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Cancer Prevention With Dehydroepiandrosterone and Non-Androgenic Structural Analogs

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Abstract There is increasing evidence that the adrenocortical steroid, dehydroepiandrosterone (DHEA), is an important mammalian hormone. Administration of DHEA to laboratory mice and rats inhibits development of experimental tumors of the breast, lung, colon, liver, skin and lymphatic tissue. In the two-stage skin tumorigenesis model in mice, DHEA treatment inhibits tumor initiation, as well as tumor promoter-induced epidermal hyperplasia and promotion of papillomas. There is much evidence that DHEA produces its antiproliferative and tumor preventive effects by inhibiting glucose-6-phosphate dehydrogenase and the pentose phosphate pathway. This pathway is an important source of NADPH, a critical reductant for many biochemical reactions that generate oxygen free radicals, which may act as second messengers in stimulating hyperplasia. The therapeutic use of DHEA in humans may be limited by its sex hormonal side effects. DHEA is metabolized *in vivo* to both testosterone and estrone, producing both androgenic and estrogenic effects in laboratory animals. We have developed a synthetic steroid, 16α-fluoro-5-androsten-17-one, which does not demonstrate the androgenic or estrogenic activity of DHEA, yet retains the antiproliferative and cancer preventive activity of the native steroid.

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

Reducing the food intake of laboratory mice and rats produces the most marked cancer preventive effect of any known regimen—it inhibits the development of spontaneous [1,2], chemically induced [1,3], and radiation-induced [4,5] tumors. Not only does underfeeding inhibit tumorigenesis, it also retards the rate of development of numerous age-related physiologic and pathologic changes and apparently retards the rate of aging [6]. Numerous theories have been proposed to account for the mechanism by which food restriction exerts its remarkable beneficial effects, but no satisfactory explanation has emerged [6].

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DEHYDROEPIANDROSTERONE

Over the past several years, this laboratory and others have demonstrated that administration of the adrenocortical steroid, dehydroepiandrosterone (DHEA), to laboratory mice and rats inhibits tumor development in the breast [7], lung [8], skin [9], liver [10], colon [11] and lymphatic tissue [12]. DHEA treatment of mice and rats also reduces the rate of weight gain [13,14]. This reduction of weight gain is not simply a result of impaired food intake but an apparent consequence of the stimulation of thermogenesis with a concomitant reduction in metabolic efficiency [15]. In addition to protecting animals against cancer, DHEA treatment produces many of the beneficial effects of food restriction, including an inhibition of experimentally induced atherosclerosis [16,17], suppression of age-related

proteinuria development [18], and inhibition of autoimmune disease development [19]. The anti-weight effect of DHEA, as well as its ability to produce many of the beneficial effects of food restriction, led us to hypothesize that elevated levels of DHEA in food-restricted rodents might partly mediate the tumor-suppressing and age-retarding effects of underfeeding [20].

We found previously that 12-*O*-tetradecanoylphorbol-13-acetate (TPA) stimulation of mouse epidermal hyperplasia (as measured by an increase in [^{3}H]thymidine incorporation or an increase in epidermal DNA content of a 2x2 cm^2 area of skin) is blocked by one to two weeks of food restriction prior to TPA application [21]. This relatively rapid response of the hyperplastic effect of the tumor promoter to underfeeding enables testing of experimental manipulations that might alter the responsiveness of skin tumor promotion to underfeeding.

ROLE OF ADRENOCORTICAL STEROIDS IN MEDIATING TUMOR-SUPPRESSING EFFECT OF UNDERFEEDING

As long ago as 1948, Boutwell *et al.* [22] reported that food restriction of mice appeared to activate adrenocortical activity, as evidenced by thymic involution, a decrease in blood lymphocyte counts, an enhanced gluconeogenesis rate, and an increase in the ratio of adrenal gland weight to body weight. We have found about a two-fold increase in plasma corticosterone levels in food-restricted CD-1 mice (28% less than *ad libitum* fed) after 11 weeks [23].

Our studies with CD-1 mice found that food restriction abolished TPA stimulation of epidermal [^{3}H]thymidine incorporation and markedly suppressed TPA promotion of papillomas. Adrenalectomy of mice prior to initiating food restriction completely reversed the inhibitory effect of underfeeding on TPA stimulated [^{3}H]thymidine incorporation and promotion of tumors, indicating clearly that the adrenal gland plays a critical role in inhibiting tumor promoter-induced hyperplasia and tumor promotion in food-restricted mice [23].

We also found that adrenalectomy completely reverses the tumor-inhibitory effect of food restriction in a lung tumor model. In this study, lung adenomas were induced in male A/J mice by a single oral dose of 0.5 mg of 7,12-dimethylbenz(*a*)anthracene. One week later the mice were either adrenalectomized or sham operated and thereafter fed *ad libitum* or given 27% less food than the *ad libitum*-fed group. Fourteen weeks later the mice were sacrificed and the number of alveolar adenomas counted. Food restriction reduced the number of adenomas four-fold in the sham-operated mice, whereas adrenalectomy completely abolished the effect of food restriction. Adrenalectomy also enhanced the number of alveolar adenomas; both the *ad libitum* and food-restricted adrenalectomized mice had twice the number of adenomas as the *ad libitum* sham-operated mice [unpublished observation].

Two adrenocortical steroids, corticosterone and DHEA, inhibit TPA-stimulated epidermal hyperplasia and TPA promotion of skin tumors in mice [9,24]. We hypothesize that overproduction of these steroids in response to food restriction accounts for the tumor inhibitory effect of underfeeding [25].

EFFECT OF GLUCOCORTICOID RECEPTOR ANTAGONIST

The synthetic steroid mifepristone, RU-486, is a potent progestin and glucocorticoid antagonist [26]. RU-486 has a very high binding affinity for the glucocorticoid receptor (about 31 times that of corticosterone and at least as high as potent agonists such as dexamethasone [27]). When administered *in vivo* to rats (at 10–25 mg/kg, po), RU-486 completely prevents the thymolytic activity of corticosterone and dexamethasone [28].

The availability of RU-486 enables testing of the hypothesis that elevated levels of glucocorticoid steroids contribute to the inhibition by food restriction of TPA-promoted skin tumors. We first determined that orally administered RU-486 reversed the suppression produced by corticosterone treatment in TPA-stimulated epidermal hyperplasia. Hyperplasia was measured by quantitating the epidermal DNA content of a 2x2 cm^2 section of mouse skin 48 hours after TPA application. RU-486 was administered po as a solution in sesame oil or given in the diet to yield a dose of approximately 25 mg/kg. As shown in Table I, 200 μg of topically applied corticosterone abolished the TPA stimulation in epidermal DNA content, and RU-486 completely reversed the inhibitory effect of corticosterone. This dose of RU-486 also had no effect on inhibition produced by

TABLE I. Effect of RU-486 Administration on Corticosterone-induced Suppression in TPA-Stimulated Epidermal DNA Content

Treatment Group	DNA Content of 2x2 cm^2 of Mouse Epidermis (μg)
Control (no TPA)	73.9 ± 9.1
TPA	132 ± 7.4
TPA + RU-486	138 ± 20.2
TPA + Corticosterone	59.8 ± 8.6
TPA + Corticosterone + RU-486	129 ± 12.7*

The backs of 6–7 week old CD-1 female mice were shaved, and only those mice showing no hair growth were used. Two days prior to TPA treatment, mice were given Purina 5015 chow or chow containing RU-486 (at a level yielding a dose of 23–24 mg/kg body weight). Two days later, mice were treated topically with 200 μg corticosterone in 0.2 ml acetone, or with acetone vehicle, one hour before TPA treatment, (2 μg in 0.2 ml of acetone). Mice received a second treatment of 200 μg corticosterone or acetone vehicle 24 hours later, and 24 hours after this, the mice were sacrificed. The epidermis was isolated from a 2x2 cm^2 piece of skin, and the DNA content was determined [38]. Each value is the mean ± S.D. for five separately treated mice.

* > TPA + Corticosterone group, $p < 0.001$.

Table II. Effect of RU-486 Administration on Food Restriction-induced Inhibition in TPA-Stimulated Epidermal DNA Content

Treatment Group	DNA Content of 2x2 cm^2 of Mouse Epidermis (μg)
Control	49.7 ± 12.3
TPA	138 ± 20.1
TPA + RU-486	151 ± 37.2
Food Restricted	49.6 ± 7.2
Food Restricted + TPA	39.7 ± 9.5
Food Restricted + TPA + RU-486	121 ± 23.5*

Six to seven week old female CD-1 mice were singly housed and given *ad libitum* access to Purina 5015 chow or food-restricted with daily allotments of 62% of the food consumed by the *ad libitum* fed animals. RU-486-treated mice received RU-486 daily in the chow to give a dose of 25 mg/kg body weight. Eight days after initiating food restriction and RU-486 treatment, mice were treated on their shaved backs with 2 μg TPA in 0.2 ml acetone or with acetone vehicle. Forty-eight hours later, the mice were sacrificed and epidermal DNA content of a 2x2 cm^2 section of mouse skin was determined [38]. Each value is the mean ± S.D. of five separately treated mice.

* > Food Restricted + TPA group, $p < 0.001$.

DHEA treatment in TPA-induced epidermal hyperplasia (data not shown).

As mentioned previously, food restriction of CD-1 mice blocks TPA-stimulated epidermal hyperplasia and inhibits TPA promotion of papillomas. We found that RU-486 administration, at doses that completely reversed corticosterone-induced suppression of TPA-stimulated epidermal hyperplasia, significantly reversed the inhibition produced by eight days of food restriction in TPA-stimulated hyperplasia. We have consistently observed this effect of RU-486, whether the drug is administered po in sesame oil or in the diet, and these data strongly suggest that elevated corticosterone levels in food-restricted mice significantly inhibit TPA-induced epidermal hyperplasia (Table II).

We then determined if RU-486 administration in the diet significantly reversed the suppression produced by food restriction in papilloma development, and much to our surprise, we found no effect of RU-486 treatment on food restriction-induced suppression in skin tumor formation (Fig. 1). Since it seemed very likely to us that any agent which reversed epidermal hyperplasia suppression induced by food restriction would also reverse tumorigenesis inhibition, we determined the effect of RU-486 administration on TPA-induced hyperplasia following four weeks of food restriction. We found that RU-486 treatment during a four-week period of food restriction had no effect on suppressing TPA-induced hyperplasia produced by underfeeding (Table III).

In humans, RU-486 blocks the inhibitory feedback effect of cortisol on corticotrophin secretion, leading to an increase in plasma cortisol and corticotrophin levels [29]. Thus, it is possible that plasma corticosterone levels were sufficiently elevated in long-term RU-486-treated animals to overcome the RU-486 blockade of glucocorticoid receptors. Alternatively, elevated levels of DHEA

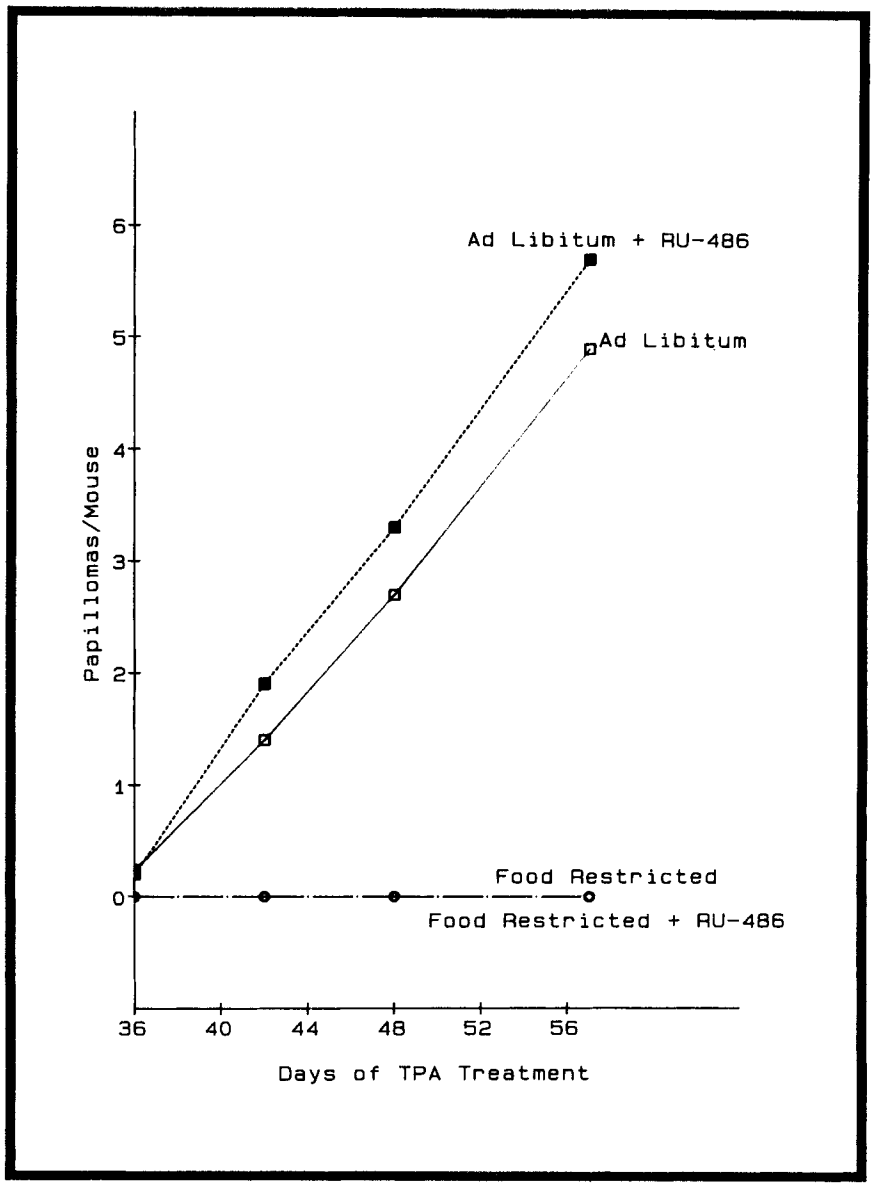

Fig. 1. Effect of RU-486 administration on food restriction-induced suppression in TPA promotion of mouse skin papillomas. Female CD-1 mice were initiated on the shaved backs with 200 nmol 7,12-dimethylbenz(*a*)anthracene in 0.2 ml acetone. Seven days later, the mice were given *ad libitum* access to chow or restricted with daily allotments of 56% of the food consumed by the *ad libitum*-fed mice. One group of *ad libitum*-fed mice and one group of food-restricted mice also received RU-486 in the diet to yield a daily dose of drug of 24 ± 1.5 mg/kg body weight. Eight days after carcinogen initiation, all mice were treated with twice weekly applications of 2 μg TPA in 0.1 ml acetone. Mice were palpated weekly for papillomas, and the mean number of tumors per group was determined. There were 23 mice in each of the RU-486-treated groups, and 25 mice in each of the non-RU-486-treated groups.

Table III. Effect of RU-486 Administration on Food Restriction-induced Inhibition in TPA-Stimulated Epidermal DNA Content (Four Week Food Restriction)

Treatment Group	DNA Content of 2x2 cm^2 of Mouse Epidermis (μg)
Control	53.3 ± 12.1
TPA	120 ± 20.4
Food Restricted	42.7 ± 5.6
Food Restricted + TPA	43.5 ± 8.5
Food Restricted + TPA + RU-486	39.0 ± 13.8

The experimental conditions were the same as in Table II except that mice were food restricted and treated with RU-486 for four weeks instead of eight days.

or a DHEA-like steroid in the RU-486 food-restricted mice may contribute to the inhibition of TPA-induced epidermal hyperplasia and TPA promotion of tumors. The inhibition in TPA-induced hyperplasia and tumor promotion produced by DHEA administration, unlike the inhibition produced by corticosterone, is not reversed by RU-486 treatment (unpublished results).

BIOLOGICAL SIGNIFICANCE OF DEHYDROEPIANDROSTERONE

DHEA, along with its sulfate ester, is secreted by the human adrenal cortex in quantities at least as great as all the other adrenocortical steroids combined [30]. There is increasing evidence that this substance may be an important adrenocortical hormone [31]. The plasma level of DHEA sulfate is highest in the second decade, then declines markedly [32]. Epidemiological studies suggest that low plasma levels of this steroid may predispose individuals to develop certain cancers [33,34] and die from cardiovascular disease [35].

DHEA is a potent uncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase [36], the rate-controlling enzyme in the pentose phosphate pathway. This pathway is an important source of NADPH as well as ribose 5-phosphate. Inhibition of this pathway by DHEA apparently accounts for the antiproliferative and antitumor promoting actions of the steroid [37,38]. Probably as a result of a reduction in NADPH levels, DHEA, also inhibits mixed-function oxidase activation of chemical carcinogens [39], the NADPH-dependent production of oxygen free radicals from paraquat [40], and the oxidative burst generation of O_2^- [41].

There is increasing evidence that oxygen free radicals contribute to the development of many age-related diseases, including cancer [42], atherosclerosis [43], and the familial autosomal form of amyotrophic lateral sclerosis, as well as other neurodegenerative diseases [44]. Oxygen free radicals, aside from directly damaging DNA and producing mutations [45], may also serve as second messengers stimulating hyperplasia [46]. The importance of oxygen free radicals in tumor promotion is underscored by the marked suppression of TPA promotion of skin tumor formation by treatment with a low molecular weight copper chelate with superoxide dismutase activity [47].

We have postulated that the glucocorticoid steroids suppress inflammation, an important source of oxygen free radicals; DHEA inhibits the pentose phosphate pathway and the generation of NADPH, a critical reductant for reactions generating oxygen free radicals [45,48–50]. Together they may mediate the tumor suppressing and possibly the age-retarding effects of underfeeding [25].

DEHYDROEPIANDROSTERONE ANALOGS

The striking biological effects of DHEA in laboratory animals suggest that this steroid, or specific structural analogs, could become an important class of drug in humans. DHEA was efficacious in treating systemic lupus erythematosus and type II diabetes in early clinical trials [51-53]. However, DHEA has side effects that may limit its use in humans. It is metabolized into both testosterone and estrone and produces estrogenic and androgenic effects in laboratory rats [54]. Pharmacological doses of DHEA given to six postmenopausal women for 28 days produced a marked elevation of plasma testosterone (9-fold) and dihydrotestosterone (20-fold) [55]. DHEA treatment induced insulin resistance in these women and significantly lowered plasma HDL levels, very likely as a result of the andro-

genic state induced. DHEA is also a peroxisome proliferator; its long-term administration to Fischer rats induces a high incidence of hepatocellular carcinoma [56].

We have developed the synthetic steroid, 16α-fluoro-5-androsten-17-one, which does not demonstrate the androgenic, estrogenic, or peroxisome-proliferating properties of DHEA, yet has retained the antiproliferative, cancer preventive, anti-obesity and anti-diabetic actions of the native steroid [54,57,58]. Compounds such as 16α-fluoro-5-androsten-17-one may become important therapeutic agents in humans.

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