

Effect of long-term topical application of dehydroepiandrosterone (DHEA) and oral estrogens on morphology, cell proliferation, procollagen A1 and androgen receptor levels in rat skin

Louise Berger, Mohamed El-Alfy, Céline Martel and Fernand Labrie*

Research Center in Molecular Endocrinology, Oncology and Human Genomics, Laval University Hospital Research Center (CRCHUL), Quebec City, Canada

Abstract

Background: After cessation of estrogen secretion by the ovaries at menopause, all estrogens and almost all androgens acting in the skin of postmenopausal women are synthesized locally from dehydroepiandrosterone (DHEA), a prohormone of adrenal origin that progressively declines with age.

Objective: To better understand the effects of DHEA on the skin, ovariectomized (OVX) rats were treated for 9 months with local topical application of DHEA compared with oral conjugated equine estrogens.

Materials and methods: Morphological evaluation, immunohistochemistry for androgen receptor (AR) and Cdc47 proliferation marker, and in situ hybridization for procollagen A1 were performed on dorsal skin.

Results: Local topical DHEA application increased the thickness of the granular cell layer and total epidermis in OVX animals, whereas systemic estrogens had no significant effect. Although DHEA did not affect total dermal thickness, a 190% increase in dermal procollagen A1 mRNA was observed. Moreover, DHEA treatment decreased hypodermal thickness by 47% and increased skin muscle thickness by 58%. In the epidermis, DHEA induced a non-significant increase in cell proliferation, whereas AR labeling was increased in both the epidermis and dermis by DHEA.

Conclusions: Although estrogens did not significantly modify any of the above-mentioned parameters, the androgenic action of DHEA induced significant changes in all skin layers, without any sign of toxicity or lack of tolerance to DHEA after a 9-month local application of 4% (80 mg/kg) DHEA on the skin.

Keywords: androgen receptor; dehydroepiandrosterone (DHEA); intracrinology; menopause; skin atrophy.

Introduction

The skin is the largest organ in the body and represents the organ where aging-related changes can be more easily seen. Skin is a target organ as well as a site of formation of a series of hormones (1, 2), in particular sex steroid hormones, which have a profound influence on skin development and composition; adequate levels of steroids are required to maintain its structural integrity and functional capacity (3). In women's skin, menopausal changes lead to thinning and wrinkling as well as loss of elasticity and firmness (4). These signs of aging are believed to be caused by decreased collagen synthesis and increased collagen degradation (5), as well as a decrease in interfibrillar ground substance and blood vessels and irregularities in the size and shape of epidermal cells (6). Some of these changes can occur rapidly at the onset of menopause; for example, approximately 30% of dermal types I and III collagen are lost within the first 5 years after menopause (7, 8).

The beneficial influence of systemic estrogens and their derivatives, alone or in combination with progesterone, on the skin of postmenopausal women is well documented and includes increase in skin thickness, collagen content, elasticity, epidermal hydration and surface lipids (9, 10). Nevertheless, the findings of recent clinical studies have raised issues about the safety of long-term systemic replacement of these two hormones in postmenopausal women (11). Local topical application of dehydroepiandrosterone (DHEA) to postmenopausal women has shown a reduction of the hypodermal layer thickness and an increase in sebum production (12, 13). Moreover, improvements of skin hydration, epidermal thickness, sebum production and reduction of pigmentation after oral administration of DHEA have also been observed (14).

Most of these above-mentioned effects could be explained by the action of androgens and estrogens produced by local intracrine transformation of DHEA, through the action of steroidogenic enzymes of the skin (1). Indeed, in the human as well as in the rodent model used in the present study, cells from all skin compartments express all or most of the androgen- and estrogen-synthesizing enzymes required for local intracrine formation of androgens and estrogens (1, 15–26). In postmenopausal women, all sex steroids, except a small amount of testosterone of ovarian origin, are produced in the

*Corresponding author: Dr. Fernand Labrie, Director, Research Center in Molecular Endocrinology, Oncology and Human Genomics, Laval University and Laval University Hospital Research Center (CRCHUL), 2705 Laurier Boulevard, Quebec City, QC, G1V 4G2, Canada
Phone: +1-418-653-0055, Fax: +1-418-653-1794,
E-mail: flabrie@attglobal.net
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skin from the intracrine transformation of adrenal precursors, especially DHEA (1, 16, 18). These steroids exert their action through their cognate androgen receptor (AR) and estrogen receptor (ER). The former is present in epidermal and follicular keratinocytes, sebocytes, sweat gland cells, dermal papilla cells, dermal fibroblasts and in the endothelial cells of blood vessels (27, 28), and localization of ER alpha and beta in the skin and its appendages has also been extensively described (29, 30). Although actions of estrogens and, to a lesser extent, of androgens, have been described in the skin, the effects of DHEA on the different skin layers have not been characterized in detail. Short-term studies with DHEA have been performed in the ovariectomized (OVX) mouse model and in the OVX rat in an attempt to answer this question (24, 31, 32).

In the human, it is known that the adrenal secretion of DHEA progressively decreases from the age of 30 years to represent only 40% of young age levels at the time of menopause (33, 34). In the present study, we have used the OVX rat as a model to study the effect of local topical application of DHEA for 9 months on all dorsal skin layers. Meanwhile, these effects were compared with the effects of orally administered conjugated equine estrogens (CEE) used as an equivalent of systemic estrogen replacement therapy. As the rat adrenal does not secrete DHEA or DHEA-sulfate (35), the topical administration of DHEA in OVX animals, while preventing first pass of the orally administered steroid through the liver, is the only source of sex steroids in this model, thus facilitating interpretation of the data.

Materials and methods

Animals and treatments

Female Sprague-Dawley rats, 10–12 weeks old (CrI:CD[®](SD)Br VAF/Plus[™]) (Charles River Laboratory, St Constant, QC, 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 and 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, USA). The experiment was conducted in accordance with the Canadian Council on Animal Care (CCAC) Guide for Care and Use of Experimental Animals in an animal facility approved by the CCAC and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

A total of 56 rats were randomly distributed into four groups of 14 animals (five rats per group were used for histology) as follows: (i) INTACT control; (ii) ovariectomized control (OVX); (iii) OVX+CEE (0.5 mg/kg/day orally); and (iv) OVX+DHEA (80 mg/kg/day topically on the skin). On the first day of the study, the animals of groups 2, 3, and 4 were bilaterally ovariectomized (OVX) under isoflurane anesthesia. The DHEA, obtained from Schweizerhall Inc. (Piscataway, NJ, USA) at purity near 100%, was solubilized in a solution of 50% ethanol and 50% propylene glycol and was topically applied (0.5 mL per rat) on a shaved area of 4 × 4 cm of the dorsal skin. The DHEA was administered at a pharmacological dose, with DHEA blood levels ranging between 70 and

100 nM (34). CEE was administered by oral gavage (0.5 mg/kg/day in 0.5 mL per rat) as a suspension in 0.4% methylcellulose. CEE was a product of Wyeth-Ayerst Canada Inc. (Montreal, QC, Canada) for intravenous human use containing 24.8 mg per vial of total CEE of which 54.4% was sodium estrone sulfate and 28.7% sodium equilin sulfate. Dosage selection for CEE corresponds to the minimal dose sufficient to reverse OVX-induced uterine atrophy, as determined after estradiol and CEE dose-response studies (unpublished data). Treatment was initiated on day 2 of the study and the compounds were administered once daily for 36 weeks. All animals from the INTACT and OVX control groups received the two vehicles by oral gavage and topical application. The animals were sacrificed under ketamine/xylazine-induced anesthesia by intracardiac perfusion with 10% neutral buffered formalin.

Histological procedures

A piece of approximately 1.5 cm² of dorsal skin was collected at the treatment site from each animal, laid and flattened on a piece of carton, then immersed in 10% neutral buffered formalin for 24 h. The fixed skin was then trimmed to two rectangular pieces of approximately 1.5 cm of length × 0.3 cm of width. After processing in paraffin, the two skin pieces of a given animal were vertically embedded in the same paraffin block. For each animal, 4 μm-thick paraffin sections were prepared for hematoxylin-eosin staining (H/E), immunohistochemistry and in situ hybridization (ISH).

Histomorphometry

For each animal, the thickness of the different skin layers (H/E) and the labeling for procollagen A1-bound mRNA (ISH) were measured on the sections of the two skin pieces, for all the animals of each group. Images were captured with a DC-330 3CCD color camera (Dage-MTI, Michigan City, IN, USA) and quantified using Image-Pro Plus 3.0 software (Media Cybernetics, Bethesda, MD, USA). For thickness measurements, the 20× objective was used for the epidermis and the 2.5× objective for the dermis, hypodermis, and skin muscle (Leica, Richmond Hill, ON, Canada). For each skin layer, a total of 8–16 representative images per animal were captured and 4–8 thickness measurements for each image were obtained from the two skin pieces. For procollagen A1 labeling measurements (number of labeled pixels), a 5× objective was used to capture 3–4 images from the dermis per skin section; then, one measurement for each image was performed. To prevent measuring the background silver grains of ISH labeling, a representative OVX control antisense slide was used to preselect and adjust certain parameters of the image analysis program to measure the area of the labeled fibroblasts while excluding the background.

Immunohistochemistry

Immunostaining was performed using Zymed SP kits (San Francisco, CA, USA). Paraffin sections were deparaffinized in toluene and rehydrated through ethanol. Endogenous peroxidase activity was eliminated by preincubation with 3% H₂O₂ in methanol for 30 min. A microwave retrieval technique using 0.01 M citrate buffer for 15 min was applied (36). After cooling the slides, non-specific binding was blocked using 10% goat serum for 40 min. Sections were then incubated for 1.5 h at room temperature with AR (1:250, N-20, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) or Cdc47 (1:400, NeoMarkers, Fremont, CA, USA) antibodies. After washing in phosphate buffered saline, sections were incubated with biotinylated anti-rabbit or anti-mouse secondary antibody for 10 min and thereafter with streptavidin-peroxidase for another 10 min (Zymed,

San Francisco, CA, USA). Diaminobenzidine was used as the chromogen to visualize the biotin/streptavidin-peroxidase complex, under microscope monitoring. Counterstaining was performed using #2 Gill's hematoxylin for 30 s.

For controls, immunoabsorption with an excess of the peptide used to raise the antibody, or substitution with non-immune rabbit IgG, was performed. Only a minimal background staining was observed on the negative control slides. To evaluate the immunostaining labeling, a semiquantitative analysis of AR and Cdc47 labeling was performed in the epidermis, for each skin section. In this skin layer, to evaluate the increase in the number of labeled nuclei and their staining intensity, a combined score ranging from + to +++ (Cdc47) or + to ++++ (AR) was initially used for comparison between the treated and control groups. Then, these results were transformed into numbers (1–3 for Cdc47 and 1–4 for AR) in order to be presented in a histogram. In the dermis, hypodermis, and *panniculus carnosus* (skin muscle), a qualitative evaluation for AR and Cdc47 labeling was performed.

In situ hybridization

A 49-bp fragment corresponding to nucleotides 4402–4450 of the full-length rat procollagen alpha 1 type 1 gene (GeneBank access no. Z78279) was inserted in the vector pCR-Blunt II-TOPO (Invitrogen, Burlington, ON, Canada). Antisense and sense riboprobes were generated by incubation of the linearized templates (2 µg, using *Bam*HI and *Xho*I, respectively) with 65 µCi of [³⁵S] UTP (1250 Ci mmol⁻¹; NEN, Newton, MA, USA), in presence of T7 or SP6 RNA polymerase, for 60 min at 37°C, according to the manufacturer's specifications (Riboprobe Combination System, Promega, Madison, WI, USA). After deparafinization and rehydration, skin sections were treated with proteinase K (1 µg mL⁻¹ in 100 mM Tris-HCl, 50 mM EDTA, pH 8.0, for 30 min at 37°C) and with acetic anhydride (0.25% × 10 min). After dehydration and drying of the sections, a prehybridization buffer (60% formamide, 0.35 M NaCl, 12 mM Tris base, pH 8.0, 1 mM EDTA, 1.2 × Denhardt's solution, 400 µg mL⁻¹ tRNA, and 12% dextran sulfate) was applied on the sections for 2 h at room temperature, followed by the hybridization buffer, containing the probe [prehybridization buffer plus 50 mM dithiothreitol (DTT) and 2 × 10⁶ cpm of ³⁵S-labeled RNA per slide] for an overnight incubation at 53°C. To remove the excess probe, the sections were washed twice in 50% formamide, 2 × SSC (saline-sodium citrate buffer) at 58°C for 90 min, with an RNase A treatment (20 µg mL⁻¹ × 30 min at 37°C) between the two washes. The slides were dehydrated in graded alcohol, air-dried, and then dipped in a photographic emulsion (LM-1, Amersham Biosciences, Buckinghamshire, UK) and exposed in the dark at 4°C for 8 days.

Slides were developed (Kodak D-19 Developer and Kodak Rapid Fixer, Kodak, Rochester, NY, USA) and counterstained with H/E. The negative controls consisted of the hybridization with the sense probe: only a weak background signal was detected on these sections.

Statistical analysis

For the scoring of Cdc47 and AR immunoeexpressions and for measurement of the labeling for procollagen A1 in the different layers of dorsal skin, data are presented as means ± SEM of five animals per group. Statistical significance was determined according to the multiple range test of Duncan-Kramer (37).

Results

DHEA induced a series of morphological changes in the various skin layers. Histopathological assessment revealed no sign which could suggest toxicity, thus indicating good tolerance of all skin layers to relatively high concentrations of DHEA applied on the skin for 9 months.

Epidermis

The epidermis of the rat dorsal skin consists of a *stratum basale*, a relatively thin *stratum spinosum*, the *stratum granulosum*, and the *stratum corneum*. Nine months after OVX, there was a moderate thickening (43%) of the epidermis with no significant change of the dermis or hypodermis (Figures 1 and 2). After 9 months of DHEA treatment of OVX animals, the total epidermal thickness was significantly higher by 41% than that of OVX animals. The *stratum granulosum* of the DHEA-treated group was thicker and the most prominent among all groups, with large granular cells containing numerous large keratohyalin granules (Figure 1). The epidermis of the DHEA-treated rats also shows a significant increase in the thickness of the *stratum corneum* (Figure 1D), thus contributing to an increase of total epidermis thickness (41%, Figure 2A). However, because of the inherent distortion of the *stratum corneum* resulting from tissue processing, no attempt was made to quantitate its thickness. CEE had no significant effect on the thickness of the epidermis.

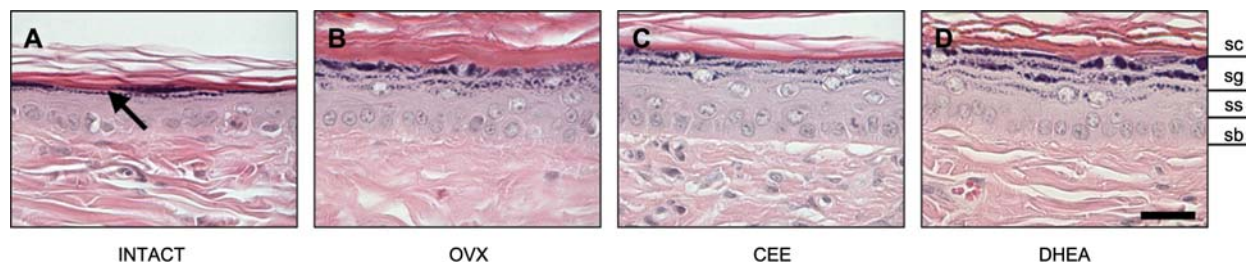


Figure 1 Paraffin sections of the keratinized stratified squamous epithelium of female rat dorsal skin epidermis and a small part of the upper dermis. The four skin layers are delineated on the right as: sc, *stratum corneum*; sg, *stratum granulosum*; ss, *stratum spinosum*; and sb, *stratum basale*. The epidermis, including *stratum granulosum* (arrow) is thinner in the INTACT group. The thickness of *stratum granulosum* and the size of its granular cells are the highest in the DHEA-treated group, when compared with the other three groups. Magnification (200×). Scale bar = 30 µm.

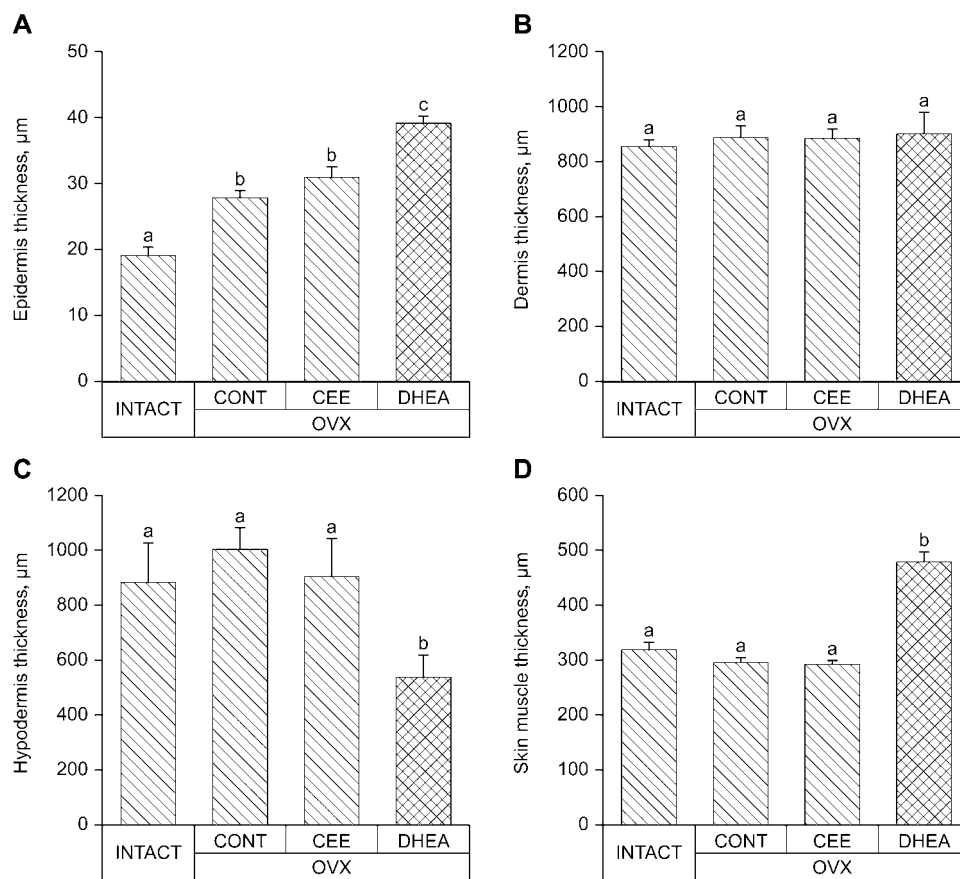


Figure 2 Thickness of the different skin layers of the female rat after ovariectomy (OVX) and CEE or DHEA treatment of OVX animals. (A) In OVX control animals, a 47% increase in epidermal thickness was observed when compared with the INTACT control group. DHEA further increased epidermal thickness by 41% when compared with the OVX group and by 27% when compared with the CEE group. (B) No change was observed in the dermal thickness of the four examined groups. (C) Only DHEA induced a significant decrease (47% vs. OVX) in the thickness of the hypodermis. (D) Of all groups, a marked thickening of the skin muscle layer (58% vs. OVX) was observed only in the DHEA-treated group. Means \pm SEM, $n=5$ per group. Groups sharing the same letter are not significantly different at $p<0.05$.

When Cdc47 was used as marker of cell proliferation, labeling in the epidermis was found to be significantly higher in the control OVX, OVX+CEE and OVX+DHEA-treated groups compared with the INTACT group. The highest level of cell proliferation was observed in the DHEA group with a non-significant 33% increase over OVX control. The immunolabeling for AR, by contrast, showed a 130% increase over INTACT control in the DHEA group, the difference being highly significant when compared with all other groups ($p<0.01$) (Figure 3B). Interestingly, Cdc47 immunolabeling for cell proliferation was restricted to the basal cell layer, whereas AR labeling was localized in both the basal and suprabasal cell layers (data not shown).

Dermis

The only morphological difference between the four groups seen in the dermis was the enlarged sebaceous glands of the DHEA-treated animals (Figure 4D), whereas the dermal thickness displayed similar values in the four groups (Figure 2B). Immunostaining for cell proliferation revealed few stained fibroblasts in all groups. The expression of AR in

the fibroblast nuclei was weak or absent in the dermis of INTACT, OVX, and OVX+CEE groups, whereas it was significantly higher in the dermis of DHEA-treated animals (data not shown).

In all groups, ISH for procollagen A1 displayed a clear cytoplasmic silver grain labeling in the dermal fibroblasts (Figure 5). Quantitative analysis of silver grain-labeled areas revealed a higher level of labeling in the DHEA group when compared to the other three groups and a statistically significant 190% increase when compared with the OVX control group (Figure 6). We have no explanation for the higher labeling in the OVX compared with INTACT control groups (Figure 6).

Hypodermis

After 9 months of treatment, a marked decrease of the thickness of the hypodermis was observed only in the DHEA-treated group, when compared with the other three groups, a 46% decrease in thickness being found when compared with the OVX control group (40%) (Figure 2C). CEE treatment did not induce any significant change. After immuno-

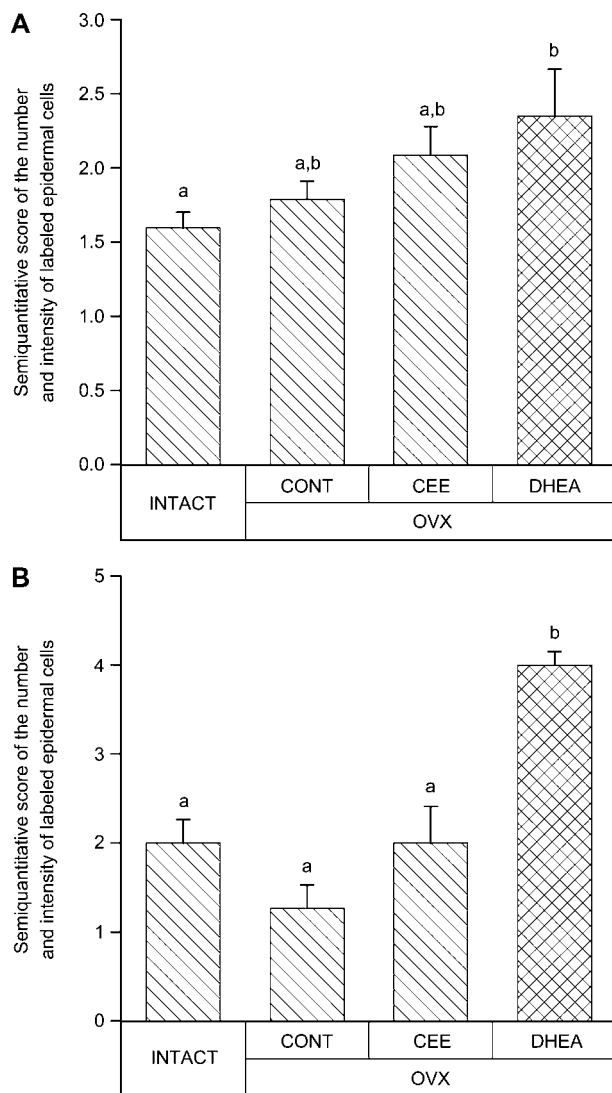


Figure 3 Semiquantitative scoring evaluation of the Cdc47 cell proliferation marker and AR in the epidermis of intact and OVX rats treated with CEE and DHEA. (A) When the number and staining intensity of proliferating cells were compared by scoring on a scale of 0–3, the highest score obtained was in the DHEA-treated group, although the difference was not significant. (B) Similarly, when the number and staining intensity of AR-labeled nuclei were compared by scoring on a scale of 0–4, the DHEA-treated group was found to be the highest, the value being significantly different from the three other groups. Means \pm SEM, $n = 5$ per group. Groups sharing the same letter are not significantly different at $p < 0.05$.

staining for Cdc47 or AR, a similar pattern of rare scattered labeled adipocyte nuclei was detected in all groups (data not shown).

Panniculus carnosus (skin striated muscle)

A marked increase of the skin muscle layer thickness was observed in the DHEA-treated group, when compared with all other groups, whereas CEE had no effect (Figure 2D). No immunolabeling for cell proliferation or for AR was detectable in any group (data not shown).

Discussion

Skin aging, namely cellular atrophy, epidermal thinning, declining collagen dermal content, and decreased skin thickness, leading to dryness, laxity, and wrinkling, as well as impaired wound healing, have usually been attributed to estrogen deprivation caused by cessation of ovarian estrogen secretion at menopause (7, 9, 38). Conversely, the effects of peri- and postmenopausal changes in androgens on the skin have received little attention in the literature. As an exception, decreased sebaceous gland size and hair growth in the axillary and pubic regions have been suggested as consequences of a decline in tissue concentration of androgens (39).

Although the use of estrogens in postmenopausal women is limited by their associated risks (11), the accumulation of documented favorable effects of DHEA in the skin increases the interest for the use of topical DHEA in limited skin areas for the prevention and treatment of postmenopausal skin aging, as well as impaired wound healing (12, 14, 24, 40–42). The interest in the action of DHEA pertains to its tissue- and even cell-specific conversion to physiological amounts of intracellular estrogens and/or androgens, according to the specific enzymatic battery, steroid receptors and cofactors present in each cutaneous tissue compartment (1, 43) and the excretion of these steroids as glucuronidated inactive metabolites (34). The process of intracrinology permits a more physiological sex hormone biosynthesis and local control of hormone tissue action (43). In the present study, we report specific effects of DHEA on the dorsal skin of OVX rats for each cutaneous compartment, the effect of DHEA being potentially due to a combination of androgenic and estrogenic effects.

Epidermis

The main features suggesting an androgenic effect of DHEA in the observed increased epidermal thickening are, firstly, predominance of the granular cell layer in the DHEA-treated animals, a feature which was less apparent with CEE treatment, and, secondly, the intense AR immunolabeling in epidermal cell nuclei, a well-known AR upregulation effect of androgens (44). In agreement with our results, previous studies have shown increased epidermal thickening with prominent granular cell layers in the rat (45, 46). Moreover, in cultured human epidermis, DHEA and testosterone treatment significantly increased the number of granular cell layers, despite a negligible effect on epidermal thickness (47, 48).

A role of estrogens synthesized from aromatization of androgens derived from DHEA cannot be excluded in the observed epidermal thickening. Indeed, an experiment in the OVX mouse (32) has shown a significant epidermal thickening after estradiol treatment, whereas with dihydrotestosterone (DHT), a non-aromatizable androgen, epithelial thickness was not significantly different from the OVX control group, thus supporting the possibility of an estrogenic effect in the increased epidermal thickening induced by DHEA. The trend for increased epidermal cell proliferation with DHEA treatment is compatible with other studies

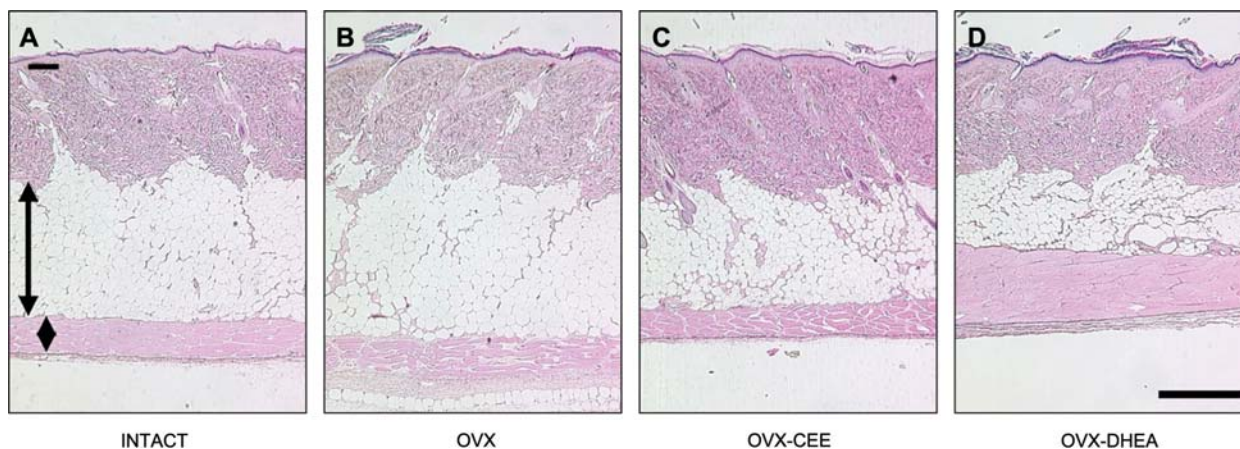


Figure 4 Low magnification (50 \times) microphotographs of the whole female dorsal skin of the four examined groups. In the first picture representing the INTACT group (A), the black band in the upper part separates the epidermis from the dermis. The long vertical double-headed arrow represents the hypodermal thickness, whereas the lozenge ends delineate the thickness of the skin muscle layer. As clearly seen in (D), the hypodermal thickness is the thinnest, whereas the muscle layer is the thickest in the DHEA-treated group. Scale bar = 100 μ m.

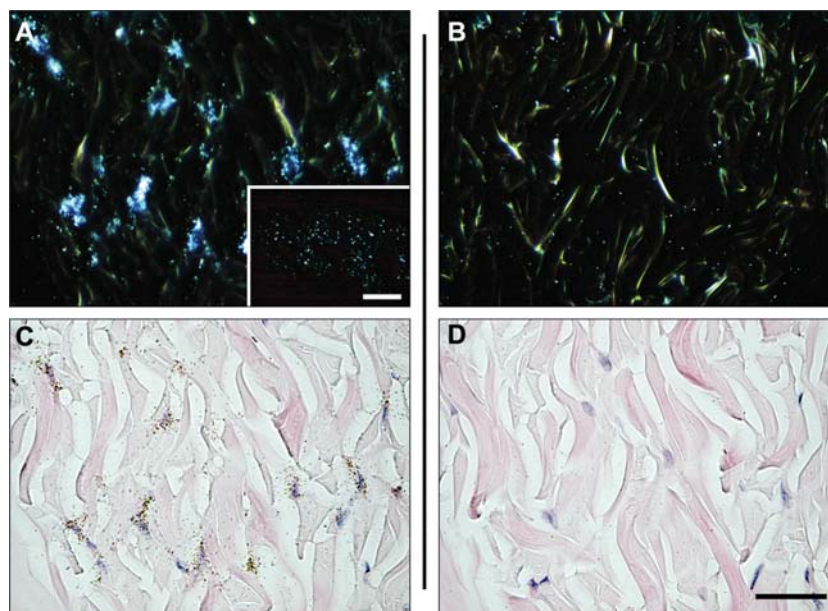


Figure 5 Autoradiographs of 35 S-labeled procollagen A1 antisense (left) and sense (right) riboprobes (49 bp) hybridized in situ to the cytoplasm of dermal fibroblasts of an OVX rat treated with DHEA (as an example) taken at high magnification (400 \times), in dark (upper row) and bright (lower row) field. Measurement of the labeled area was performed on antisense slides, in dark field microscopy at low magnification (25 \times), as shown on the insert. In a consecutive serial section, hybridized with the sense probe, only scattered background silver grains could be detected. Upper scale bar = 300 μ m and lower scale bar = 30 μ m.

describing similar results when using testosterone treatment in OVX (49) or castrated rats (50), or estradiol in human cultured keratinocytes (51). Similarly, in a wound healing rat cell culture model of re-epithelialization, a high DHT treatment dose, while hindering the keratinocyte migration step, did not affect proliferation (52).

In the present study, the increase in epidermal thickness observed in the OVX group could have masked the potential CEE-induced thickness increase. However, the difference in

administration routes could have influenced the extent of the observed epidermal thickening: indeed, DHEA was topically applied at a 4% concentration and the evaluated skin area was the application site, whereas CEE was administered orally, thus exerting a “diluted” systemic effect, although the dose was also in the pharmacologic range.

Contradictory results have been reported regarding the efficacy of topical estrogen treatment in increasing epidermal thickness, with positive results reported when used alone

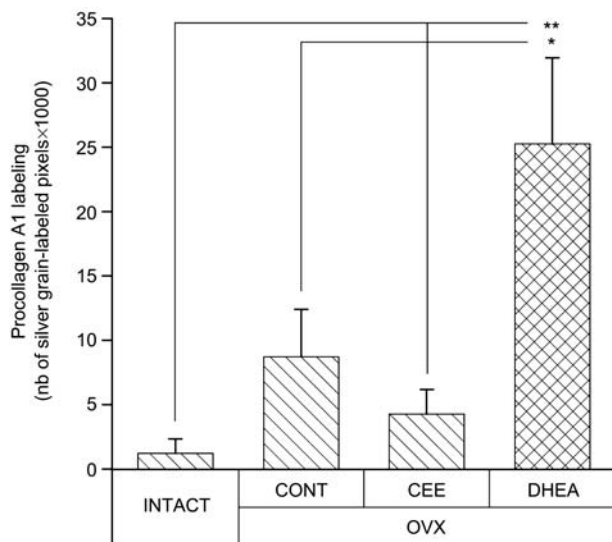


Figure 6 Graph representing the number of pixels from labeled fibroblasts over the total area, in the dermis of the four examined groups. Only the DHEA-treated group clearly displays a significant high level of procollagen A1 mRNA expression compared with the other three groups. Means \pm SEM, $n=5$ per group. * $p<0.05$, ** $p<0.01$.

(53) or combined with systemic hormone replacement therapy (54) or no effect observed on epidermal and dermal thickness (55). Meanwhile, re-epithelialization of acute wounds in elderly women can be accelerated with systemic estrogen treatment (56). A large randomized, placebo-controlled trial reported an increase in epidermal thickness after 12 months of oral DHEA administration to postmenopausal women (14). Altogether, the available data suggest that the androgenic component of DHEA is likely to exert a significant role in the increase in epidermal thickness, when compared with that of the CEE group, although an estrogenic component in DHEA action cannot be ruled out.

Dermis

The present data show that a pharmacological dose of DHEA, in the rat model, exerts a beneficial effect on the skin dermis by markedly increasing (190%) the expression of procollagen A1 mRNA. Similarly, a 160% increase in $\alpha 1$ procollagen mRNA, along with a decrease in collagenase mRNA, was observed after treatment of cultured human skin fibroblasts with a pharmacological dose of DHEA (57). In the human, a significant increase in type I procollagen mRNA and protein, as well as an increased inhibition of collagen degradation, was observed in men after a 1-month topical application of 5% DHEA (40), whereas total skin collagen was found to be decreased in women with osteoporosis and increased after treatment with androgens (58).

In agreement with the present data, treatment with oral DHEA accelerated wound healing in OVX rats through increased collagen 1 deposition and a lowered inflammatory response (24). Although these effects were suggested to occur via DHEA conversion to estrogens (24), our findings

suggest an androgenic effect in the stimulatory action of DHEA on procollagen A1 expression, in the context of a normal hormonally deprived skin model. Supporting this explanation, studies in animal models and in the human have demonstrated the stimulatory effect of testosterone and DHT on dermal collagen content (32, 58, 59), whereas, in wound healing, the role of androgens could be different (60, 61). However, neutral or a beneficial role of androgens in stimulating the production of type 1 collagen protein have been described in scratch-wounded and intact rat dermal fibroblasts, respectively (59).

The androgenic effect of DHEA on dermal collagen synthesis is also supported by the finding of increased expression of AR in our DHEA-treated animals, consistent with the report of significantly reduced levels of dermal collagen in knockout AR-deficient mice, when compared with their wild type littermates (62). Altogether, the available data suggest that DHEA could use different pathways to induce different effects in the dermis, not only according to each of the skin cell compartments but also in response to the different skin physiological or pathological states.

Hypodermis

In the present study, DHEA treatment caused an important decrease in the thickness of the hypodermal fat layer, when compared with the OVX group, whereas CEE had no effect. These results suggest a predominant androgenic effect of DHEA on the decrease in hypodermal thickness. Similar results were observed after 2 weeks of treatment with DHT or DHEA in OVX mice, whereas a more modest decrease was observed after estradiol treatment (32). Moreover, in a 27-week OVX rat study, 3% DHEA topical treatment led to reduced adiposity and decreased retroperitoneal fat (63). These results are in agreement with a 12-month study in postmenopausal women who received DHEA percutaneously, where a decrease in subcutaneous fat was observed (13).

Panniculus carnosus (skin skeletal muscle)

The present data show a marked increase in the thickness of the skin skeletal muscle induced by DHEA. To the best of our knowledge, this finding has never been reported. It has recently been shown that the rat skeletal muscles possess the key steroidogenic enzymes that can produce testosterone, DHT and estradiol from DHEA by intracrine mechanism (64, 65). The absence of effect of CEE treatment suggests that the stimulatory effect of DHEA on the skin muscle is exclusively androgenic.

In conclusion, the effects observed in different skin models suggest the design of future clinical studies to confirm the possibility of important effects of DHEA on skin aging.

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