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DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: Its role during aging

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Human and some other primates are unique since their adrenals secrete large amounts of dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S), which are converted into androstenedione (4-dione) and then into potent androgens and estrogens in peripheral tissues, therefore providing autonomous intracrine control to target tissues that can adjust the formation and metabolism of active sex steroids according to local requirements. Knowledge in this area has recently made rapid progress with the elucidation of the structure of most of the tissue-specific cDNAs and genes that encode the steroidogenic enzymes responsible for the transformation of these inactive precursor steroids into androgens and/or estrogens. It is estimated that 30 to 50% of total androgens in men are synthesized in peripheral intracrine tissues from inactive adrenal precursors while, in women, peripheral estrogen formation is even more important, the best estimate being 75% before menopause and 100% after menopause. The marked reduction in the formation of DHEA-S by the adrenals during aging, especially before the age of 50 years, results in a dramatic fall in the formation of active sex steroids in peripheral target tissues, a situation which is thought to be associated with a long series of age-related decreases such as insulin resistance, obesity, osteoporosis, cardiovascular diseases, loss of muscle mass, cancer and other diseases. We have demonstrated for the first time a series of medically important beneficial effects of DHEA administered for 12 months to post-menopausal women. Most interestingly, the bone mineral density significantly increased. This relatively rapid change was associated with an increase in plasma osteocalcin, a marker of bone formation, while a decrease in bone resorption reflected by a decrease in urinary hydroxyproline excretion was observed in parallel. In addition, the estrogenic stimulation of vaginal cytology in the absence of any sign of stimulatory effect on the endometrium is also of potentially major interest for the prevention and management of menopause. Furthermore, the inhibitory effect of DHEA on the growth of human breast cancer xenografts in vivo in nude mice supports the beneficial use of DHEA as hormone replacement therapy in women. (*Steroids* **63**:322–328, 1998)
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Intracrinology

A discovery of major importance in the field of sex steroids is that human and some other primates are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids DHEA, its sulfate (DHEA-S), and androstenedione (4-dione), which are converted into potent androgens and estrogens in peripheral tissues (Figure 1). The secretion of DHEA and DHEA-S by the adrenals increases during the adrenarche in children at the age of 6 to 8 years, and elevated values of circulating DHEA-S and DHEA are maintained throughout adult life, providing high levels of substrates for conversion into potent androgens and estrogens in peripheral tissues.

In fact, plasma DHEA-S levels in adult men and women are 100 to 500 times higher than those of testosterone and 1000 to 10,000 times higher than those of estradiol, thus providing a large reservoir for conversion into androgens and/or estrogens in peripheral intracrine tissues. The term intracrinology was coined in 1988[1] to describe the synthesis of active steroids in cells of peripheral target tissues in which the action of these steroids is exerted without release in the extracellular space and general circulation.[2]

Knowledge in this area has recently made rapid progress, with the elucidation of the structure of most of the tissue-specific cDNAs and genes that encode the steroidogenic enzymes responsible for the transformation of DHEA-S and

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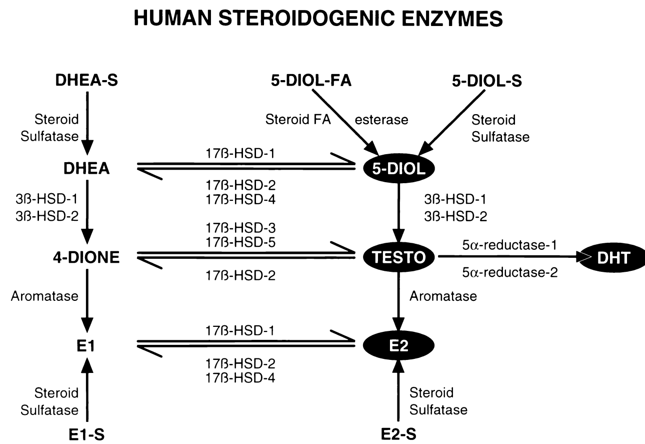

Figure 1 Human steroidogenic enzymes in peripheral intracrine tissues (reproduced with permission, Reference 5),

DHEA into androgens and/or estrogens in peripheral tissues.[3–7]

The marked reduction in the formation of DHEA-S by the adrenals during aging[8,9] results in a dramatic fall in the formation of androgens and estrogens in peripheral target tissues, a situation that is thought to be associated with age-related diseases such as insulin resistance[10] and obesity.[11] Moreover, low circulating levels of DHEA-S and DHEA have been found in patients with breast[12] and prostate[13] cancer. DHEA has also been found to exert antioncogenic activity in a series of animal models.[14] On the other hand, a stimulatory effect of DHEA on the immune system has been described in postmenopausal women.[15] Moreover, the oral administration of DHEA has been reported to have beneficial effects in older men and women.[16–18]

The rate of formation of each sex steroid thus depends upon the level of expression of specific androgen- and estrogen-synthesizing enzymes in each tissue. Through intracrine activity, locally produced androgens and/or estrogens exert their action inside the same cells in which synthesis takes place without the need for release and dilution into the extracellular compartment and the general circulation[2,19] (Figure 2).

As illustrated in Figure 1, the formation from DHEA of the most potent natural androgen dihydrotestosterone (DHT) or the most potent natural estrogen 17β-estradiol (E_2) involves several enzymatic activities, namely 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD), 17β-HSD, 5α-reductase, and/or aromatase. Because the molecular structure of the key non-P-450-dependent enzymes in sex steroid formation has not been elucidated and knowing that local formation of sex steroids plays a major role in both normal and tumoral hormone-sensitive tissues, an important proportion of our research program has recently been devoted to this exciting and therapeutically promising area (for reviews, see References 3, 6, 20). It is important to mention that 40% of all cancers, namely breast, prostate, ovarian, and uterine cancers, are sex steroid-sensitive and are thus prime candidates for approaches based upon control of intracrine activity. The field of intracrinology should generate major interest in the pharmaceutical industry in terms of developing specific inhibitors of enzymatic activity that could potentially be combined with more potent and specific antiestrogens and antiandrogens.[21,22]

The implication is that the adrenals of humans and some other primates secrete large amounts of adrenal steroids, especially DHEA sulfate, that are metabolized into active androgens and estrogens in peripheral mammalian. It is estimated that 30 to 50% of total androgens in men are synthesized in peripheral intracrine tissues from inactive adrenal precursors whereas in women peripheral estrogen formation is even more important, the best estimate being 75% before menopause and 100% after menopause.[2,23,24]

This investigation led to the first x-ray structure determination of a mammalian steroidogenic enzyme. The structure of type 1 17β-HSD from human placenta was determined at 2.20Å resolution by a combination of isomorphous replacement (with a single mercury derivative) and molecular replacement techniques. The core of the structure is the seven-stranded parallel β-sheet (βA to βG), surrounded by six parallel α-helices (αB to αG), three on each side of the β-sheet.[5]

The isolation of multiple 17β-HSDs strongly favors the proposal that intracellular formation and degradation of androgens and estrogens play a crucial role in the regulation of cell function and proliferation. The isoenzymes of the 17β-HSD family represent key steps in the regulation of the intracellular concentration of active androgens and estrogens, thereby controlling specific cell activity and function. Due to their pivotal role in the pathways of sex steroid formation, the various types of 17β-HSDs provide each peripheral cell with the required mechanisms to control its own development, growth, and function (See Reference 5 for review).

Most of the decline in circulating DHEA occurs before the age of 60 years

To gain a better knowledge of the role of DHEA and DHEA-S transformation in both men and women, we ana-

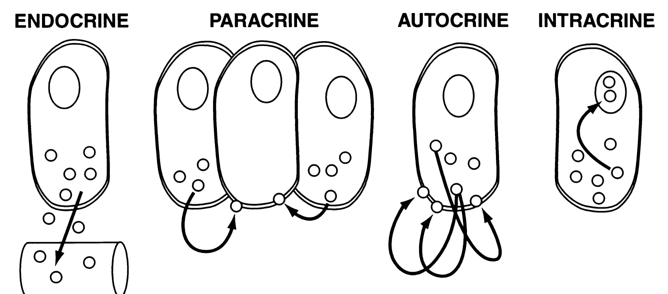

Figure 2 Schematic representation of endocrine, paracrine, autocrine, and intracrine secretion. Classically, endocrine activity includes the hormones secreted in specialized glands for release into the general circulation and transport to distant target cells. In addition, hormones released from one cell can influence neighboring cells (paracrine activity) or can exert a positive or negative action on the same cell (autocrine activity). Intracrine activity describes the formation of active hormones that exert their action in the same cells without release into the pericellular compartment (reproduced with permission, Reference 2).

lyzed the serum levels of 18 conjugated C21- and C19-steroids.[25] We thereby wanted to assess precisely the changes occurring in the serum concentration of these steroids of particular interest over a range of ages from the peak value of adrenal secretion of DHEA and DHEA-S (20–30 yr) to the near lowest values found at 70 to 80 years.

The data obtained show a dramatic decline in the circulating levels of DHEA, DHEA-sulfate (DHEA-S), androst-5-ene-3β,17β-diol (5-diol), 5-diol-S, 5-diol fatty acid esters, and androstenedione in both men and women between the ages of 20 and 80 years. In the 50- to 60-year-old group, serum DHEA has already decreased by 74% and 70% from its 20- to 30-year-old peak values in men and women, respectively (Figure 3). The serum concentrations of the conjugated metabolites of DHT, namely androsterone (ADT)-G, androstane-3α,17β-diol (3α-diol-G), androstane-3β,17β-diol (3β-diol-G), and ADT-sulfate are the most reliable parameters of the total androgen pool in both men and women whereas serum testosterone and dihydrotestosterone can be used as markers of testicular secretion in men and interstitial ovarian secretion in women, respectively. The serum concentration of these various conjugated androgen metabolites decreased by 40.8 to 72.8% between the 20 to 30 and 70 to 80 age groups in men and women, thus suggesting a parallel decrease in the total androgen pool with age. As estimated by measurement of the circulating levels of these conjugated metabolites of DHT, it is noteworthy that women produce approximately 66% of the total androgens found in men: in women, most of these androgens originate from the transformation of DHEA and DHEA-S into testosterone and DHT in peripheral intracrine tissues while, in men, the testes and DHEA + DHEA-S provide approximately equal amounts of androgens at the age of 50 to 60 years. An additional potentially highly significant observation is that the majority of the marked decline in circulating adrenal C_{19} steroids and in their resulting androgen metabolites takes place between the age groups of 20 to 30 years and 50 to 60 years with smaller changes observed after the age of 60 years.[25]

It seems appropriate at this stage to represent schematically the distribution of androgen precursors, active androgens (T and DHT) and their metabolites in blood and peripheral tissues (Figures 4 and 5).

Application of DHEA as hormonal replacement therapy in women

One of the most serious problems that is associated with aging is osteoporosis, which causes morbidity and mortality mainly through increased fracture rates.[26] Estrogen replacement therapy, which is commonly used against osteoporosis does, however, require the addition of progestins to counteract the endometrial proliferation induced by estrogens used alone. Moreover, since both estrogens and progestins are thought to increase the risk of breast cancer[27,28], we have studied the effect of 12-month administration of DHEA to 60- to 70-year old women on bone mineral density and other parameters of bone formation and turnover as well as on

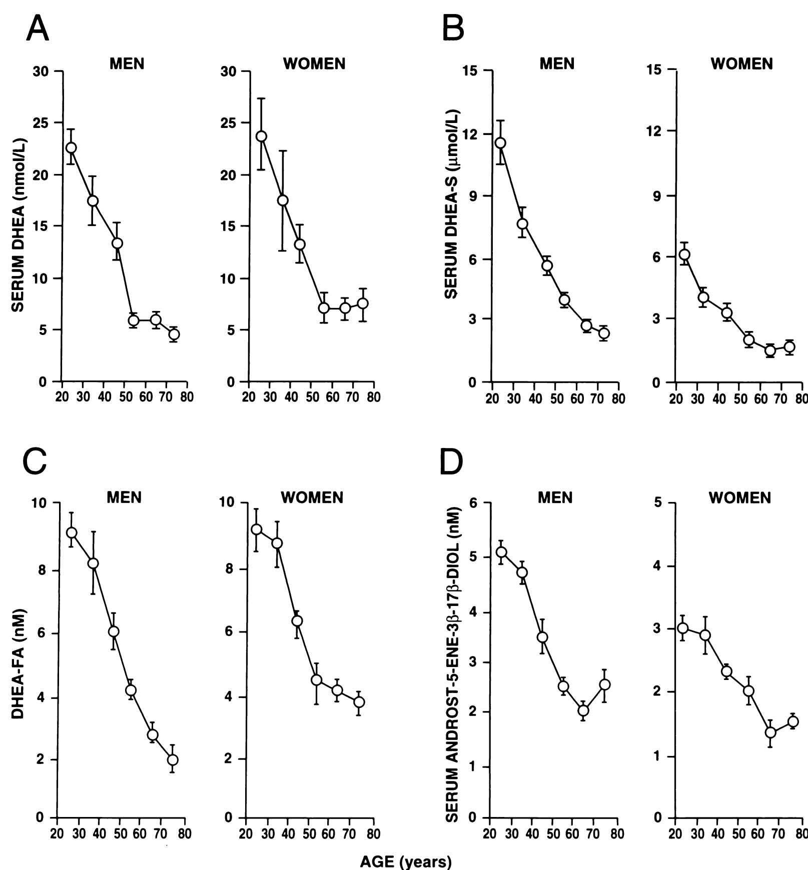

Figure 3 Effect of age (20- to 30-year-old versus 70- to 80-year-old) on serum concentration of DHEA (A), DHEA-S (B), DHEA-fatty acid esters (DHEA-FA (C), and 5-diol (D) in men and women (reproduced with permission, Reference 36).

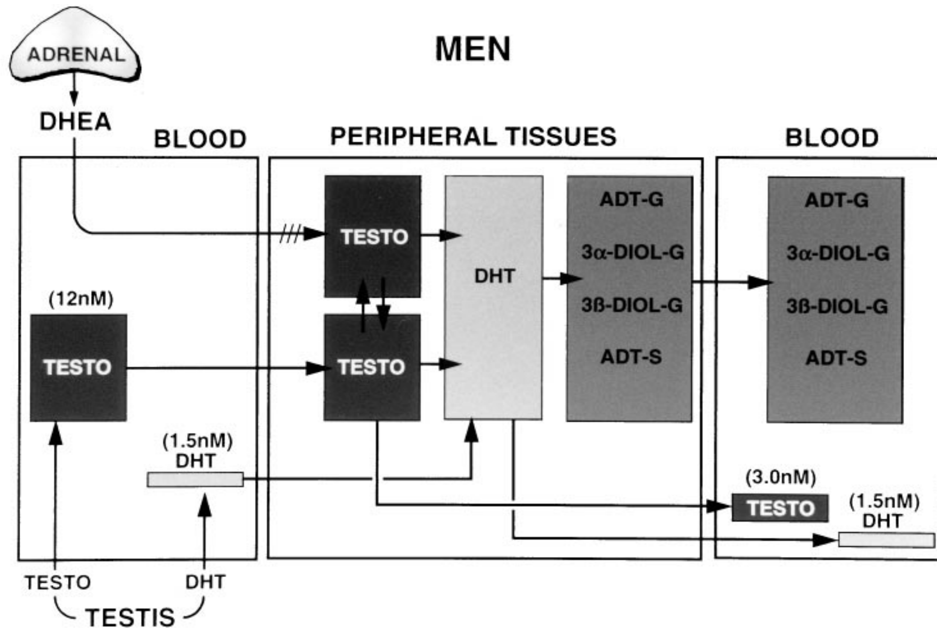

Figure 4 Distribution in men of the active androgens T and DHT, the sex steroid precursor DHEA and the main metabolites of androgens (ADT-G, 3α-diol-G, and 3β-diol-G) in the circulation and peripheral intracrine tissues. The height of the bars is proportional to the concentrations of each steroid or its derivatives in individual compartments.

vaginal and endometrial histology. DHEA was administered percutaneously to avoid first passage of the steroid precursor through the liver.[17,18]

Vaginal epithelial maturation was stimulated by DHEA administration in 8 of 10 women with a maturation value of zero at the onset of therapy, and a stimulatory effect was seen in all three women who had an intermediate vaginal maturation index before therapy. The estrogenic effect of DHEA observed in the vagina was not observed in the endometrium which remained atrophic in all women. Most interesting, the bone mineral density significantly increased at the hip from 0.744 ± 0.021 g/cm^2 to 0.759 ± 0.025 g/cm^2 after 12 months of treatment ($p < 0.05$). These changes in bone mineral density were associated with a significant 20%

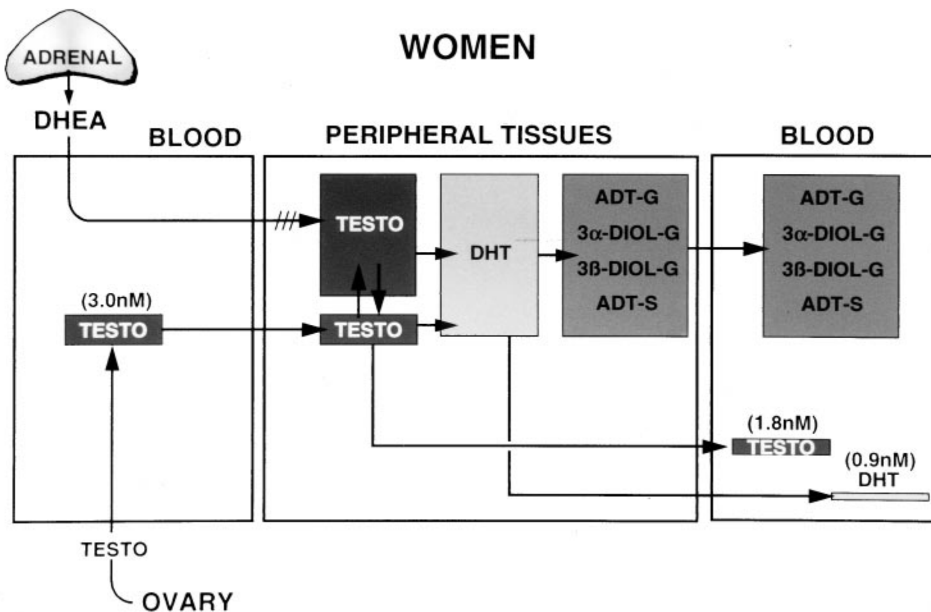

Figure 5 Distribution in women of the active androgens T and DHT, the sex steroid precursor DHEA, and the main metabolites of androgens (ADT-G, 3α-diol-G and 3β-diol-G) in the circulation and in peripheral intracrine tissues. The height of the bars is proportional to the concentrations of each steroid or its derivatives in individual compartments.

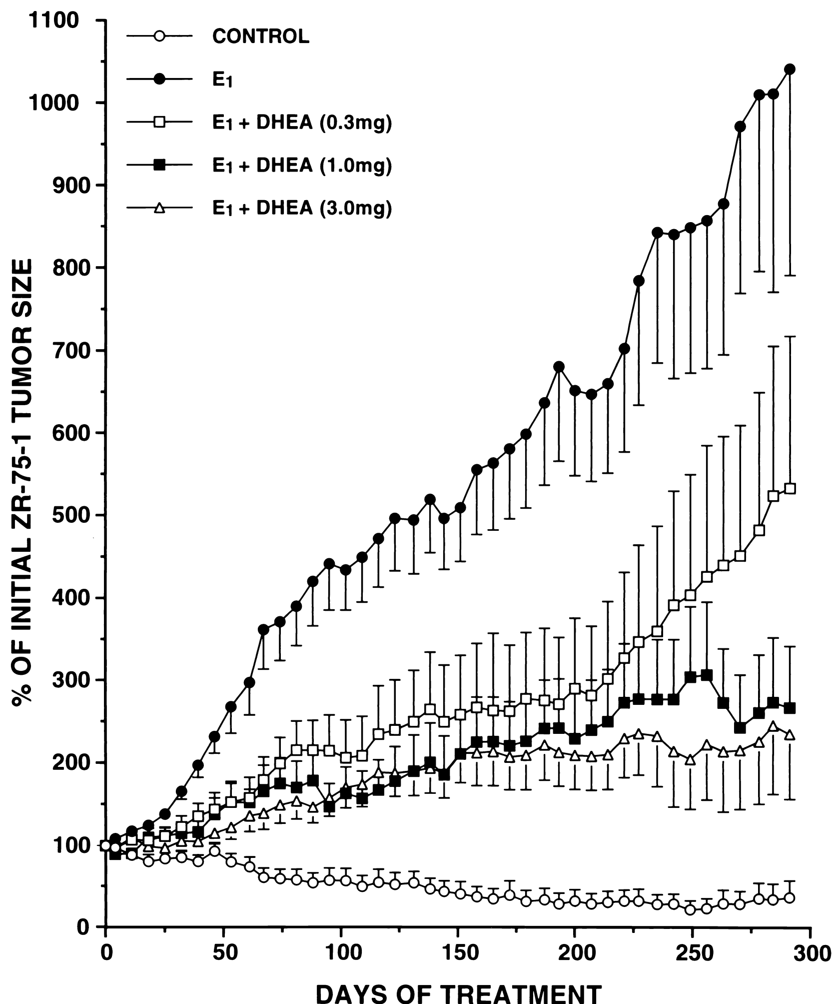

Figure 6 Effect of increasing oral doses of DHEA (0.3, 1.0, or 3.0 mg) for 9.5 months on average ZR-75–1 tumor size in ovariectomized (OVX) nude mice supplemented with estrone. The initial tumor size was taken as 100% OVX mice receiving the vehicle alone were used as additional controls. Estrone was administered subcutaneously at the dose of 0.5 μg once daily whereas DHEA was dissolved in 50% ethanol: 50% propylene glycol and applied on the dorsal skin area twice daily in a volume of 0.02 mL. Comparison is also made with OVX animals receiving the vehicle alone.

decrease ($p < 0.01$) in plasma bone alkaline phosphatase and a 28% decrease in the urinary hydroxyproline/creatinine ratio. A 2.1-fold increase over control ($p < 0.01$) in plasma osteocalcin was concomitantly observed.[18] The present data describe for the first time a series of medically important beneficial effects of DHEA therapy in postmenopausal women through transformation of the precursor steroid DHEA into androgens and/or estrogens in specific peripheral intracrine tissues without significant adverse effects. The stimulatory effect on the vaginal epithelium in the absence of stimulation of the endometrium is of particular interest since it eliminates the need for progestin replacement therapy. On the other hand, the stimulatory effect on bone mineral density accompanied by an increase in serum osteocalcin, a marker of bone formation, suggests stimulation of bone formation by the androgenic action of DHEA, a finding of particular interest for both the prevention and treatment of osteoporosis.

Considering the major concern related to the stimulatory effect of estrogens on endometrial proliferation with the related risk of endometrial carcinoma[29,30], an endometrial biopsy was performed before starting treatment and after 12 months of DHEA administration. The endometrial atrophy seen in all women at start of treatment remained unaffected by 12 months of DHEA administration.

The present study describes for the first time a series of medically important beneficial effects of DHEA administered for 12 months to postmenopausal women. Possibly the most important effect could be the DHEA-induced stimulation of bone mineral density. The relatively rapid change in bone mineral density is accompanied by an increase in the value of a marker of bone formation, namely serum osteocalcin concentration, while a decrease in bone resorption reflected by a decrease in urinary hydroxyproline excretion was observed in parallel. In addition, the estrogenic stimulation of vaginal cytology in the absence of any sign of stimulatory effect on the endometrium is also of potentially major interest for the prevention and management of menopause. Our data also confirm the beneficial effects of DHEA on energy and well-being reported previously.[16]

As far as serum lipids are concerned, we have observed a small but not statistically significant decrease in serum triglycerides, cholesterol, and lipoprotein components up to 12 months of percutaneous administration of DHEA.[17] The present data certainly suggest that DHEA treatment has no deleterious effects but rather indicate a trend toward positive effects on the serum lipid and lipoprotein profile, although a larger cohort of subjects is needed to reach definitive conclusions. On the other hand, it should be mentioned that conjugated equine estrogens raise triglyceride levels

and show a deterioration of the insulin response.[31,32] In fact, our data show an inhibitory effect of DHEA treatment on fasting blood glucose and insulin levels[17], thus suggesting an advantage over equine estrogens on glucose metabolism.

The present data clearly suggest the interest of a new approach to hormone replacement therapy having potentially improved efficacy and tolerance. It is possible that DHEA replacement therapy could not only correct but also prevent the multiple problems associated with menopause, a phenomenon preceded and accompanied by a decreased formation of both androgens and estrogens during aging in women.

Prevention and/or inhibition of breast cancer growth

Since, as mentioned above, androgens inhibit breast cancer, we have studied the possibility that DHEA could inhibit the growth of the human breast cancer ZR-75–1 cell line in vivo in nude mice. To avoid the inhibitory effects of DHEA on gonadotropin secretion, and to assess the direct effects of DHEA-derived steroids on breast cancer growth, we have used ovariectomized animals supplemented with estrone.

Estrone by itself caused a 9.4-fold increase in ZR-75–1 tumor area after 9.5 months of treatment whereas the daily oral administration of 15, 50, or 100 μg of the pure antiestrogen EM-800[33–35] in estrone-supplemented mice led to inhibitions of 88, 93, and 94%, respectively. DHEA, at the doses of 0.3, 1.0, and 3.0 mg, inhibited tumor weight by 67, 82, and 85%, respectively (Figure 6). The combination of a daily 15-μg oral dose of EM-800 with the three doses of topical DHEA produced 84 to 94% inhibitions of estrone-stimulated ZR-75–1 tumor growth, these values being not significantly different from the 88% inhibition achieved by EM-800 alone.

The inhibitory effect of DHEA on the growth of human breast cancer xenografts supports the use of DHEA as hormone replacement therapy in women. Moreover, since the administration of DHEA did not interfere with the inhibitory effect of EM-800 on ZR-75–1 tumor growth, combined treatment with DHEA and EM-800 could possibly be a convenient therapy in symptomatic postmenopausal women with breast cancer.

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