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Chronic administration of dehydroepiandrosterone (DHEA) to female monkey and rat has no effect on mammary gland histology

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Abstract

Dehydroepiandrosterone (DHEA), the major steroid precursor of androgens and estrogens produced in peripheral tissues in primates, has been shown to exert chemopreventive effect on the development of carcinogen-induced rat mammary tumors. Since little is known on the effect of DHEA administration on mammary gland physiology and histology, we have studied the effect of long-term administration of DHEA to normal female monkey and rat on mammary gland histology as well as on serum DHEA, DHEA sulphate (DHEA-S), testosterone and estradiol levels. In monkeys, DHEA treatment (2 or 10 mg/(kg b.w. day)) induced a dose-related increase in serum DHEA and DHEA-S (above 20-fold) levels. At the highest dose of DHEA, serum testosterone levels were significantly increased (three- to fourfold), while serum estradiol concentration was not modified. DHEA treatment did not modify the histological characteristics of monkey mammary glands. In the rat, following DHEA administration (10 or 100 mg/(kg b.w. day)), a dose-related marked increase in serum DHEA and DHEA-S was observed. Serum testosterone was also increased in DHEA-treated animals, while no significant changes in serum estradiol levels were detected. As in the monkey, the histology of the female rat mammary gland remained unchanged following long-term treatment with any of the two doses of DHEA.

Keywords: DHEA; Mammary gland; Intracrinology; Androgens; Estrogens; Histology

1. Introduction

It is well documented that dehydroepiandrosterone (DHEA) and its sulfated derivative DHEA-S produced by the adrenals in primates are converted into potent androgens and/or estrogens in peripheral tissues [1,2]. The local synthesis and action of sex steroids in peripheral tissues have been named intracrinology [2,3]. In women it is estimated that intracrine formation of estrogens is of the order of 75% before menopause and 100% after menopause [2,4]. Moreover, in women the majority of androgens are synthesized locally, DHEA administered orally or percutaneously to postmenopausal women have been shown to produce beneficial effects on the bone, skin, glucose metabolism, muscle mass, adiposity and well-being [5–8].

Estrogens play a predominant role in the growth of the mammary gland as well as breast cancer. In postmenopausal women, the role of peripheral estrogen formation is clearly illustrated by the major benefits of aromatase inhibitors in breast cancer therapy [9–11], these important benefits being necessarily due to the inhibition of local estrogen synthesis. There is also much evidence that androgens exert an opposite (inhibition) role on breast epithelial cell proliferation. In the rhesus monkey model, this primate being the only species which secretes DHEA in large amounts, the administration of the pure antiandrogen flutamide significantly increased by twofold the proliferation index of epithelial cells [12]. Moreover, testosterone administration completely blocked the 3.5-fold stimulation of epithelial cell proliferation induced by estradiol in the mammary gland of ovariectomized monkeys [12].

Androgens have been successfully used for the treatment of breast cancer in women in adjuvant therapy with an efficacy comparable to that achieved with other types of

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endocrine manipulation [13–16]. In animal models, DHEA has been shown to inhibit mammary gland carcinogenesis. Treatment with increasing doses of DHEA induced a progressive inhibition of the development of DMBA-induced mammary carcinoma in the rat [17]. On the other hand, we have already reported that chronic treatment (12 months) of ovariectomized rats stimulated alveolar and ductal growth as well as the secretory activity of the acinar cells [18], an effect believed to be species-specific and limited to the rat. Since this stimulatory effect was completely inhibited by the concomitant administration of the pure antiandrogen flutamide, it was concluded that it was related to the conversion of DHEA to androgens.

In order to clarify the potential influence of DHEA on the mammary gland in intact animals, we studied the effect of chronic administration of DHEA to intact female monkey and rat on mammary gland histopathology and circulating estradiol and testosterone levels.

2. Materials and methods

2.1. Animals

Monkeys: Twelve young adult to adult female cynomolgus monkeys (*Macaca fascicularis*) 6–18-year-old were received from Charles River Biomedical Resources Foundation (Houston, TX; two monkeys) and from Health Canada-Health Protection Branch (Ottawa, Ont.; 10 monkeys). At dosing initiation, the mean body weight of the female monkeys was 5.0 kg (range: 2.9–7.4 kg). The morning meal of monkeys consisted of four certified primate cookies (Lab Diet Certified High-Fiber Primate Diet 5K91®; PMI Nutrition International Inc., St. Louis, MO) and half a banana. The afternoon meal consisted of four cookies and was provided 6 h (±1 h) after the morning meal distribution. Water was available *ad libitum*.

Rats: Female Sprague–Dawley rats (Crl:CD®(SD) IGS BR) 6–7-week-old were obtained from Charles-River, Inc. (St-Constant, Que., Canada). At dosing initiation, the mean body weight was 174 g (range: 149–195 g). Rats received 17 g/day of a certified commercial rodent diet (powdered feed) (Lab Diet Certified Rodent Diet No. 5002; PMI Nutrition International Inc., St. Louis, MO), and water was available *ad libitum*.

Monkeys and rats were allowed to acclimatize to laboratory conditions for at least 5 days prior to dosing initiation. The animals were housed individually in stainless steel cages of appropriate dimensions and they were on a 12 ± 1-h light/dark light cycle (lights on at 07:15 h). Temperature in the monkey and rat rooms was 23 ± 3 °C and 22 ± 3 °C, respectively, while relative humidity was 50 ± 20% for both species. Food consumption was recorded daily while body weights were recorded once a week throughout the rat and monkey studies. These studies were conducted in a Canadian Council on Animal Care (CCAC)-accredited facility in accordance with the CCAC Guide for the Care and Use of Experimental Animals, United States Food and Drug Administration Good Laboratory Practice Regulations (21 CFR Part 58), and the Standard Operating Procedures of the Laboratory of Molecular Endocrinology.

2.2. Treatments

Monkeys: Monkeys were assigned to three groups of four animals each on a stratified random basis (using a computer-generated program) according to the most recent body weights. The monkeys were allowed to feed for 20–30 min following morning meal distribution and they were then dosed orally by nasogastric intubation once daily with vehicle alone (0.4% (w/v) aqueous methylcellulose; group 1) or with dehydroepiandrosterone (DHEA; Micron Technologies, Exton, PA, USA) suspended in the vehicle at a dose of 2 mg/(kg b.w. day) (group 2) or 10 mg/(kg b.w. day) (group 3). Each monkey received a certified primate snack (treat) post-dose. The monkeys were dosed for 359 days. Individual dose volumes were calculated and adjusted every 2 weeks according to the most recent body weights. Doses used in this study were selected according to a previous pharmacokinetic study of DHEA in monkeys [19].

Rats: Two hundred and twenty-eight rats were assigned to 3 groups of 76 animals each (10 for tissue collection, 48 for Study Day 1 steroid analysis and 18 for Study Day 175 steroid analysis) on a stratified random basis (using a computer-generated program) according to the most recent body weights. Animals were dosed orally by gavage once daily with vehicle alone (0.4% (w/v) aqueous methylcellulose; group 1) or with DHEA suspended in the vehicle at a dose of 10 mg/(kg b.w. day) (group 2) or 100 mg/(kg b.w. day) (group 3). The animals were dosed for 1 day or 175 days. Individual dose volumes were calculated and adjusted weekly according to the most recent body weights. Doses were selected according to the results obtained in some previous rat studies performed with DHEA [18,20].

2.3. Steroid analyses

Monkeys: Blood samples for steroid analysis were collected at the femoral vein from each monkey on Study Day (SD) 1 predose and at target intervals of 1, 2, 3, 4, 6, 12 and 24 h post-dose as well as on SD 184 and SD 359 at the same target intervals mentioned above.

Rats: Blood samples for steroid analysis were collected at the jugular vein on SD 1 predose and at target intervals of 1, 2, 3, 4, 6, 12 and 24 h post-dose from six rats per time point. On SD 175, blood samples were collected at the same target intervals as on SD 1 but each animal was used for four-scheduled blood collections instead of being used only once.

Blood samples collected from rats and monkeys were processed for serum preparation and kept at −80 °C until