Effect of Dehydroepiandrosterone on Bone Mass, Serum Lipids, and Dimethylbenz(a)anthracene-Induced Mammary Carcinoma in the Rat*

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ABSTRACT

The present study investigated the effect of dehydroepiandrosterone (DHEA) on bone mass and serum lipids in the rat with dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma. The animals received DHEA once daily, percutaneously, at the dose of 5, 10, or 20 mg for 9 months following a single dose of 20 mg DMBA at 50–52 days of age. Bone mineral content (BMC) and bone mineral density (BMD) of total skeleton, lumbar spine, and femur were measured by dual energy x-ray absorptiometry. A 9-month treatment with DHEA increased BMC and BMD of total skeleton by 14.2% to 14.5% (all P < 0.01) and 6.7% to 8.3% (all P < 0.01), respectively. Similarly, femoral BMC and BMD were stimulated by 13.6% to 14.7% (all P < 0.05) and by 8.1% to 9.5% (all P < 0.01), respectively. In addition, BMD of lumbar spine was increased by 10.4% to 10.8% (all P < 0.05), whereas the 9.4% to 11.1% increment in BMC of lumbar spine was not statistically significant. Treatment with DHEA led to 26% (NS), 60% (P < 0.01), and 62% (P < 0.01) decreases in serum triglyceride levels at the same doses. On the other hand, no significant change in serum cholesterol concentrations was observed. Two hundred and seventy-nine days after DMBA administration, the incidence of mammary carcinoma had decreased from 95% in control animals to 73% (P < 0.05), 57% (P < 0.01), and 39% (P < 0.01) at the daily percutaneous doses of 5, 10, and 20 mg of DHEA, respectively. Moreover, the mean tumor number per tumor-bearing animal and the mean tumor area per tumor-bearing animal were also reduced by the same treatments. DHEA increased serum total alkaline phosphatase activity and decreased urinary calcium excretion, but had no effect on the urinary ratio of hydroxyproline to creatinine and urinary phosphorus excretion.

These data show that DHEA exerts a stimulatory effect on bone mass and an inhibitory effect on serum triglycerides, as well as a preventive effect on the development of mammary carcinoma induced by DMBA in the rat. Such data suggest that while decreasing the risk of breast cancer, DHEA replacement therapy could also exert beneficial effects on the bone and lipid metabolism in women receiving DHEA replacement therapy. (Endocrinology 138: 3387–3394, 1997)

Dehydroepiandrosterone-Sulfate (DHEA-S) is the major secretory product of the human adrenal gland, and its concentration in the serum is higher than that of any other steroidal hormone in men and women. In fact, the serum DHEA-S concentration is 200–1,000 times higher than that of testosterone in adult men and 5,000–25,000 times higher than that of 17β-estradiol in adult women. Following a peak value reached during the third decade, it is well documented that serum DHEA and DHEA-S markedly decrease with age in both men and women. By the age of 70 yr, serum DHEA-S levels have decreased to approximately 20% of their maximal levels (1–9).

DHEA-S and DHEA are metabolized into active androgens and/or estrogens in target intracrine tissues, where they exert their action inside the same cells in which synthesis takes place and without being released to the extracellular space (8–12). This new area of endocrinology has been called intracrinology (10, 12). The level of transformation of the inactive precursors DHEA-S and DHEA is dependent on the intracellular activities of the steroidogenic enzymes involved, namely steroid sulfatase, 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase, 17β-hydroxysteroid dehydrogenase, 5α-reductase, and aromatase (7–9, 12). It has been calculated that approximately 50% of androgens in adult men derive from the peripheral conversion of DHEA-S and DHEA into the androgens testosterone and dihydrotestosterone (DHT), whereas in women the best estimate of the proportion of estrogens and androgens synthesized from DHEA and DHEA-S is 75% before menopause and close to 100% after menopause (12).

A number of studies suggest that the marked decline in the serum levels of DHEA could be involved in the pathogenesis of diseases associated with aging, including cancers, and a series of other conditions such as obesity, autoimmune disease, fatigue, loss of muscle mass, insulin resistance, poor immune response, and reduced longevity (13–17). Long-term administration of DHEA has been shown to protect against some cancers in animal models of tumorigenesis, including skin, liver, lung, and colon carcinomas (18–24).

Postmenopausal osteoporosis is a common complication associated with significant morbidity and mortality and an increasing negative impact resulting from aging of the population (25). Postmenopausal women are also at a high risk for coronary heart disease (26), which has been at least partially attributed to an increase in serum lipids (27). Estrogen replacement therapy in postmenopausal women is currently considered the standard therapy at menopause, specifically to decrease the rate of bone loss as well as to protect against the risk of coronary heart disease (28). However, there are a number of undesirable effects associated with chronic estro-
Animals ad libitum to clot at 4 C overnight before centrifugation at 3000 rpm for 30 min. The animal received Purina rodent chow and tap water ad libitum. The animal studies were conducted in a Canadian Council on Animal Care (CCAC)-approved facility in accordance with the CCAC Guide for Care and Use of Experimental Animals.

Induction of mammary tumors by DMBA

Mammary carcinomas were induced by a single intragastric administration of 20 mg of DMBA (Sigma Chemical Co., St. Louis, MO) in 1 ml corn oil at 50–52 days of age. Two months later, tumor measurement was performed biweekly. The two largest perpendicular diameters of each tumor were recorded with calipers to estimate tumor size as described (32). Tumor site, size, and number were recorded.

Treatments

The rats were randomly divided into the following groups, each containing 20 animals with the exception of the control group, which contained 40 animals: vehicle-treated control (group 1) or DHEA (administered percutaneously, once daily) at the doses of 5 mg (group 2), 10 mg (group 3), or 20 mg (group 4) in 0.5 ml 50% ethanol-50% propylene glycol for 282 days. Treatment was initiated 3 days before the oral administration of DMBA. DHEA was purchased from Steroidals (Wilton, NH). Many of the control animals and some of the DHEA-treated rats were killed by decapitation under isoflurane-induced anesthesia 6 months after DMBA administration because the size of tumors was too large. The information about tumors (size and number) of these rats was included in the analysis of the incidence of tumors, average tumor number per tumor-bearing animal, and average tumor size per tumor-bearing animal. The remaining animals (9 rats for the control group and 13–18 rats for each other group) continued to receive treatment for another 3 months and were killed at the end of the experiment, which was 279 days after DMBA administration. The uteri, ovaries, and vaginae were immediately removed, freed from connective and adipose tissue, and weighed.

Sample collection and processing

Twenty-four-hour urinary samples were collected from the first nine rats of each group transferred into metabolic cages (Allentown Caging Equipment Co., Allentown, NJ) one week before the end of the experiment. Two 24-hour urinary samples were collected and analyzed on different days for each rat to minimize the influence of daily variation. Therefore, each value shown represents the mean of two measurements performed on two different days. Toluene (0.5 ml) was added into the collecting tubes to prevent urine evaporation and bacterial growth. The urine volume was recorded, and trunk blood was collected and allowed to clot at 4 C overnight before centrifugation at 3000 rpm for 30 min.

Analysis of urine and serum biochemical parameters

Fresh samples were used for the assay of urinary creatinine, calcium, and phosphorus, as well as serum total alkaline phosphatase activity (TALP), cholesterol, and triglycerides. These biochemical parameters were measured automatically with a Monarch 2000 Chemistry System (Instrumentation Laboratory Co., Lexington, MA) under good laboratory practice conditions. Urinary hydroxyproline was measured as described (33).

Bone mass measurements:

Rats were anesthetized with an ip injection of ketamine hydrochloride and diazepam at doses of 50 and 4 mg/kg BW, respectively. The whole skeleton and femur were scanned using dual energy absorptiometry (DEXA; QDR 2000–7.10C, Hologic, Waltham, MA) equipped with a regional high resolution software. The scan field sizes were 28.110 × 17.805 and 5.0 × 1.902 cm, the resolution was 0.1511 × 0.0761 and 0.0254 × 0.0127 cm, and the scan speeds were 0.3508 and 0.056 mm/sec for the total skeleton and femur, respectively. Both bone mineral content (BMC) and bone mineral density (BMD) of total skeleton, lumbar spine, and femur were determined on the scan images of total skeleton and femur.

RIAs

Serum steroid concentrations were measured by RIAs following methanol and diethyl ether extraction and chromatography on LH-20 columns as described in detail elsewhere (34).

Statistical analysis

The data are presented as means ± SEM, and statistical significance was calculated according to the multiple range test of Duncan-Kramer (35). Analysis of the incidence of development of mammary tumors was performed using the Fisher’s exact test (36).

Results

Effect on bone mass

BMC of total skeleton in rats treated with DHEA at the doses of 5, 10, and 20 mg were 14.2% to 14.5% higher (P < 0.01 at all doses) than that observed in control animals (Fig. 1A). Similarly, increases ranging from 6.7% to 8.3% (P < 0.01 for all groups) were found on BMD of total skeleton in the same animals (Fig. 1B). Daily treatment with DHEA caused 13.6% to 14.7% (P < 0.05 for all groups) stimulations of femoral BMC as well as 8.1% to 9.5% (P < 0.01 for all groups) stimulations of femoral BMD at the three doses of DHEA (Fig. 2). In addition, BMD of lumbar spine was increased by 10.4% to 10.8% (P < 0.05 for all groups) by DHEA, whereas the 11.1%, 9.4%, and 10.1% increases recorded for BMC of lumbar spine did not reach the level of statistical significance (Fig. 3).

Effect on bone mineral metabolism

As shown in Table 1, treatment with DHEA decreased urinary calcium excretion by 41% (P < 0.05) at the highest dose of 20 mg, whereas no significant effect was observed at the two lower doses. On the other hand, the urinary ratio of hydroxyproline to creatinine and urinary phosphorus excretion were not significantly affected by DHEA treatment. However, DHEA treatment stimulated serum total ALP levels by 26% (NS), 74% (P < 0.05), and 62% (P < 0.05), respectively, at the doses of 5, 10, and 20 mg. DHEA had no effect on urinary creatinine excretion (data not shown).
Effect on serum lipid levels

The daily 5-mg dose of DHEA induced a statistically non-significant 26% decrease in serum triglyceride levels, whereas 60% ($P < 0.01$) and 62% ($P < 0.01$) reductions were achieved by the daily 10- and 20-mg doses, respectively (Fig. 4). In contrast, the DHEA treatment failed to significantly alter serum cholesterol concentrations (Fig. 4).

Effect on the development of rat mammary carcinoma induced by DMBA

As illustrated in Fig. 5, 279 days after DMBA administration, 95% of control rats had developed palpable mammary carcinoma. In contrast, treatment with increasing doses of DHEA caused a progressive inhibition of the development of tumors, and the incidence was thus
reduced to 73% (P < 0.05), 57% (P < 0.01), and 38% (P < 0.01), respectively, with the 5-, 10-, and 20-mg doses of DHEA. It is of interest to see in Fig. 6 that tumor number per tumor-bearing animal decreased from 4.7 ± 0.5 tumors in the control group to 2.9 ± 0.4 (P < 0.05), 3.4 ± 0.6 (NS), and 2.4 ± 0.5 (P < 0.05) tumors in the above-indicated groups, respectively. On the other hand, the average tumor area per tumor-bearing animal was reduced from 12.8 ± 1.3 cm² in control animals to 9.7 ± 2.2 (NS), 10.2 ± 2.1 (NS), and 5.2 ± 1.1 (P < 0.05) cm² by the same treatments.

**Serum steroid levels**

As shown in Table 2, daily treatment with DHEA at the doses of 5, 10, and 20 mg for 9 months resulted in marked increases in serum DHEA levels. Although supraphysiological serum levels of androstenedione, testosterone, and DHT were observed at the daily 20-mg dose of DHEA, the values achieved at the 5- and 10-mg doses of DHEA were within the physiological range. Serum 17β-estradiol values, in contrast, were within the physiological range at all doses of DHEA.

**Effect on tissue and BW**

Table 3 shows total body, uterine, vaginal, ovarian, and adrenal weights measured at the end of the experiment. Nine-month treatment with the 5-, 10-, and 20-mg doses of DHEA stimulated vaginal weight by 11.3% (NS), 13.9% (P < 0.05), and 15.8% (P < 0.01), respectively. In contrast, the 20-mg dose of DHEA led to 15.3% (P < 0.01) inhibition of uterine weight, whereas no significant effect was observed at the two lower doses. Ovarian and adrenal weights were not significantly affected by DHEA treatment at the doses used.

**Discussion**

The higher incidence of osteoporosis in women is usually thought to be secondary to the fall in ovarian function at menopause, whereas the marked decline in DHEA-S and DHEA secretion by the adrenals is also likely to play an important role (7). The two major risk factors related to osteoporosis in women are believed to be a low bone mass already present at menopause and a fast rate of bone loss after menopause. The present data showing that DHEA increases both BMC and BMD of total skeleton as well as of femur and lumbar spine in the rat, suggest that DHEA given as a preventive measure could possibly reduce the risk of fracture by increasing bone mass at menopause.

The concentration of serum tALP activity has been used as a marker of bone formation (37, 38), whereas the urinary excretion of calcium has been used as a marker of bone resorption (37, 39). Because serum tALP activity was elevated, whereas the urinary excretion of calcium was reduced after long-term treatment with DHEA, one could speculate...
that DHEA exerts dual actions on bone, namely stimulating bone formation while at the same time inhibiting bone resorption. The effect of DHEA on bone resorption could require a higher dose than that on bone formation, because only the highest dose (20 mg) of DHEA decreased the urinary excretion of calcium, whereas the middle dose (10 mg) of DHEA caused a significant elevation of the serum tALP concentration.

It is recognized that both androgens and estrogens can preserve bone mass at menopause. However, the protective effect of androgens and estrogens on bone is mediated through different mechanisms. Androgens are thought to mainly induce stimulation of bone formation (40), whereas estrogens act primarily by reducing bone loss and secondarily bone turnover (41, 42). The present data are in agreement with the findings of a stimulation of femoral bone density on the ovariectomized rat after 12 weeks of 0.3% DHEA in the diet (43). It is possible that the protective effect of DHEA observed in the present study is achieved mainly via androgens synthesized in bone tissue (12), thus increasing bone formation. Such a mechanism of action of DHEA is well supported by the observation that the stimulatory effect of DHEA on bone mass can be blocked almost completely by the simultaneous administration of the antiandrogen flutamide (44).

DHEA given at 100 nM (a supraphysiological concentration) has been found to reduce the basal levels of c-fos messenger RNA in normal human osteoblastic cells, whereas 10 nM DHT or 10–20 nM testosterone had no effect (45). Although specific inhibitors of androgen and/or estrogen action were not used in that study, and the absence of effect of androgens on that parameter, the suggestion was made that DHEA as well as DHEA-S were converted into testosterone and DHT in the osteoblastic cells. In fact, estrogens as well as androgens have been found to stimulate the expression of c-fos in human osteoblastic (46) and osteocarcinoma (47) cells. Boccuzzi et al. (48) reported an inhibition of DMBA-induced tumor growth in intact rats by DHEA, whereas a stimulatory effect was found in ovariectomized animals. It should be mentioned that the dose used in that study (2 mg, orally, twice daily) was extremely low (see Ref. 49), as confirmed by the serum levels measured (0.4–0.5 ng/ml), thus making it somewhat unlikely that DHEA could have a significant role in the effects reported. It should also be mentioned that the association between low circulating levels of DHEA and DHEA-S and breast cancer described in some studies (18, 50–52) was not observed in other reports (53, 54).

In agreement with the role of androgens in DHEA action, the presence of androgen receptors in cells of osteoblast origin has been reported (55, 56). Moreover, testosterone can be converted to DHT in bone cells in vitro (57). It should also be mentioned that treatment with DHT stimulates endochondral bone development in orchidectomized rats (58), whereas both testosterone and DHT increase the transcript-
TABLE 2. Effect of daily percutaneous administration of 5, 10, or 20 mg DHEA for 9 months on serum DHEA, androstenedione (4-DIONE), androst-5-ene-3β,17β-diol (5-DIOL), testosterone (TESTO), dihydrotestosterone (DHT), and estradiol levels in the rat

<table>
<thead>
<tr>
<th>Serum steroids (nmol/liter)</th>
<th>Control (0)</th>
<th>DHEA (mg)</th>
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<tbody>
<tr>
<td>DHEA</td>
<td>2.49 ± 0.32</td>
<td>200 ± 38.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4-DIONE</td>
<td>&lt;1.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.57 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>5-DIOL</td>
<td>1.44 ± 0.18</td>
<td>171 ± 39.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TESTO</td>
<td>&lt;0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.44 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DHT</td>
<td>&lt;1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.96 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Estradiol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>293 ± 35.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>334 ± 31.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM. <sup>a</sup> P < 0.05; <sup>b</sup> P < 0.01 vs. respective control. <sup>c</sup> Minimal detectable level. <sup>d</sup> pmol/liter.

DHEA was administered percutaneously, thus avoiding the first pass through the liver, and possibly leading to different effects than those observed when DHEA is administered orally and for shorter time periods. In the human, oral noncontraceptive estrogens lower low-density lipoprotein (LDL) and have various effects on HDL (Ref. 69 and references cited therein). Estrogen is thought to increase HDL by increasing apoprotein A-1 synthesis in the liver and reducing the activity of hepatic lipase, the enzyme that catabolizes HDL. Circulating LDL levels are decreased by an estrogen-stimulated increase in LDL receptors and an estrogen-inhibited decrease in apoprotein B synthesis in the liver (69). Estrogen increases the production of triglyceride-rich very low-density lipoprotein (VLDL), therefore, frequently increasing triglyceride levels by 30%, an observation that is associated with the rapid clearance of VLDL, with no resultant increase in the remaining particles or LDL. Such an effect is therefore not considered to be atherogenic (27, 69, 70). In contrast to the effect of estrogens on human HDL, estrogens decrease HDL in the rat (71). This difference is due to the presence of ApoE, an apoprotein with high affinity for the LDL receptor, which is present at a much higher level in the rat as compared with human HDL (71).

In contrast, androgens have, in general, opposite effects on serum lipid metabolism in the human (72, 73). Androgen decreases VLDL and triglyceride levels, probably by increasing lipoprotein lipase activity, a lipolytic enzyme found primarily in adipose tissue, and, perhaps, by enhancing hepatic VLDL synthesis as well. Androgen also decreases HDL<sub>2</sub> and total HDL levels, probably by increasing the activity of the enzyme hepatic triglyceride lipase. Compared with normal women, those suffering from polycystic ovary syndrome, who have increased levels of free testosterone and insulin, also have increased triglyceride and VLDL-cholesterol (C), and decreased HDL-C levels. In these women, the dyslipidemia appears to be related to both androgen excess and hyperinsulinemia (74). In the present study, because treatment with DHEA lowered serum triglyceride levels, the effect of DHEA on serum lipid metabolism appears closer to the effect of androgens rather than the effect of estrogens.

Although the present data clearly show that long-term administration of DHEA increases bone mass and decreases serum triglyceride levels while preventing mammary carcinoma induced by DMBA in the rat, the precise mechanisms remain to be determined. As mentioned earlier, DHEA can be metabolized into androgens and/or estrogens to exert its actions in a specific fashion in each peripheral target intracrine tissue (12). Such specific activities of DHEA are achieved through the action of the steroidogenic enzymes.
specifically expressed in such tissues (7–9). It is also possible that DHEA elicits part of its inhibitory effects by decreasing the function of ovarian estrogen secretion. This mechanism of action seems unlikely at the doses of DHEA used, because ovarian weight was not affected significantly by DHEA treatment. Finally, DHEA could potentially act directly through binding to a specific receptor. However, until now, no such protein has been reported in the liver, bone, or mammary gland, although a still uncharacterized DHEA binding protein has been reported in murine T lymphocytes (75) and rat brain (76). Our findings that the stimulatory action of DHEA in the rat ventral prostate and seminal vesicles (10, 11) and bone can be completed reversed by simultaneous administration of the antiandrogen flutamide (44) strongly suggest that the action of DHEA, at least in these tissues, is mediated by its conversion into testosterone and DHT and specific activation of the androgen receptor.

In agreement with our previous data (31), the present study shows that DHEA prevents carcinogenesis induced by DMBA in the rat. Treatment with DHEA delays the carcinogenesis and decreases the incidence of palpable mammary carcinomas following DMBA administration. The mechanisms by which DHEA prevents DMBA-induced carcinogenesis are not fully understood. However, it has been found that androgens exert a direct antiproliferative activity on the growth of ZR-75–1 human breast cancer, and such inhibitory effect of androgens is additive to that of an antiestrogen in vitro (77, 78) and in vivo in nude mice (79). Moreover, androgens have been shown to inhibit the growth of DMBA-induced mammary carcinoma in the rat and that such inhibition is reversed by the simultaneous administration of the antiandrogen flutamide (79). As mentioned above, DHEA is well known to possess androgenic activity, and treatment with DHEA induces androgen-sensitive gene expression in the rat ventral prostate (10, 11). Taken together, these data strongly suggest that DHEA exerts its chemopreventive action through its conversion to androgens and activation of the androgen receptor.

The present data clearly demonstrate that treatment with DHEA, in addition to inhibiting the development of DMBA-induced mammary carcinoma in the rat, increases bone mass and decreases serum triglyceride levels. Such data suggest that the androgenic action of DHEA has the potential of exerting in parallel beneficial effects on three important aspects of women’s health, namely prevention of breast cancer, osteoporosis, and atherosclerosis. Although the present data obtained in the rat are encouraging, comparable data in the human remain to be obtained at physiological levels of DHEA and under chronic treatment conditions in a large population of subjects. However, it should be mentioned that the stimulatory effects of DHEA on bone mineral density have already been obtained in postmenopausal women treated with percutaneous DHEA for 12 months (80).

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References

DHEA AND BONE, LIPIDS, AND DMBA-INDUCED MAMMARY TUMORS

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