Evaluation of Dietary Dehydroepiandrosterone for Chemoprotection against Tumorigenesis in Premalignant Colonic Epithelium of Male F344 Rats

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ABSTRACT

Epidemiological and experimental studies suggest that dehydroepiandrosterone (DHEA), an adrenal cortical steroid, has chemoprotective properties. Rat colonic epithelium which had been induced to a premalignant state by the colonic carcinogen azoxymethane was used as a model for patients at high risk of colorectal carcinoma, and the efficacy of dietary DHEA for chemoprotection against tumorigenesis was evaluated. Ten-week-old male F344 rats (n = 100) were given 10 weekly s.c. injections of azoxymethane at a dose of 10 mg/kg/week. One day after the final dose of carcinogen, DHEA was added to the diet of 50 rats (0.5% DHEA chow), and the other rats were used as pair-fed controls. DHEA-fed rats lost body weight throughout the 17-week study, in contrast to their pair-fed controls. Serum DHEA in DHEA-fed rats at the end of the study was 6 times that of controls (120 ± 30 versus 20 ± 14 pmol/ml), and serum DHEA sulfate was 23 times that of controls (1311 ± 13 versus 55 ± 13 pmol/ml). Addition of DHEA to the diet produced no significant chemoprotection in our model. Tumor-related mortality was somewhat increased in DHEA-fed rats (20% versus 6% in week 16 of DHEA feeding, P not significant). The cumulative prevalence of left colonic tumors, identified by weekly colonoscopic examinations, was somewhat lower in DHEA-fed rats than in controls during weeks 10 through 13 (17% versus 33% in week 12, P not significant), but in week 14 the prevalence in DHEA-fed rats became similar to that in controls (39% versus 41%). Growth curves of autochthonous left colonic tumors, as assessed for 8 weeks by computed image analysis of colonoscopic photographs, were similar for DHEA-fed and control rats. Prevalence, mean frequency, multiplicity, and diameter of colonic tumors at necropsy of colonoscopically negative rats in week 17 were somewhat lower in the DHEA-fed rats (e.g., prevalence of 47% versus 67%), but the differences from controls were not significant. Parameters of colonic epithelial proliferation after tritiated thymidine incorporation in DHEA-fed rats were similar to those in control rats (labeling index of 8.3 ± 0.7% versus 8.4 ± 0.6% in week 17), despite higher serum DHEA and DHEA sulfate levels. Our findings indicate that DHEA did not have significant postinduction chemoprotective activity against azoxymethane-induced colonic tumorigenesis in this model utilizing pair-fed controls. Further preclinical studies appear to be needed before dietary DHEA can be recommended for chemoprotection trials in patients with premalignant colorectal epithelium.

INTRODUCTION

DHEA is a naturally occurring adrenal cortical steroid which is a normal precursor for androgens and estrogens (for review on DHEA, see Refs. 1 and 2). The sulfate conjugate of DHEA serves as the principal precursor for placental estrogen synthesis, and DHEA is a potent uncompetitive inhibitor of glucose-6-phosphate dehydrogenase activity. DHEA appears to have chemoprotective properties, although its mechanism of action is uncertain. After peaking in the third decade of life, DHEA levels decline with advancing age, as the incidences of most cancers rise. Subnormal blood levels of DHEA have been found in patients with various cancers (1–4). More importantly, a prospective study has associated low levels of plasma DHEA and its urinary metabolites with the subsequent development of breast cancer (5). Administration of DHEA to animals has been found to be protective against experimental chemical carcinogenesis in the skin (6–8), lung (9, 10), liver (10–18), thyroid gland (10), adrenal gland (15), and pancreas (18). Dietary DHEA also suppressed spontaneous breast tumorigenesis in rodents (19).

Colorectal carcinoma is the second most common cause of cancer deaths in the United States (20, 21), leading to interest in the identification of chemoprotective agents (22, 23). Experimental models of colonic carcinogenesis have been used widely for preclinical studies of potential chemoprotective agents (24–27). Dietary DHEA was found to suppress colonic carcinogenesis in one model using dimethylhydrazine-treated female mice when DHEA was administered before, during, and after the course of colonic carcinogen (28). Based on these findings, the authors suggested that DHEA could be a chemopreventive agent in patients with premalignant lesions of the colon.

Clinical conditions which result in high risk of colorectal carcinoma are associated with crypt epithelial hyperproliferation in grossly normal colorectal mucosa (29–31). These conditions include inherited diseases, such as adenomatous polyposis syndrome and hereditary nonpolyposis colorectal cancer syndrome (Lynch syndrome), and the presence of colorectal adenomas, which are precursors to carcinoma. Hyperproliferative colorectal epithelium in these conditions is manifested by increased epithelial DNA synthesis and increased activity of ornithine decarboxylase, a regulatory enzyme in polyamine biosynthesis (32). These markers of hyperproliferation are also manifested in premalignant colonic epithelium induced in experimental animals by administration of colonic carcinogens such as dimethylhydrazine and its metabolite AOM (33, 34).

Malignant tumors will eventually develop in the hyperproliferative colonic epithelium of animals treated with colonic carcinogens. The hyperproliferative epithelium during the postinduction phase thus provides a model premalignant condition of the colon which can be used to test the effectiveness of candidate chemoprotective agents (35–38). Compounds which inhibit the development of tumors in experimental premalignant colonic epithelium could have particular value in patients with premalignant conditions of the colorectum. We therefore administered DHEA in the diet during the postinduction phase of...
AOM-treated male F344 rats and assessed the effects on colonic tumorigenesis and markers of crypt epithelial proliferation.

MATERIALS AND METHODS

Animals and Housing. Guidelines for the care and use of laboratory animals promulgated by the Division of Comparative Medicine of The Johns Hopkins Medical Institutions were followed (39). Ten-week-old male Fischer 344 rats (n = 100) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The rats were housed in plastic cages with wood chip bedding, in animal quarters with controlled temperature (21–22°C), humidity (30–50%), and light (12-h cycles). During the initial weeks prior to DHEA administration, the rats were given ad libitum Purina Rat Chow 5002 (Ralston Purina, St. Louis, MO). Distilled drinking water was supplied by an automatic system.

Carcinogen and Administration Procedures. All carcinogen-handling procedures were carried out in accordance with regulations promulgated by the Office of Safety and Environmental Health of The Johns Hopkins Medical Institutions (40). A single batch of AOM (CAS: 25843-45-2; Ash Stevens, Detroit, MI) was used. After 2 weeks of acclimatization, all rats were given weekly s.c. injections of AOM, at a dose of 10 mg/kg/week.

Dietary DHEA Administration and Pair-feeding Procedures. DHEA in powder form was purchased from Diosynth (Chicago, IL). DHEA (0.5%) in Purina Rat Chow 5001 and control chow without DHEA were prepared in biscuit form by Bio-Serv (Frenchtown, NJ). On the day after the last (tenth) injection of AOM, the rats were distributed into weight-matched pairs and housed individually for pair-feeding. On the first day of DHEA chow, the DHEA-fed rats showed a marked decline in chow consumption, compared to the rats receiving control chow. For the remainder of the study, therefore, the weight of chow consumed each day by the DHEA-fed rat in each pair was determined and its matched control was then fed that weight of control chow.

Colonoscopic Procedures. Beginning 5 weeks after the addition of DHEA to the diet, all rats underwent weekly colonoscopic examination to the splenic flexure, as in our previous studies (41, 42). An Olympus BF-B2 pediatric fiberoptic bronchoscope was used. When an autofluorescent tumor was identified in the left colon by surveillance colonoscopy, the date was recorded for determination of cumulative tumor prevalence in the DHEA-fed and control groups. In order to document the size of the tumor, the rat was anesthetized and photographs of the tumor and an adjacent scale (passed through the biopsy channel of the endoscope) were taken. Tumor volume was assessed by computerized image analysis of the colonoscopic photographs. Tumor growth was assessed for 8 weeks and the rat was then killing.

DNA Labeling and Necropsy Procedures. All carcinogenically negative rats were killed in week 17 of DHEA feeding. Ten representative rats in each group were decapitated 1 h after an i.p. injection of [3H]thymidine (0.5 mCi/kg, approximately 40 Ci/mmol; Amersham, Chicago, IL). Serum was collected for DHEA and DHEA sulfate analysis. The entire gastrointestinal tract was removed and examined for tumors, as in our previous studies (43). For autoradiographic assessment of epithelial proliferation, specimens of grossly normal mid-left colon were processed as in our previous studies (42, 43).

Serum DHEA and DHEAS Assays. Serum levels of DHEA and DHEAS were measured in pooled serum samples, using chromatographic-enzymic reference methods. In brief, serum samples, spiked with tracer amounts of [3H]DHEA or [3H]DHEAS, were applied to C18 SEP-PAK cartridges. DHEAS was selectively eluted with 45% methanol and DHEA with 100% methanol, following elution of DHEAS. Fractions containing DHEAS were hydrolyzed, and the resulting unconjugated DHEA was isolated by high performance liquid chromatography and assayed using 3β-hydroxysteroid dehydrogenase, as previously described (44). Fractions of serum containing DHEA were purified by high performance liquid chromatography and assayed with 17β-hydroxysteroid dehydrogenase (45). Reported values for DHEA and DHEAS were corrected for total recovery of tracer (80–90%).

Statistical Analysis. Four rats, 3 controls and 1 DHEA-fed, died before week 10 and had no tumors at necropsy. These rats were excluded from statistical analysis. The sample size had the statistical power to detect a large effect of DHEA on tumor prevalence, which could encourage clinical trials: 80% power to detect a reduction from 40% to 15%. Because of the statistical inefficiency of dichotomous variables, other continuous endpoints were also assessed, including tumor size and parameters of epithelial proliferation. Prevalences of tumors were compared by Fisher exact test. Differences between means were calculated by two-tailed t test for paired and unpaired data, Wilcoxon matched-pairs signed-rank test, and Mann-Whitney U test as appropriate. DAISY statistical software (Rainbow Computing, Northridge, CA) was used.

RESULTS

Dietary administration of DHEA resulted in a dramatic fall in chow intake and body weight during the first week of the study (Fig. 1). DHEA chow intake returned to the baseline level after 2 weeks but then declined slowly for the remainder of the study. The decline in chow intake was paralleled by a slow decline in body weight in the DHEA-fed group. Chow intake and body weight in the control group were similar to those in the DHEA-fed group during the first week. Despite pair-feeding, however, the control group subsequently began to regain body weight until the last 4 weeks of the study, when the mean weight declined slightly. The lower body weight in DHEA-fed rats, which has been attributed in previous studies to reduced efficiency of food utilization (46–48), resulted in higher chow intake/g body weight than in the pair-fed control group. DHEA administration was also reflected in elevated serum DHEA and DHEAS levels; DHEA-fed rats had a mean serum DHEA level 6 times that of the controls (120 ± 30 versus 18 ± 14 pmol/ml at necropsy in week 17) and a mean serum DHEAS level 23 times that of the controls (1311 ± 13 versus 55 ± 13 pmol/ml).

The endpoints used in our study were: tumor-related mortality; occurrence and growth of left colonic tumors; prevalence, frequency, multiplicity, and size of gastrointestinal tract tumors at necropsy; and parameters of colonic epithelial proliferation. Dietary DHEA administration produced no reduction in tumor-
related mortality (Fig. 2). The percentage of rats surviving was lower in the DHEA-fed group in the latter weeks of the study (e.g., 80% versus 95% in week 16), although the differences were not statistically significant. The cumulative frequency of left colonic tumors identified by colonoscopy showed a transient lag in the DHEA-fed group, as compared with controls (Fig. 2). For example, in week 12 the prevalence of left colonic tumors in DHEA-fed rats was about one half that of controls (17% versus 33%), but the differences were not statistically significant and the prevalences became similar only 2 weeks later. Growth of autochthonous left colonic tumors was not affected by DHEA, inasmuch as the growth curves were similar in the two groups (Fig. 3). Tumorigenesis in the entire gastrointestinal tract, assessed at necropsy in rats with no colonoscopically identified colonic tumors, was not significantly affected by DHEA. The prevalence, mean frequency, and mean diameter of colonic tumors were generally somewhat smaller in the DHEA-fed group (Table 1), but the differences were not statistically significant. In addition, parameters of colonic epithelial proliferation (Table 2), which serve as markers for risk of tumorigenesis (29), were similar in the left colon of the DHEA-fed and control groups. Thus, dietary DHEA had no significant chemoprotective activity in our model.

Table 1 Tumor results at necropsy in colonoscopically negative rats (week 17)
The differences between the DHEA-fed and control groups were not statistically significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DHEA-fed group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>Prevalence (colonie)</td>
<td>7/15 (47%)</td>
<td>10/15 (67%)</td>
</tr>
<tr>
<td>Small bowel tumors</td>
<td>8/15 (53%)</td>
<td>10/15 (67%)</td>
</tr>
<tr>
<td>Frequency (colonie)</td>
<td>0.8 ± 0.3*</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Small bowel tumors</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Multiplicity</td>
<td>1.7 ± 0.2</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Colonie tumors</td>
<td>3.1 ± 0.7</td>
<td>5.0 ± 1.3</td>
</tr>
<tr>
<td>Small bowel tumors</td>
<td>7.5 ± 1.0</td>
<td>6.6 ± 0.5</td>
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</tbody>
</table>

* Mean ± SE.

DISCUSSION

A previous study by Nyce et al. (28) suggested that DHEA had antineoplastic activity against experimental colonic carcinogenesis; colonic tumors and their histopathological precursors, anal tumors, as well as colonic epithelial proliferation and its response to carcinogen, were all reduced by DHEA feeding. Based on their findings, Nyce et al. suggested that DHEA could find application as a chemopreventive agent in patients with premalignant lesions of the colon. The animal model used by Nyce et al. differed substantially from that in our study, as summarized in Table 3.

The differences in animal species and gender, chemical carcinogen, timing of DHEA administration relative to carcinogen, and pair-feeding of control animals could have contributed to the disparate results in the two studies. DHEA has, however, been effective in male rats in other models of chemical carcinogenesis (10, 11, 15, 17, 18), and AOM is a metabolite of dimethylhydrazine with relatively similar tumorigenic characteristics (49). Of potentially greater importance, Nyce et al. (28) began treatment with DHEA prior to administration of dimethylhydrazine and continued DHEA throughout the experiment. In contrast, in the current study DHEA was not given until AOM administration was completed. Thus, we evaluated only postinduction effects of DHEA, as opposed to the preinduction, induction, and postinduction phase effects observed by Nyce et al. While these investigators found that DHEA did not alter
Table 3

<table>
<thead>
<tr>
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<th>Nyce et al. (28)</th>
<th>Present study</th>
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<tbody>
<tr>
<td>Animals</td>
<td>8–12-week-old female BALB/c mice</td>
<td>10-week-old male F344 rats</td>
</tr>
<tr>
<td>Carcinogen</td>
<td>Dimethylhydrazine, 21 mg/kg/week, for 22 weeks</td>
<td>Azoxy methane, 10 mg/kg/week, for 10 weeks</td>
</tr>
<tr>
<td>DHEA administration</td>
<td>0.6% in Purina Breeder's Chow; no pair-feeding</td>
<td>0.5% in Purina Rat Chow 5001; pair-fed controls</td>
</tr>
<tr>
<td>Timing of DHEA relative to carcinogen</td>
<td>2 weeks before, during, and after carcinogen (preinduction, induction, and postinduction phases)</td>
<td>After carcinogen only (postinduction phase)</td>
</tr>
</tbody>
</table>

DNA adduct levels, they did find that DHEA inhibited both baseline and carcinogen-stimulated colonic epithelial proliferation, a finding which has been associated with diminished colonic tumorigenesis (33). Moreover, inhibition of DNA adduct levels produced by another carcinogen (dimethylnitrosamine) and decreased binding to DNA of still other carcinogens (dimethylbenz(a)anthracene and aflatoxin B1) have been found in DHEA-treated rats, along with enhanced hepatic metabolism of the carcinogens (50–52). Thus, the absence of preinduction and induction phase effects of DHEA is a possible explanation for the absence of chemoprotective activity in our model.

A second major difference between the two studies is our use of pair-fed controls. Nyce et al. used control mice which were fed ad libitum, and the chow intake of their DHEA-fed mice was substantially less than that of controls. Caloric restriction has been shown to reduce experimental colonic carcinogenesis (53) and could have contributed to the suppression of tumorigenesis in their study, independent of any direct chemoprotective effect of DHEA. In contrast, our controls were pair-fed on the basis of chow consumption by the DHEA-fed rats, allowing similar chow consumption. However, our controls had lower chow intake/g body weight than our DHEA-fed rats (Fig. 1). This relative reduction in chow intake in controls occurred because of the lower body weights in the DHEA-fed rats, attributable to reduced efficiency of food utilization produced by DHEA (46–48). As a consequence, it is theoretically possible that tumorigenesis was also suppressed in our pair-fed control rats, as compared with control rats fed ad libitum, thereby obscuring DHEA suppression of tumorigenesis. This explanation appears unlikely, however, because tumor outcome in our pair-fed control group was similar to the ad libitum fed control group in our previous study using the same experimental model (41). The precise explanation of the differing results in these two studies of DHEA and experimental colonic carcinogenesis is uncertain. Our findings do indicate that caution must be exercised in the interpretation of chemoprotection experiments when the candidate protective agent alters weight gain or body weight independently of food intake.

Chemoprotective agents which could halt the advancement of premalignant colorectal epithelium to malignancy (tertiary chemoprevention) have great potential value, particularly in patients with predisposing or premalignant conditions. Experimental colonic carcinogenesis models similar to our model have been used to simulate such patients and to identify promising compounds (35–37, 54). These compounds include difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase activity, and indomethacin and piroxicam, nonsteroidal anti-inflammatory drugs which inhibit prostaglandin synthesis. The failure of DHEA to provide chemoprotection in our model is not without precedent in other models of chemical carcinogenesis. Specifically, experimental tumorigenesis in lung (16), pancreas (18), and ovary (55) and hepatocarcinogenesis (56) have been reported to be enhanced by DHEA. Nonetheless, chemoprotective activity in our model simulating patients at high risk of colorectal carcinoma could have provided additional impetus for clinical trials of DHEA. Our findings suggest that additional preclinical evaluation is needed.

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