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Effects of intravaginal dehydroepiandrosterone on vaginal histomorphology, sex steroid receptor expression and cell proliferation in the rat☆

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Abstract

Objective: Recent clinical studies have shown that postmenopausal hormone therapy with estrogen plus progestogen increases breast cancer risk. Moreover, intravaginal estrogen-containing pills, creams and rings lead to significant systemic exposure to estrogen, thus indicating the need for a completely novel approach to alleviate vaginal atrophy in postmenopausal women.
Design: We have studied the effect of intravaginal application of dehydroepiandrosterone at daily doses of 0.33 mg, 0.66 mg or 1 mg in ovariectomized animals for 2 weeks, with the objective of inducing local beneficial effects in the vagina without significant systemic action.
Results: After 2 weeks, serum dehydroepiandrosterone, androst-5-ene-3β,17β-diol and dehydroepiandrosterone-sulfate were increased over a 4 h time period, but serum testosterone, estradiol, estrone and dihydrotestosterone remained below detectable levels. The suppository vehicle alone produced minimal epithelial thickening limited to the vaginal distal half. The morphological effects of dehydroepiandrosterone on vaginal mucosa were observed at the lowest dose and consisted mainly of a typical androgenic effect of epithelial mucification. No change in morphological features related to cell proliferation was observed at any dehydroepiandrosterone dose on uterus, mammary gland and skin. At the highest dose, body weight showed a significant decrease, thus indicating a systemic effect on lipid accumulation. Immunohistochemistry for androgen, estrogen alpha and progesterone receptors did not reveal any significant systemic effects in the uterus, mammary gland and skin except some suggestion of increased androgen receptor labeling in mammary gland and skin at the highest dehydroepiandrosterone dose.
Conclusion: The present data show that intravaginal dehydroepiandrosterone can exert beneficial effects limited to the vagina.

Keywords: DHEA dehydroepiandrosterone; Hormone replacement therapy; Intracrinology; Menopause; Tissue-specific hormone replacement therapy (TS-HRT); Vaginal atrophy

1. Introduction

At postmenopause, vaginal dryness and related urogenital disorders such as pruritus, inflammation and dyspareunia, resulting in sexual dysfunction, affect about 50% of women at the age of 50–60 years and over 70% after the age of 70 years [1,2]. While estrogen loss is believed to be the most common cause of vaginal symptoms, there are good reasons to believe that postmenopausal urogenital atrophy and impaired sexual function are also related to low androgens [3,4]. In fact, at postmenopause, women suffer not only from the cessation of ovarian estrogen secretion, but also from the gradually declining levels of androgens produced locally by intracrine mechanisms from adrenal DHEA [5–7]. Indeed, serum DHEA and DHEA-S progressively decrease from the age of 30 to 50 years [8,9]. The decrease in serum DHEA is parallel to the decrease in total androgens, a 60% loss of total androgenic activity being already present at time of menopause [9]. There is an increasing interest in systemic androgens or combined estrogen–androgen replacement therapy [10,11].

The use of systemic exogenous estrogens is now generally restricted to the shortest possible duration of treatment of acute menopause symptoms. Indeed, estrogens, alone or combined with progestogen, have been shown to increase the risk of breast cancer [12–15] and urinary incontinence [16,17]. Moreover, local vaginal estrogen preparations are usually prescribed to provide relief, but the endometrium may be stimulated by the

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unopposed estrogen [18] and the risk of breast cancer is present [19,20]. Indeed, a series of studies have shown that estrogens administered locally in the vagina reach the systemic circulation [20–26]. In fact, although intravaginal estrogen formulations were developed to avoid systemic exposure to estrogens, a series of data have clearly demonstrated that such preparations intended for exclusive local estrogen action lead to relatively high serum estrogen levels, clearly indicating that their use is an issue related to an increased risk of breast cancer and possibly also uterine cancer. As an example, Vagifem, an estradiol tablet, when administered at the 25 mcg dose, led to serum E2 levels of 80 pmol/l at 2 h with values below 50 pmol/l at 14 h and later [26]. Based upon recent advances in our understanding of human sex steroid physiology, especially in postmenopausal women [5–9], the use of replacement with physiological doses of DHEA has become a possibility in order to provide women with the appropriate levels of both androgens and estrogens synthesized locally in specific tissues by intracrine mechanisms while avoiding or minimizing systemic effects [27–29].

In a previous experiment, we have used the ovariectomized (OVX) rat model to assess the effects on the vaginal mucosa of a pharmacological dose of micronized DHEA applied on the dorsal skin [30]. In that study, reversal of vaginal epithelial atrophy with formation of a thick mucified epithelium—a typical androgenic effect in the rodent [31,32], was observed. Another long-term study using the OVX rat model has shown that intravaginal application of DHEA achieved a significant morphological effect at a dose 10 times lower than that found to be active following application of DHEA on the dorsal skin, although the morphological analysis was limited to the superficial layer of the vaginal mucosa [33]. Those results led us to investigate the lowest possible physiological dose of DHEA which could be applied intravaginally to induce physiological morphological effects on the three layers of the vaginal wall.

In two clinical studies, either a single dose or 6-month intravaginal daily treatment with 150 mg DHEA were used to evaluate pharmacokinetic parameters and/or to obtain regression of low-grade cervical dysplasia, in premenopausal women [34,35]. None of the two studies documented any significant adverse effect of such high dose of DHEA on vaginal tissue or at the systemic level. To the best of our knowledge, no preclinical or clinical trial has been performed using intravaginal DHEA to study its potential beneficial effects on morphology of the three layers of the vaginal wall.

The present study uses the OVX rat as a model to examine the effects of intravaginal administration of physiological doses of DHEA in a suppository formulation. In addition to the vagina, the effects of DHEA are analysed on the morphology of other sex steroid-sensitive tissues, body weight, and serum levels of DHEA and its metabolites. Morphological examination of the vagina and other potential target tissues, namely the uterus, mammary gland and dorsal skin was used to discriminate local and systemic effects. Using immunohistochemistry (IHC), cell proliferation and steroid receptor labeling were examined in the above-mentioned organs to further detect any systemic effect of DHEA.

Since the rat adrenal does not secrete DHEA or DHEA-S [5,36], the intravaginal administration of DHEA in OVX animals, while preventing first pass through the liver, was the only source of sex steroids in the model used, thus facilitating interpretation of the data. Furthermore, since the rodent tissues are known to possess the steroidogenic enzymes – including 17β-hydroxysteroid dehydrogenases and aromatase – required for local transformation of DHEA into androgens and estrogens [37], and since the morphological effects of these hormones on the vaginal mucosa have been well described [30], the OVX rat is an appropriate model for the study of the effects of intravaginal DHEA on the three layers of the vaginal wall.

2. Materials and methods

2.1. Animals and treatments

Ten to twelve week-old female Sprague–Dawley rats (Crl:CD®(SD)Br VAF/Plus™) (Charles River Laboratory, St-Constant, Canada) weighing approximately 220–270 g at start of the experiment were used. The animals were acclimatized to the environmental conditions (temperature: 22 ± 3 °C; humidity: 50 ± 20%; 12-h light/12-h dark cycles, lights on at 07:15 h) for at least 1 week before starting the experiment. The animals were housed individually and were allowed free access to water and rodent food (Lab Diet 5002, Ralston Purina, St. Louis, MO). The experiment was conducted in accordance with the CCAC Guide for Care and Use of Experimental Animals in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

A total of 32 female rats were randomly distributed into 7 groups as follows: (1) four intact controls at metestrus ($n = 3$) or diestrus ($n = 1$); (2) three intact controls at estrus; (3) three ovariectomized control (OVX); (4) four OVX + placebo suppository; (5) six OVX + 0.33 mg DHEA/suppository; (6) six OVX + 0.66 mg DHEA/suppository; (7) six OVX + 1 mg DHEA/suppository. DHEA was obtained from Scheweizerhall Inc. at a purity of 100%. On the first day of the study, the animals of all groups (except groups 1 and 2) were bilaterally ovariectomized (OVX) under isoflurane-induced anesthesia. On day 5 of the study (first dosing day), the suppository (volume of 50 μl, once per day for 14 days) was gently inserted in the vagina and pushed 2–3 mm with a small rounded-end glass rod. On the same day, blood samples (0.5 ml) were collected, under isoflurane anesthesia, from the jugular vein of 3 assigned rats from groups 5, 6 and 7, at 30 min, 1, 2, 4 and 7 h after suppository applicator. All blood samples were centrifuged and frozen for further measurement of DHEA metabolites performed separately for each blood sample.

Twenty-four hour after the last dosing, overnight fasted animals were sacrificed by exsanguination at the abdominal aorta under isoflurane anesthesia. The vagina, uterus, inguinal mammary glands and dorsal skin were collected and immediately immersed in 10% neutral buffered formalin for 24 h and then trimmed and processed as described below.