>13 (31)</td>
</tr>
<tr>
<td>Carcinoma in situ (CIS) only</td>
<td>3 (7)</td>
<td>7 (16)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Adenocarcinoma or carcinoma in situ</td>
<td>32 (71)</td>
<td>19 (44)</td>
<td>20 (48)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dorsolateral prostate region (originating from dorsolateral or anterior prostate or seminal vesicle)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, macroscopic</td>
<td>5 (11)</td>
<td>2 (5)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>0</td>
<td>2 (5)</td>
<td>1 (2)</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, macroscopic size</td>
<td>6 (13)</td>
<td>2 (5)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Seminal vesicle only (clearly confined to this gland; ± CIS in dorsolateral/anterior prostate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, all (± carcinoma in situ)</td>
<td>2 (4)</td>
<td>5 (12)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Macroscopic size</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Microscopic size only</td>
<td>2 (4)</td>
<td>4 (9)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Adenocarcinoma or carcinoma in situ</td>
<td>2 (4)</td>
<td>5 (12)</td>
<td>2 (5)</td>
</tr>
</tbody>
</table>

*p < 0.01 for trend to increased chemopreventive efficacy with increased dose.

*p < 0.01 versus control (Group 1).

*p < 0.05 versus control (Group 1).

*p < 0.05 for trend to increased chemopreventive efficacy with increased dose.
The mechanism or mechanisms underlying the suppression of body weight gain induced by chronic dietary administration of fluasterone are unclear. However, at least three possible mechanisms can be identified as follows: non-specific toxicity; pharmacological effects involving increased thermogenesis, anti-glucocorticoid activity or other mechanisms; and weak androgenicity.

It is possible that non-specific toxicity may underlie at least part of the reductions in body weight gain observed in groups treated with fluasterone. Reductions in body weight gain are commonly observed as a non-specific indication of toxicity in subchronic and chronic bioassays in rodents. As was the case in the present study, modest reductions in mean body weight are often not accompanied by any clinical evidence of toxicity, nor by any morphological evidence of organ-specific toxicity or identifiable changes in organ function. Thus, although clinical observations failed to identify evidence of toxicity in animals receiving chronic dietary supplementation with fluasterone, modest systemic toxicity cannot be discounted as a possible mechanism underlying the observed suppressions in body weight gain.

Although systemic toxicity may be responsible for a portion of the observed decreases in body weight gain, pharmacological mechanisms also exist through which these decreases may be effected. Schwartz and Pashko have reported that both fluasterone and its parent compound, DHEA, alter thermogenesis through a mechanism that may involve brown fat metabolism (20). As such, alterations in thermogenesis associated with exposure to fluasterone could be considered to be a pharmacological property of the drug, rather than a toxic effect per se. However, because thermogenic mechanisms were not investigated in the present studies, we have no evidence to either support or refute this hypothesis.

An alternate pharmacological mechanism through which fluasterone may reduce body weight gain is through the suppression of glucocorticoid synthesis and/or action. It has recently been reported that DHEA downregulates the expression and oxoreductase activity of 11α-hydroxysteroid dehydrogenase type 1 (11α-HSD Type 1) in liver and adipose tissue (27). 11α-HSD Type 1 activates glucocorticoids from their inactive keto forms (28); transgenic mice overexpressing 11α-HSD Type 1 develop obesity (28), while suppression of 11α-HSD Type 1 with a novel small molecule inhibitor reduces weight gain in mice (29). Such a body weight effect could also occur through the inhibition by fluasterone of 11α-HSD Type 1 activity, with subsequent suppression of glucocorticoid action.

Although it is considered relatively unlikely, the possibility also exists that the observed reductions in body weight gain reflect the very modest androgenic activity of fluasterone. A universal finding in prostate cancer chemoprevention studies in the MNU/Wistar-Unilever rat model is that chronic exposure to testosterone is associated with reductions in body weight gain. For example, in the present study, group mean body weight at study termination in the dietary control group receiving MNU + chronic testosterone exposure was 397 g; this terminal body weight compares with a mean weight of 512 g in the dietary control group that received neither MNU nor testosterone. Although the results of the androgenicity studies presented herein suggest that fluasterone has very limited androgenic activity, the effects of this agent on body weight gain in the prostate cancer chemoprevention bioassay are consistent with androgenicity.

Further support for the possible androgenicity of fluasterone as a mechanism for the observed reduction in body weight gain comes from its differential activity in control animals versus those receiving MNU + testosterone. Whereas the high and low doses of fluasterone reduced terminal group mean body weight in otherwise untreated rats by 24.6 and 15.6%, respectively, the same fluasterone dose levels reduced mean body weight in rats receiving MNU + testosterone by 15.6 and 8.6%. The effects of androgens on body weight gain generally demonstrate a non-linear dose–response. As such, the fact that greater effects on body weight were seen in rats receiving no exogenous androgen are compatible with the hypothesis that the observed reduction in group mean body weight in groups receiving fluasterone is an androgen-associated phenomenon.

It should be noted, however, that chronic dietary administration of the parent compound, DHEA, at a dose that was comparable with the high dose of fluasterone in the present study had no effect on group mean body weight in previous cancer chemoprevention studies in the Wistar-Unilever rat prostate cancer model (1). Because the results of the present androgenicity studies demonstrate that fluasterone is less androgenic than is DHEA, and DHEA itself had no significant effect on group mean body weight in its prostate cancer chemoprevention bioassay, it is not unreasonable to conclude that the very limited androgenicity of fluasterone is unlikely to have a significant effect on animal body weight in the present study.

The chemopreventive efficacy for fluasterone in the rat prostate is of potential significance for the ultimate clinical utilization of DHEA and analogs for cancer prevention. In previous studies conducted in our laboratories, fluasterone was found to be a less potent inhibitor of rat mammary carcinogenesis than is the parent compound, DHEA (3,18). However, when administered at equivalent doses, the efficacy of these two agents as inhibitors of prostate carcinogenesis is comparable. In view of the significant concerns related to the toxicity of DHEA in humans, the results of the present study suggest that the less androgenic analog, fluasterone, may be a superior agent for possible use in clinical prostate cancer chemoprevention studies.

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References


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