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Cancer Chemoprevention With the Adrenocortical Steroid Dehydroepiandrosterone and Structural Analogs

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Abstract Dehydroepiandrosterone (DHEA) is an adrenocortical steroid that produces broad-spectrum cancer chemopreventive action in mice and rats. In the mouse two-stage skin tumorigenesis model, DHEA treatment inhibits tumor initiation, as well as tumor promoter-induced epidermal hyperplasia and promotion of papillomas. There is considerable evidence that DHEA exerts its anti-proliferative and tumor-preventive action through the inhibition of glucose-6-phosphate dehydrogenase and the pentose phosphate pathway, which generate NADPH (required for mixed-function oxidase activation of chemical carcinogens, as well as for deoxyribonucleotide synthesis) and ribose 5-phosphate (also required for deoxyribonucleotide synthesis). Long-term DHEA treatment of mice also reduces weight gain (apparently by enhancing thermogenesis), and appears to produce many of the beneficial effects of food restriction, which have been shown to inhibit the development of many age-associated diseases, including cancer. Using the mouse two-stage skin tumorigenesis model, we found that adrenalectomy completely reverses the anti-hyperplastic and antitumor-promoting effects of food restriction. It is not unlikely that food restriction stimulates enhanced levels of adrenocortical steroids, such as the anti-inflammatory glucocorticoids and DHEA, which in turn mediate the tumor-inhibitory effect of underfeeding.

Key words: Adrenal gland, dehydroepiandrosterone, glucose-6-phosphate dehydrogenase

The human adrenal cortex secretes three classes of steroid hormone: glucocorticoid, mineralocorticoid, and the so-called adrenal androgens, dehydroepiandrosterone (DHEA) and DHEA-sulfate. Although the biological significance of the first two classes of steroids is well recognized, the physiological role of DHEA is obscure. DHEA *per se* is not androgenic, and only through conversion to steroids such as testosterone does it exert androgenicity. Over the past several years, a number of investigators [1,2] have demonstrated that DHEA has striking biological effects in laboratory animals unrelated to the actions of androgens, suggesting that the steroid may be an important adrenocortical hormone in mammals. This laboratory and others have found that oral administration of DHEA to laboratory mice and rats produces broad-spectrum cancer-preventive action, including inhibition of spontaneous mammary cancers [3] and chemically induced tumors of the lung [4], colon [5], thyroid [6], and liver [7]. Topical application of either DHEA or various synthetic congeners of DHEA on the backs of mice inhibits 7,12-dimethylbenz(*a*)anthracene (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin papillomas at both the initiation and promotion stages [8], as well as suppresses the formation of skin papillomas and carcinomas produced by multiple applications of DMBA [9].

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE INHIBITION

In addition to its cancer-preventive action, DHEA treatment of laboratory animals produces anti-obesity [10], anti-diabetic [11], anti-atherosclerotic [12], and immunomodulating effects [13,14]. Although the mechanism by which DHEA produces these diverse biological actions is not clear, one effect of DHEA is apparently critical to the anti-proliferative and cancer-preventive activity. DHEA is a potent uncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH), the rate-limiting enzyme in the pentose phosphate pathway [15]. This pathway is an important source of NADPH and ribose 5-phosphate. The coenzyme NADPH is an essential reductant for several enzymes that generate oxygen free radicals [16–20]. Probably as a result of G6PDH inhibition and lowering of the NADPH pool, DHEA inhibits mixed-function oxidase activation of chemical carcinogens [21, 22], the NADPH-dependent production of oxygen free radicals from paraquat [20], and the oxidative burst generation of superoxide anion O_2^- by stimulated neutrophils [23]. Topical DHEA treatment on the backs of mice inhibits DMBA initiation of skin tumors and retards the rate of binding of [^{3}H]DMBA to epidermal DNA [8], again probably as a result of inhibiting carcinogen activation as a consequence of lowering the NADPH pool.

INHIBITION OF TUMOR PROMOTION

The inhibition of tumor promotion by DHEA may also be a result of G6PDH inhibition. Both ribose 5-phosphate and coenzyme NADPH are required to synthesize ribonucleotides and deoxyribonucleotides; if DHEA exerts its anti-proliferative action through a reduction in ribo- and deoxyribonucleotide synthesis, then providing these nucleosides should reverse DHEA-induced growth inhibition. We have indeed found that DHEA-induced growth inhibition of HeLa cells is completely reversed by the addition of the deoxyribonucleosides adenine, guanine, cytosine, and thymine to the culture medium [24].

Gordon *et al.* [15] found that DHEA and 16α-bromoepiandrosterone, a much more potent G6PDH inhibitor than DHEA, inhibit the differentiation of cultured 3T3 L1 fibroblasts to adipocytes, and that this inhibition is overcome by adding the four ribonucleosides adenine, cytosine, guanine, and uracil [2]. These investigators reported that the intracellular levels of 6-phosphogluconate and other sugar phosphate intermediates of the pentose phosphate pathway are depressed by steroid treatment, and that introduction of 6-phosphogluconate into these cells via liposomes restores the level of the sugar phosphates, and partially reverses the differentiation block [25].

Garcea *et al.* [26] found that administration of nucleosides also reverses DHEA anti-proliferative effects *in vivo*. Preneoplastic foci are induced in rat liver by a single injection of dimethylnitrosamine, followed by treatment with 2-acetylaminofluorene, partial hepatectomy, and phenobarbital administration. If DHEA is administered during the phenobarbital treatment, there is a reduction both in the size of these foci and in the [^{3}H]thymidine labeling index of focus cells. A regimen of three daily intraperitoneal injections of the four ribo- or deoxyribonucleosides completely reverses the DHEA-induced inhibition of both the focus size and the [^{3}H]thymidine labeling index of focus cells [26].

This laboratory reported that topical application of the DHEA analog 16α-fluoro-5-androsten-17-one to mouse skin inhibits both TPA-stimulated epidermal hyperplasia and TPA-promoted skin papillomas, and that a mixture of the four deoxyribonucleosides administered in drinking water completely reversed the inhibition in hyperplasia and tumor formation [27]. Figure 1 shows the inhibition in TPA-induced hyperplasia in CD-1 mouse skin by topical application of the DHEA analog 16α-fluoro-5-androsten-17-one and reversal of the inhibition by deoxyribonucleoside administration. In this experiment, 7-week-old female CD-1 mice were given tap water with or without the four deoxyribonucleosides (2.1 μmol of each deoxyribonucleoside per ml of water). Fresh water containing deoxyribonucleosides was added every 2–3 days, and the amount of water consumed was determined. The backs of the mice were shaved. Three days after shaving and five days after initiating deoxyribonucleoside treatment, 2 μg of TPA in 0.2 ml of acetone was applied to the backs. Two hundred μg of 16α-fluoro-5-androsten-17-one in 0.2 ml of acetone (or acetone vehicle) was applied one hour prior to TPA treatment and again 24 hours later. Forty-

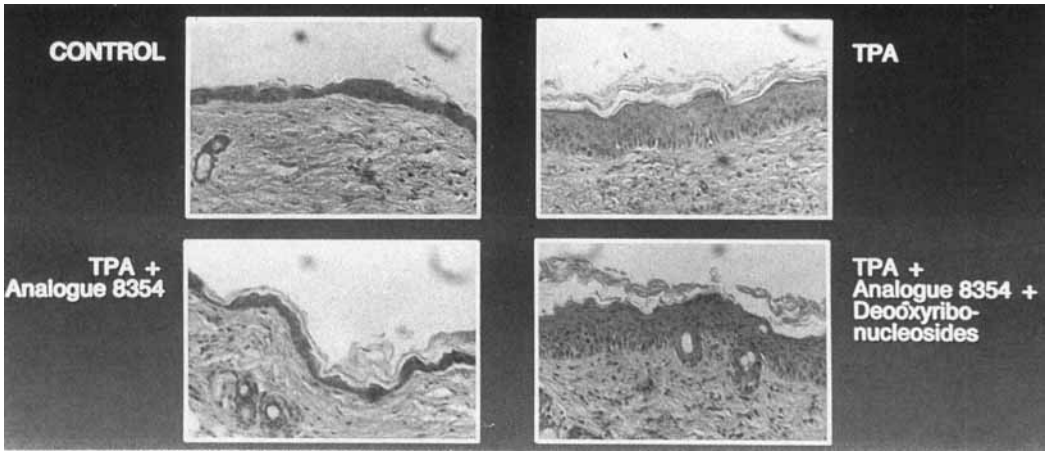

Fig. 1. Effect of deoxyribonucleoside administration on inhibition of TPA-induced hyperplasia in mouse skin by 16α-fluoro-5-androsten-17-one. Magnification is 200×.

eight hours after TPA treatment, the mice were sacrificed with an overdose of CO_2, and the skin was removed and processed for histological examination. As shown in Figure 1, the marked hyperplasia produced by TPA treatment appears virtually eliminated by 16α-fluoro-5-androsten-17-one, whereas deoxyribonucleoside administration restores the hyperplastic response.

All these studies strongly suggest that the anti-proliferative and antitumor-promoting action of the DHEA class of steroids results from suppressing nucleic acid synthesis as a consequence of the inhibition of G6PDH and the pentose phosphate pathway.

PHYSIOLOGICAL ROLE OF DHEA

DHEA treatment of laboratory mice and rats produces an anti-weight effect, apparently as a result of thermogenic stimulation and subsequent reduced metabolic efficiency [28]. Reducing the rate of weight gain of laboratory mice and rats through food restriction produces remarkable biological effects, including an inhibition in spontaneous and experimentally induced cancer formation [29], as well as a retardation of aging [30]. Long-term administration of DHEA to laboratory animals produces many of the beneficial effects of food restriction, including inhibition of tumor development in many different organs [1], inhibition of experimentally induced atherosclerosis [12], suppression of age-related proteinuria [31], inhibition of autoimmune disease development [32], and prolongation of the mean and maximal lifespan of mice [L. Pashko and A. Schwartz, manuscript in preparation].

Over 40 years ago, Boutwell *et al.* [33] reported that food restriction in mice activates adrenocortical activity as demonstrated by thymic involution, a decrease in blood lymphocyte counts, an enhanced gluconeogenesis rate, and an increase in the ratio of the adrenal gland to body weight. We hypothesize that elevated levels of DHEA contribute to the tumor-preventive and age-retarding effects of food restriction [34], and indeed have found that adrenalectomy of CD-1 mice completely reverses the antitumor-promoting effect of food restriction in the two-stage skin tumorigenesis model in CD-1 mice [35] (Figs. 2 and 3). Two classes of adrenocortical steroids, the glucocorticoids and DHEA, both repress TPA promotion of skin tumors in mice [8,36]; it is not unlikely that elevated levels of these steroids mediate the tumor-inhibitory effects of food restriction.

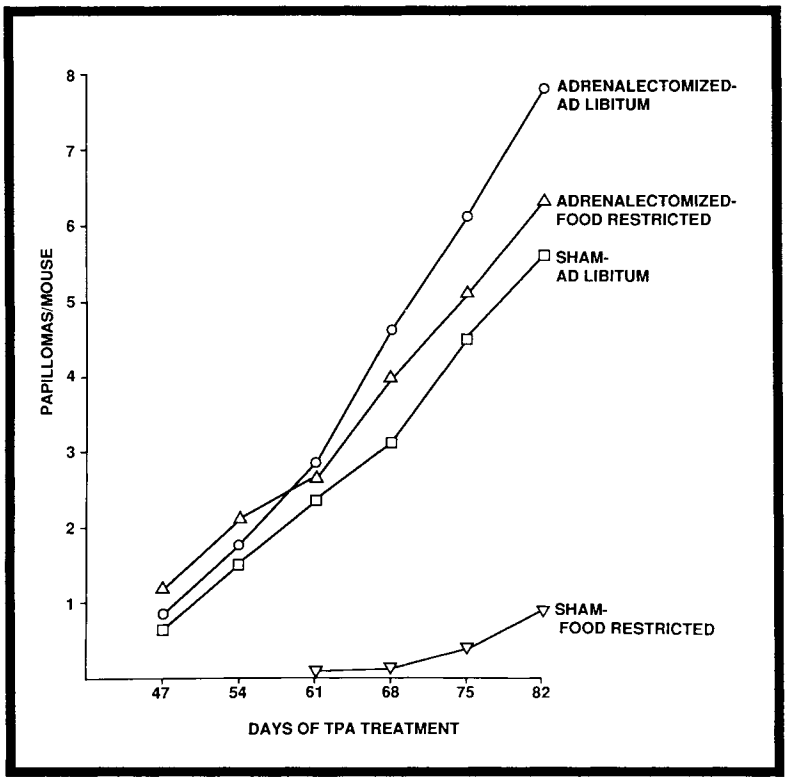

Fig. 2. Inhibition of skin papilloma development by food restriction and its reversal by adrenalectomy. Mice were initiated with DMBA and several days later were adrenalectomized or sham operated. One week after the operations, mice were fed *ad libitum* or food was restricted (27% reduction). Eight days after initiating food restriction, mice were treated topically twice weekly with TPA for the duration of the experiment. The mean number of papillomas versus time of TPA treatment are shown. The number of mice per group were: (1) sham operated, *ad libitum* fed, 42; (2) sham operated, food restricted, 38; (3) adrenalectomized, *ad libitum* fed, 42; and (4) adrenalectomized, food restricted, 40.

STRUCTURAL ANALOGS

The therapeutic use of DHEA in humans could produce undesirable sex hormonal effects. DHEA metabolizes to 4-androstene-3,17-dione, which is further converted to testosterone by 17β-hydroxysteroid dehydrogenase or metabolizes through a series of enzymatic reactions to estrone and 17β-estradiol. Treatment of young female rats with DHEA stimulates uterine enlargement as a consequence of estrogen conversion [37]. Administration of DHEA to castrated male rats increases seminal vesicle weight as a result of androgen formation [38]. Administration of pharmacological doses of DHEA to postmenopausal women for 28 days produced a 9-fold and 20-fold rise in plasma testosterone and dihydrotestosterone levels respectively, with a marked decline in high-density lipoprotein levels, as well as induction of insulin resistance, probably as a result of the induced androgenic state [39].

DHEA administration to mice and rats also produces hepatomegaly and stimulates peroxisome proliferation. DHEA, like other peroxisome proliferators, produces hepatocellular carcinomas following long-term administration to rats [40]. We have developed the synthetic steroid, 16α-fluoro-5-androsten-17-one, which does not produce uterine enlargement in young female rats or increase seminal vesicle weight in castrated male rats at highly active dosages [38]. 16α-Fluoro-5-androsten-17-one also does not produce hepatomegaly, nor does it stimulate peroxisome proliferation. When administered in the diet to CD-1 mice, the steroid is about three times as active as DHEA in inhibiting DMBA-initiated and TPA-promoted papilloma development at

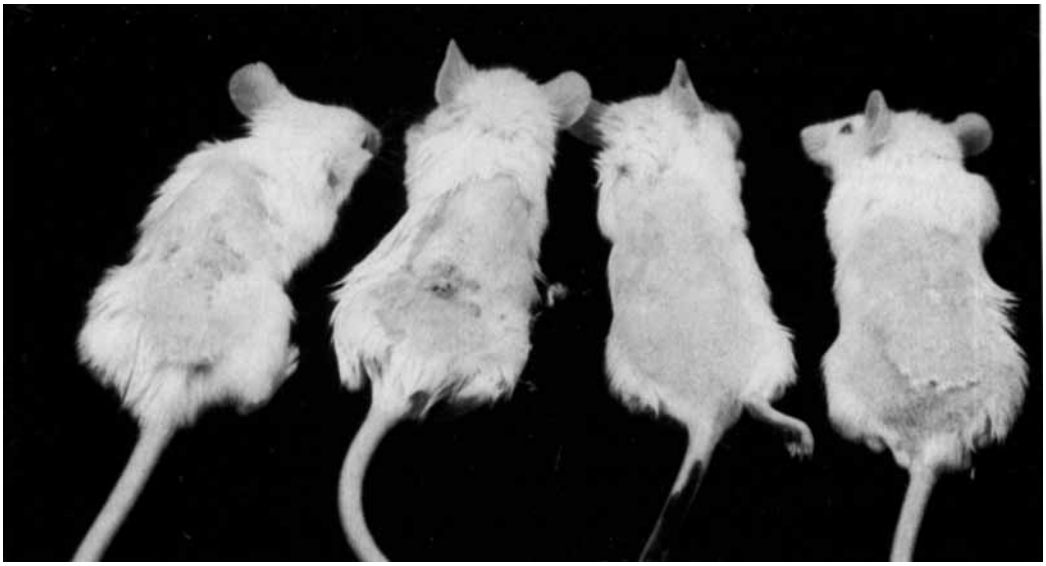

Fig. 3. Photograph of adrenalectomized, food restricted (two left mice) and sham operated, food restricted (two right mice) animals taken after 62 days of TPA treatment. Each mouse had the largest number of tumors in its respective group at the time.

both the initiation and promotion stages [41]. The steroid is also effective in inhibiting *N*-methyl-*N*-nitrosourea-induced mammary cancer development in female Sprague-Dawley rats [42] and is currently undergoing evaluation by the Chemoprevention Branch of the National Cancer Institute as a candidate for clinical trials [43].

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